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# Apples, Their Antioxidants and Benefits to Human Health

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## 1. Executive Summary

- Apples (MALUS sp., Rosaceae) are an important source of phytonutrients which possess a wide range of biological activities which may contribute to health.

- Apples are a good source of vitamin C, potassium and dietary fibre and very low in saturated fat, cholesterol and sodium. A large portion of the kilojoules from apples comes from sugars which are primarily made up of fructose (~60% of total sugars), glucose (~20%) and sucrose (~20%). As a result, the Glycaemic Index (GI) of apples is between 28 - 44 and is in the low GI category (<55).

- Many of the phytonutrients found in apples are strong antioxidants. The antioxidant activity of apples comes from components called polyphenols – such as quercetin, catechin, phloridzin, and chlorogenic acid. There is a positive correlation between total polyphenols and antioxidant capacity in apples.

- The antioxidant component of apples is concentrated in the peel, with higher levels of antioxidant capacity generally found in darker, redder and bluer coloured apples. Processing of apples into juice significantly reduces antioxidant potential – to 10% or less of the fresh apple. While a number of fruits and juices have higher *in vitro* antioxidant capacity than apples and apple juices, it is important to note that apples are the largest contributors of fruit antioxidants due to levels of consumption. Furthermore, bioavailability studies in humans which are an indication of whether these compounds are absorbed by the body suggest that consumption of apples can change some blood biomarkers in humans, which may be predictive of positive health benefits.

- Small amounts of microbially degraded phenolics may also be absorbed via the colon and exert cell signalling modulatory effects on cellular growth, proliferation and apoptosis. Hence not all the potential health benefits of dietary polyphenols is related to antioxidant effects and further research on their metabolic fate is warranted.

- Different modes of postharvest storage can affect antioxidant capacity and polyphenol content. Cold storage at 1°C increased the antioxidative capacity and polyphenol content in most of the apple cultivars but storage at 20 °C led to a decrease in polyphenol content and in antioxidant capacity. Storage under controlled atmosphere led to stable or small increases of both antioxidant capacity and polyphenol content. This underscores the importance of appropriate refrigerated storage for apples to retain antioxidants.

- Consumption of apples may affect appetite control more than pureed fruit or juice. Eating a solid fruit such as an apple at the start of a meal can reduce kilojoule intake, with a possible subsequent effect on weight reduction. These effects may be related to the solid nature of apples, the time taken to consume them and their low energy density rather than their fibre content.

- Other promising human observational studies suggest that consuming 1 apple per day is associated with a 28% reduced risk of type 2 diabetes compared with those who consumed no apples. This may be related to the potential effects of apples on weight control and their low glycaemic index.

- Amongst the strongest evidence for the role of apples in human health comes from human studies that show that whole apples have a cholesterol-lowering effect in humans, in the range of 5 - 8%. This can be achieved with a daily intake of approximately three whole apples.

- There is some suggestive evidence of benefits of apple consumption on respiratory conditions such as asthma and allergic rhinitis. Further human intervention trials with whole apples are needed before such effects can be confirmed.

- To advance the understanding of the role that apples and their phytonutrients might contribute to health and disease prevention additional targeted areas of research are recommended. These are summarised in the conclusion of this report and includes further compositional analysis of Australian apples, mechanistic studies in appropriate animal models and interventions in humans with whole apple components rather than individual polyphenolics.

## 2. Scope

This review covers human studies relating to apples or apple polyphenols and health-related outcomes. Animal studies relating to apple consumption have also been considered. There have been an array of epidemiological studies on polyphenols from a wide array of fruit other than apples on human health but these fall outside the scope of this review. *In vitro* studies (e.g. on laboratory cell lines) are also outside the scope of this project. This review will specifically focus on studies published in peer-reviewed scientific literature, including databases of clinical trials. The scientific literature covering the period from 1977 to very early 2010 was critically evaluated.

## 3. Nutritional Profiling

Nutritional information has been derived from *NUTTAB 2006*, the online version of the compiled Australian Food Composition Tables available at:
*www.foodstandards.gov.au/monitoringandsurveillance/nuttab2006*

Nutritional tables (which appear in the Appendices) cover:

Apples: raw/unpeeled and processed apples (baked, canned etc)
- Macronutrient content (protein, carbohydrate, fat, fibre, energy, sugars)
- Vitamin content
- Mineral content

Compositional tables for apples are also included for:
- Anthocyanidins
- Flavanols
- Flavonols
- Flavanones
- Proanthocyanidins

Information on specific named varieties of apples is difficult to obtain, with most databases including only generic apple data – mostly from an undefined variety but sometimes from an average of a number of varieties. Thus, there is no additional data to compare with the NUTTAB Australian data for Granny Smith and Pink Lady apples.

The food composition tables for processed apples from the USDA tables were the only other set with sufficient detail to include for confirmation and comparison with Australian data. Relevant data from the USDA food composition material, from the National Nutrient Database for Standard Reference Release 21 is included in the appendices.

The information on flavonoid and proanthocyanidin content was extracted from USDA tables which were compiled from a variety of scientific publications. The full details of these sources can be obtained from:

USDA Database for the Flavonoid Content of Selected Foods Release 21 January 2007. Available at: http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/Flav02-1.pdf

USDA Database for the Proanthocyanidin Content of Selected Food, August 2004. Available at: http://www.nal.usda.gov/fnic/foodcomp/Data/PA/PA.pdf

## 4. Recommended Daily Intake (RDI) Values

Over the past ten years, the concepts of Recommended Daily Intakes (RDIs) or Recommended Daily Allowances (RDAs) have been expanded to a more complex set of tables known as the Nutrient Reference Values. These retain the concept of RDIs (or the average daily dietary intake sufficient to meet the requirements of nearly all healthy individuals in a particular life stage and gender group) and also include the concept of an Estimated Average Requirements (EARs), or daily nutrient levels estimated to meet the requirements of half the healthy individuals in a particular life stage and gender group. Where no RDI has been determined, they are replaced by Adequate Intakes (AIs) or an assumed measurement of observed or experimentally obtained approximation of nutrient intake by groups of apparently healthy people. Current tables also include an Upper Limit (ULs) or highest level likely to produce no adverse health effects. The latest summary also includes a table of recommended intakes to reduce chronic disease risk for people 14 years and over. The latest summaries of Australian tables are available at: www.nhmrc.gov.au/PUBLICATIONS/synopses/n35syn.htm. The Australian system is in line with similar review recommendations from a wide range of countries such as the USA, Canada, UK, Germany, Austria and Switzerland.

Although apples contain a wide range of vitamins and minerals most are in small quantities and therefore, it seemed not to be productive to try to calculate contents of the apples against these now quite complex tables – for example: Thiamin AIs and RDIs 0.2-0.3 mg per day for infants through to 0.4-1.0 mg for adolescents and 0.9-1.2 mg per day for adults, and the range present in these fruits and fruit products ranges from 0 to 0.02mg.

An examination of the nutrition data which uses the USDA Food Composition Tables was used to produce Nutrition Facts style labels showing an average percentage of Daily Values (DV) against American requirements. As the composition tables use 100g as a standard measurement, we produced sample nutrition facts tables for fresh apples to check the daily values provided. It should be noted that 100g is smaller than definitions of a medium apple in different eating/nutritional guides worldwide.

Fresh apples, raw with skin contain vitamin C at 8% DV. All other available calculations equate to levels less than 5% DV.

**Composition of Apples (*Malus domestica*), raw with skin (USDA data), summary.**
Refuse: 10% core and stem

| Nutrients and units | Amount in 100g of edible portion |
|---|---|
| Water (g) | 85.56 |
| Energy (kJ) | 218 |
| Protein (g) | 0.26 |
| Total lipid, fat (g) | 0.17 |
| Carbohydrate (g) | 13.81 |
| Total dietary fibre (g) | 2.4 |
| Sugars, total (g) | 10.39 |
| Sucrose (g) | 2.07 |
| Glucose (dextrose) (g) | 2.43 |
| Fructose (g) | 5.90 |
| Lactose (g) | 0.00 |
| Maltose (g) | 0.00 |
| Galactose (g) | 0.00 |
| Starch (g) | 0.05 |

Detailed compositional tables appear in the Appendices.

## 5. Determination of Antioxidant Capacity – Criticisms and Issues

The Oxygen Radical Absorbance Capacity (ORAC) assay measures the scavenging capacity of antioxidants in nutrients or *in vivo* against the peroxyl radical, which is one of the most common reactive oxygen species (ROS) found in the body. The ORAC-hydro assay reflects water-soluble antioxidant capacity, while the ORAC-lipo assay measures lipid-soluble antioxidant capacity. The values of these two assays are additive. Trolox, a water-soluble vitamin E analog, is used as the calibration standard and the ORAC result is expressed as micromole Trolox equivalent (TE) per gram. These ORAC assays do not measure the scavenging capacity against other ROS found in the body (Bank and Schauss, 2004).

The activity and mechanism of multifunctional natural antioxidants are affected by many factors. The influence of all these factors cannot be evaluated using a single assay. For example, the relative antioxidant activity of many phenolics varies widely according to testing methods. The ranking of antioxidant activity is highly dependent on the test system and on the substrate to be protected by the antioxidants. The discrepancy in ranking of antioxidants can be explained by the multiplicity of mechanisms effective for these phenolic compounds (Frankel, 2005a, Frankel, 2005b).

In a major comparative study, Ou et al (Ou et al., 2002) reported on the antioxidant activities of common vegetables, as determined by FRAP (Ferric Reducing Antioxidant Power) and ORAC assays. The data show that the ORAC and FRAP values of individual vegetable species were highly dependent on geographical origin and harvest time. While the authors concluded that the ORAC method is chemically more relevant to chainbreaking antioxidants activity, they also noted that the ORAC assay is not a "total antioxidant activity assay", because it only measures antioxidant activity against peroxyl radicals. Biologically relevant reactive oxygen species (ROS) also include $O_2^{-\cdot}$, $HO^{\cdot}$, $ONOO^{-}$, and singlet oxygen. Since the different ROS have different reaction mechanisms, it is an over-simplification and inappropriate to claim that "total antioxidant activity" can be provided by a single assay. Comprehensive assays are required to elucidate a full profile of antioxidant activity against various ROS.

Individual phytonutrients can have many important physiological effects and, as such, they are much more than just antioxidants and therefore, antioxidant testing is not the "*be all and end all*" of phytonutrition (Maher, 2006).

## 5.1 Antioxidant Capacity of Whole Apples

The phytochemical composition of apples varies greatly between different varieties of apples, and there are also changes in phytochemicals during the maturation and ripening of the fruit. Processing can greatly affect apple phytochemicals (Boyer and Liu, 2004). The antioxidant capacity (Oxygen Radical Absorbance Capacity (ORAC)) of apples, and other fruits has been documented by the United States Department of Agriculture (United States Department of Agriculture, 2007). Different modes of post-harvest storage can affect antioxidant capacity and polyphenol content. Matthes and Schmitz-Eiberger (2009) tested post harvest storage at 20°C to simulate domestic conditions and cold storage at 1°C for 4.5 months. This study also showed a positive correlation between total polyphenols and antioxidant capacity. Cold storage at 1°C increased the antioxidative capacity and polyphenol content in most of the apple cultivars but storage at 20°C led to a decrease in polyphenol content and in antioxidant capacity. Storage under controlled atmosphere led to stable or small increases of both antioxidant capacity and polyphenol content. This underscores the importance of appropriate refrigerated storage for apples to retain antioxidants.

A study has evaluated the relationship between the antioxidant content in apple peel and the whole fruit in two growing seasons. Antioxidants were mainly localized in the apple peel, but cultivars exhibited very high biodiversity in the distribution pattern. High or very high correlation coefficients between apple peel glutathione reductase and catalase activity as well as ascorbate, glutathione, and anthocyanin concentrations and the whole fruit were observed on the basis of both fresh and dry weight basis in two growing seasons. Apple peel therefore is a good marker of antioxidant potential of apples (Lata, 2007).

The antioxidant content of peel and flesh and harvest quality characteristics of seven apple cultivars has recently been studied (Drogoudi et al., 2008). Total antioxidant activity, total phenolic and ascorbic acid content, total soluble solids, total acidity and colour parameters were measured in flesh and peel fruit tissues from the apple cultivars Fuji, Golden Delicious, Granny Smith, Jonagored, Mutsu, Starkrimson and Fyriki. In flesh tissue, Fyriki contained the highest antioxidant activity and total phenolic content (up to 82% and 67% more, respectively), while the lowest values were found in Fuji, Golden Delicious and Granny Smith. The ascorbic acid content was also greatest in the flesh tissue of Fyriki (up to 36% more). In peel tissue, the greatest antioxidant activity and total phenolic content were found in Starkrimson (up to 64% more) whereas the lowest values were found in

Golden Delicious and Granny Smith. Apple peel contains from 1.5 to 9.2 times greater total antioxidant activity and from 1.2 to 3.3 times greater total phenolic content compared with flesh. Principal component analysis and correlation analysis showed that a more nutritious peel may be darker, redder and bluer, while a more nutritious flesh may have a lighter colour and lower soluble solid content.

## *5.2 Antioxidant Capacity of Apple Juice*

The effect of processing apples into juice on polyphenolic antioxidant content and activity is very significant. Raw juice obtained from Jonagold apples by pulping and straight pressing or after pulp enzyming had an antioxidant activity that was only 10 and 3%, respectively, of the activity of the fresh apples. The levels of flavonoids and chlorogenic acid in the juice were reduced to between 50% (chlorogenic acid) and 3% (catechins). Most of the antioxidants were retained in the pomace rather than being transferred into the juice. For three apple cultivars tested (Elstar, Golden Delicious, and Jonagold), the processing methods had similar effects. The results indicate that processing can have a major impact on the partitioning of the polyphenolic content (van der Sluis et al., 2002).

The antioxidant activities of fruit juices have been estimated by measuring antioxidant status in human plasma in ten healthy men 25-26 years old. Within 30 minutes after consumption, apple juice (and orange, melon, grape, peach, plum and kiwi juices) has been shown to suppress reactive oxygen species generation in human plasma. This radical scavenging effect of fruit juices was maintained for up to 90 minutes post-consumption. However, long-term studies with more subjects are needed to provide additional supportive evidence and better characterize the antioxidant properties of natural fruit juices (Ko et al., 2005).

Antioxidant function in elderly subjects has been evaluated in 26 elderly subjects that were randomly divided into 2 groups - an apple and a pomegranate group, in which 250 mL of juice was consumed daily for 4 weeks. Changes in plasma antioxidant capacity, activity of antioxidant enzymes, contents of ascorbic acid, vitamin E, reduced glutathione, malondialdehyde, oxidized low-density lipoprotein and carbonyls, and the degree of DNA damage in mononuclear blood cells were measured. Urine samples were collected for determination of 8-hydroxy-2'-deoxyguanosine content. Increased plasma antioxidant capacity and decreased plasma carbonyl content were demonstrated after daily consumption of pomegranate juice. In comparison, apple juice consumption presented a less significant effect on antioxidant function in elderly subjects (Guo et al., 2008).

A comparison of antioxidant potency of commonly consumed polyphenol-rich beverages in the United States has been recently reported (Seeram et al., 2008). Pomegranate juice had the greatest antioxidant potency composite index of the beverages tested and was at least 20% greater than any of the other beverages tested. Antioxidant potency, ability to inhibit low density lipoprotein (LDL) oxidation, and total polyphenol content were consistent in classifying the antioxidant capacity of the polyphenol-rich beverages in the following order: pomegranate juice >red wine>Concord grape juice>blueberry juice>black cherry juice, acai juice, cranberry juice>orange juice, iced tea beverages, apple juice. The beverages included several different brands of each product.

***Summary: Antioxidant capacity***

Antioxidant capacity of apples is concentrated in the peel, with higher levels of antioxidant capacity generally found in darker, redder and bluer coloured apples. *In vitro* antioxidant potency (e.g. a high ORAC value) does not prove *in vivo* biological activity in humans as some of the most abundant polyphenols are incompletely absorbed in humans and their circulating levels may be low (see section below on *Bioavailablity and bioefficacy of polyphenols in humans*). Apples are the largest contributors of fruit phenolics in the diet as apples are the fruit consumed in the highest volume (Wolfe et al., 2008)), and apples and strawberries are the biggest suppliers of cellular antioxidant activity due to levels of consumption. Apples are also a significant source of flavonols in Australian adults (Somerset and Johannot, 2008). Appropriate cold storage of apples can maintain antioxidant and polyphenol content for several months.

## 6. Bioavailability and Bioefficacy of Polyphenols in Humans

Polyphenols are a group of chemical substances found in plants, characterized by the presence of more than one phenol unit per molecule. The largest and best studied polyphenols are the flavonoids which include several thousand compounds, among them the flavonols, flavones, anthocyanidins and isoflavanoids.

Biological activity of polyphenols is often assessed by using cultured cells as tissue models, and in almost all such studies, cells are treated with aglycones or polyphenol-rich extracts and data reported at concentrations that elicited a response. There are inherent flaws in such an approach as plasma and tissues are not exposed *in vivo* to polyphenols in these forms. Human studies have identified the nature of polyphenol conjugates *in viv*o and shown that dietary polyphenols undergo extensive modification during first-pass metabolism so that the forms reaching the blood and tissues are, in general, neither aglycones (except for green tea catechins) nor the same as the dietary source. Polyphenols are present as conjugates of glucuronate or sulfate, with or without methylation of the catechol functional group. As a consequence, the polyphenol conjugates are likely to possess different biological properties and distribution patterns within tissues and cells than do polyphenol aglycones. The polyphenol concentrations tested should also be of the same order as the maximum plasma concentrations attained after a polyphenol-rich meal, which are in the range of 0.1-10 μmol/L. For correct interpretation of results, future efforts to define biological activities of polyphenols should make use of the available data concerning bioavailability and metabolism in humans (Kroon et al., 2004).

There is scarce information on the absorption, metabolism and excretion of dietary polyphenols in humans and their fate in the gastrointestinal tract. In general, polyphenols are not completely absorbed in the small intestine (affected by the sugar residue and its position), and while flavonoids and their glycosides can be absorbed from the gastrointestinal tract, their uptake is incomplete and their circulating levels are low (Biedrzycka and Amarowicz, 2008).

There have been numerous studies on the antioxidant properties of apples that have shown contrasting results between *in vitro* studies and *in vivo* effects of apple polyphenols as antioxidants in humans. The largest and best studied polyphenols are the flavonoids. Apple flavonoids (quercetin, rutin, epicatechin, catechin) have been shown to have the highest antioxidant capacity, yet they contribute low levels of the total antioxidant capacity of aqueous apple extracts. Positive antioxidant effects *in vitro*, have in many cases not been reproducible in *in vivo* studies in humans. Furthermore, it has been reported that the increase in human plasma antioxidant capacity after apple consumption was due to a metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids (Lotito and Frei, 2004).

Evidence for *in vivo* antioxidant effects of flavonoids is therefore confusing and equivocal. This may be because maximal plasma concentrations, even after extensive flavonoid intake, may be low (insufficient to exert significant systemic antioxidant effects) and because flavonoid metabolites tend to have decreased antioxidant activity. Reports of substantial increases in plasma total antioxidant activity after flavonoid intake must be interpreted with caution, as findings may be attributable to changes in urate concentrations as indicated above. However, phenols may exert direct effects within the gastrointestinal tract, because of the high concentrations present. These effects could include binding of prooxidant iron, scavenging of reactive nitrogen, chlorine, and oxygen species, and perhaps inhibition of cyclooxygenases and lipoxygenases (Halliwell et al., 2005).

A review of 97 bioavailablity studies has shown that bioavailability differs greatly between the various polyphenols, with the most abundant polyphenols not necessarily being those that have the best bioavailability profile in humans (Manach et al., 2004). Furthermore, bioavailability of individual polyphenols from different food sources also differ significantly e.g. quercetin from apples has only 30% of the bioavailability in humans relative to quercetin from onions (Hollman et al., 1997). Therefore considerable caution is needed when extrapolating the levels of polyphenols in apples (and other foods) to human health outcomes.

While experimental studies on animals or cultured human cell lines support a role of polyphenols in the prevention of a number of disease states, it is very difficult to predict from such results the effects of polyphenol intake on disease prevention in humans. One of the reasons is that such studies have often been conducted at doses or concentrations far exceeding those studied in humans. The few clinical studies on biomarkers of oxidative stress, cardiovascular disease risk factors, and tumour or bone resorption biomarkers have often led to contradictory results. More human studies are needed to provide clear evidence of their health protective effects and to better evaluate the risks possibly resulting from exceedingly high levels of polyphenol consumption (Scalbert et al., 2005).

Williamson and Manarch have reviewed the bioavailability and bioefficacy of polyphenols in 93 intervention studies (Williamson and Manach, 2005). They reported that for some classes of dietary polyphenols, there are now a reasonable number of intervention studies to suggest effects on humans *in vivo*, on the basis of short-term changes in biomarkers. For example, procyanidins (oligomeric catechins found at high concentrations in red wine, grapes, cocoa, cranberries and apples) have effects on the vascular system, including, but not limited to, plasma antioxidant activity. Quercetin (the main representative of the flavonoid class, found at high concentrations in onions, apples, red wine, broccoli, tea, and Ginkgo biloba) influences some carcinogenesis markers and has small effects on plasma antioxidant biomarkers *in vivo*, although some studies failed to find an effect. Compared with the effects of polyphenols *in vitro*, the effects *in vivo* are significant, but more limited due to the lack of validated *in vivo* biomarkers, especially in the area of carcinogenesis, the lack of long-term studies, and a lack of understanding or consideration of bioavailability in the *in vitro* studies, which are subsequently used for the design of *in vivo* experiments. The authors also concluded that the length of human intervention studies will need to be increased, to more closely reflect the long-term dietary consumption of polyphenols (Williamson and Manach, 2005).

As polyphenols are also known to have additive or synergistic effects with other polyphenols, future studies should involve the whole apple rather than individual phytochemicals when human health effects are to be evaluated (Biedrzycka and Amarowicz, 2008).

The fate of polyphenols that are not absorbed in the small intestine, but which pass into the large intestine may also be important in disease prevention. Polyphenols that are not absorbed in the small intestine are degraded by the colonic microflora to phenolic acids, which can be absorbed into the circulatory system and subjected to phase II metabolism prior to excretion. Although the protective effect of dietary phenolics may in part be due to their antioxidant properties which result in a lowering of the levels of free radicals within the body, there are alternative mechanisms which have recently been postulated. There is now emerging evidence that the metabolites of dietary phenolics,which appear in the circulatory system in nmol/L to low mmol/L concentrations, exert modulatory effects in cells through selective actions on different components of the intracellular signalling cascades vital for cellular functions such as growth, proliferation and apoptosis. In addition, the intracellular concentrations required to affect cell signalling pathways are considerably lower than those required to impact on antioxidant capacity (Crozier et al 2009).

***Summary: Bioavailability and bioefficacy***

Antioxidant effects *in vitro* have in many cases not been reproducible in *in vivo* studies in humans. This is most likely due to dietary polyphenols undergoing extensive modification during first-pass metabolism so that the forms reaching the blood and tissues are, in general, not the same as the dietary source (i.e. not in the form used in *in vitro* studies). High ORAC values (and other *in vitro* measures of antioxidant capacity) may not be assumed to extrapolate to human health outcomes. However, there is a growing body of evidence that consumption of apples can change some blood biomarkers in humans, which may be predictive of positive health benefits. Furthermore recent data suggest that rather than antioxidant capacity, small concentrations of metabolites of dietary phenolics may exert modulatory effects in cells through intracellular signalling cascades vital for cellular functions such as growth, proliferation and apoptosis. Future studies on apple consumption should focus on whole apples rather than individual phytochemicals, as many polyphenols have additive or synergistic effects. The metabolic fate of consumed apple using a metabolomics approach may yield new insights,

# 7. Effects of Consuming Apples on Health

## *7.1 Brain Health*

### *Animal Studies*

Folate deficiency has been associated with age-related neurodegeneration. It has been reported that dietary deficiency in folate and vitamin E, coupled with pro-oxidant stress induced by dietary iron, increased amyloid-beta levels in normal adult mice. This increase was potentiated by apolipoprotein E (ApoE) deficiency as shown by treatment of transgenic mice homozygously lacking murine ApoE. Dietary supplementation with apple juice concentrate in drinking water alleviated the increase in Abeta for both mouse genotypes. These findings suggest a link between nutritional and genetic risk factors for age-related neurodegeneration, and underscore that dietary supplementation may be useful to augment therapeutic approaches (Chan and Shea, 2009).

Quercetin is a flavonoid abundant in onions, apples, tea and red wine. Dietary quercetin intake is suggested to be health promoting, but this assumption is mainly based on mechanistic studies performed *in vitro*. The effect of quercetin on stress-induced changes in oxidative biomarkers in the hypothalamus of rats has recently been studied (Haleagrahara et al., 2009). Adult male Sprague Dawley rats were subjected to forced swimming stress for 45 min daily for 14 days. The effect of quercetin at three different doses (10, 20 and 30 mg/kg body weight) on serum corticosterone and oxidative biomarkers (lipid hydroperoxides, antioxidant enzymes and total antioxidants) was estimated. Swimming stress significantly increased the serum corticosterone and lipid hydroperoxide levels. A significant decrease in total antioxidant levels and super oxide dismutase, glutathione peroxidase and catalase levels was seen in the hypothalamus after stress and treatment with quercetin significantly increased these oxidative parameters and there was a significant decrease in lipid hydroperoxide levels. These data suggest that forced swimming stress produced oxidative damage in the hypothalamus and treatment with quercetin markedly attenuated these stress-induced changes. The antioxidant action of quercetin may be beneficial for the prevention and treatment of stress-induced oxidative damage in the brain, although further studies are needed to scientifically substantiate this effect in humans.

### *Human Studies*

There have been no randomised controlled human clinical studies examining the role of apple components on brain function. An open-label clinical trial in 21 institutionalized individuals with moderate-to-severe AD who consumed two 4-oz glasses of apple juice daily for 1 month showed no change in the Dementia Rating Scale and no change in Alzheimer's Disease Cooperative Study (ADCS)-Activities of Daily Living (ADL) (Remington et al 2010). The caregivers did report an approximate 27% improvement in behavioural and psychotic symptoms associated with dementia, with the largest changes in anxiety, agitation, and delusion. However, as this was not a randomised placebo-controlled trial, these changes cannot be confirmed and may reflect either better nutrition or other factors such as consequences of greater personal care in a twice daily feeding schedule.

## *7.2 Carcinogenesis Studies*

### 7.2.1 Bladder Cancer

***Human Studies***

The association between vegetable and fruit consumption and the risk of bladder cancer among participants of the European Prospective Investigation into Cancer and Nutrition (EPIC) study has recently been investigated. Data on food consumption and complete follow-up for cancer occurrence was carried for 478,533 participants, who were recruited in 10 European countries. After a mean follow-up of 8.7 years, 1015 participants were newly diagnosed with bladder cancer. Increments of 100 g/day in fruit and vegetable consumption combined did not affect bladder cancer risk. Borderline statistically significant lower bladder cancer risks were found among never smokers with increased consumption of fruit and vegetables combined and increased consumption of apples and pears. While this provided some suggestive evidence, the data do not support an effect of fruit and vegetable consumption, combined or separately, on bladder cancer risk (Buchner et al., 2009).

***Summary: Bladder Cancer***

There is no evidence for an effect of fruit (apples and pears) and vegetable consumption, combined or separately, on bladder cancer risk.

### 7.2.2 Breast Cancer

***Animal Studies***

Freeze dried powders of Red Delicious (RD), Fuji (FJ), Golden Delicious (GD), and Granny Smith (GS) apple cultivars have been evaluated in a rat model of experimentally induced breast cancer. Significant differences were noted among cultivars in total phenolics, flavonoids, oxygen radical absorbance capacity, and growth inhibition in the MDA-MB-468 human breast cancer cell line relative to vehicle-treated cells. Although the rats fed the apple-containing diets did not have a lower incidence or multiplicity of cancers than rats fed a control diet, a finding consistent with epidemiological reports on fruit and breast cancer risk, differences among cultivars were noted, with the greatest difference in cancer multiplicity between GS and RD (1.46 vs. 2.47 cancers/rat). The rate of cell proliferation in mammary carcinomas differed between GS and RD, whereas the apoptotic rate did not. The data perhaps suggest that more diverse apple cultivars with higher phytochemical content should be evaluated (Thompson et al., 2009).

Fresh apples have been reported to suppress mammary carcinogenesis and proliferative activity and induce apoptosis (programmed cell death) in mammary tumours in Sprague-Dawley rats (Liu et al., 2009). The anticancer activity of apple extracts in a rat mammary cancer model induced by 7,12-dimethylbenz(a)anthracene (DMBA) *in vivo* was evaluated and the effects of apple extracts on inhibition of cell proliferation and apoptosis in mammary cancer tissues was studied *in vivo*. Rats were given a whole apple extracts (0, 3.3, 10.0, or 20.0 g/kg of body weight) by gavage starting 2 weeks prior to DMBA administration and continuing for 24 weeks. Rats treated with DMBA (positive control) developed mammary tumours with 71.4% tumour incidence during the 24-week study. No tumours were detected in the negative control group untreated with DMBA. A dose-dependent inhibition of mammary carcinogenesis by apple extracts was observed. Tumour multiplicity decreased with increasing apple extracts. The proportions of adenocarcinoma masses decreased with increasing apple extracts. The expression of proliferating cell nuclear antigen (PCNA), cyclin D1, and Bcl-2 decreased, and Bax expression and apoptosis increased with increasing apple extracts. These results demonstrate a strong capacity of fresh apples to suppress DMBA-initiated mammary cancers in rats. An earlier study by the same group, reported that whole apple extracts prevented mammary cancer in the rat model in a dose-dependent manner at doses comparable to human consumption of one, three, and six apples a day (Liu et al., 2005).

### *Human Studies*

A case-control study has been conducted in Brazil with 33 women recently diagnosed with breast cancer and a control group of 33 healthy women volunteers (Di Pietro et al., 2007). Personal details, health history and past dietary intake were obtained via questionnaires and interviews. The study concluded that age (> 45 years), low family income, poor educational level and past regular consumption of pork fat and fatty meat may be factors associated with an increased risk of breast cancer, while apples, watermelons, tomatoes, plain cakes and desserts appeared to be related to some degree of protection, although the effects were modest and the number of subjects relatively low.

While some small epidemiological studies have suggested a possible modest protective effect of apple consumption on breast cancer, a large study - The Women's Health Study (38,408 women, 11.5 year follow-up, 3,234 cancer cases) has reported that the results do not support a major role of 5 common flavonoids (quercetin, kaempferol, myricetin, apigenin and luteolin) or selected flavonoid-rich foods (tea, apple, broccoli, onion, and tofu) in breast cancer, colorectal cancer, lung cancer, endometrial cancer, and ovarian cancer prevention (Wang et al., 2009). This does not rule out a potential role for apples in breast cancer prevention through other nutritional and compositional characteristics of apples.

### *Summary: Breast Cancer*

Despite promising results of apple extracts in animal models of mammary carcinogenesis, a causal role of apples and breast cancer protection has not yet been established in humans. Further animal studies with apple cultivars with higher phytochemical content should be evaluated.

### 7.2.3 Colorectal Cancer

***Animal Studies***

A recent extensive review of animal studies (Koch et al., 2009) has summarized the preventative potential of cloudy and clear apple juice and different apple constituents on biomarkers related to colon carcinogenesis in obesity. Obesity bears major cancer promoting parameters (e.g., pro-inflammatory cytokines, growth factors), hence brings the model closer to the human situation. However, under the cancer promoting condition of obesity, apple juice did not show cancer-preventive bioactivity.

Candidates for potential protection from colorectal cancer have already been identified *in vitro* (e.g., procyanidins, dihydrochalcones), but the majority of *in vivo* studies have indicated the limited potential of a single substance or sub-fractions of the complex juice to achieve the observed *in vivo* activities of the complex combination of bioactive constituents in apple juice. These studies all emphasize the benefit of undertaking studies in whole foods and further studies in different animal models are warranted.

***Human Studies***

Epidemiologic cohort studies relating flavonoid intake to risk of colorectal cancer have been sparse and inconclusive. Apples are a rich source of flavonoids and peels have a stronger antioxidant activity than apple flesh. A recent re-analysis of several case-control studies in Italy has reported a consistent inverse association between apple consumption and the risk of various cancers, including colorectal cancer (Gallus et al 2005). In a separate case control study, that the highest risk of colorectal cancer was among older people and the risk of colorectal cancer was inversely correlated with the daily number of apple servings, but the most significant reductions were observed for an intake one or more apple servings daily. No other fruit tested (berries, citrus, or stone fruits) were significantly associated with altering the risk of colorectal cancer (Jedrychowski and Maugeri, 2009, Jedrychowski et al., 2009).

A more recent case-controlled study by the same group (Jedrychowski et al., 2010) evaluated the effect of regular consumption of apples on colorectal cancer risk in a population with relatively low intake of fruits and vegetables. The results showed that the adjusted risk of colorectal cancer inversely correlated with the daily number of apple servings. The reduced risk of colorectal cancer of borderline significance level was observed following consumption of at least one apple a day, but with more than one apple a day the risk was reduced by about 50%. Neither the consumption of vegetables nor other fruits (berries, citrus, or stone fruits) showed beneficial effects on the risk of colorectal cancer.

While these data appear promising, a cautionary note lies in the results of epidemiologic studies on dietary flavonoids and cancer risk that have yielded inconsistent or negative results. The association between the intake of selected flavonoids and flavonoid-rich foods and risk of cancers in a Women's Health Study has recently been studied (Wang et al., 2009). A total of 3,234 incident cancer cases were identified during 11.5 years of follow-up among 38,408 women. Intake of individual flavonoids (quercetin, kaempferol, myricetin, apigenin and luteolin) was assessed from food-frequency questionnaires. The relative risk of total and site-specific cancer across increasing intakes of total and individual selected flavonoids and flavonoid-rich foods (tea, apple, broccoli, onion, and tofu) was determined. The results did not support a major role of 5 common flavonols and flavones or selected flavonoid-rich foods in breast cancer, colorectal cancer, lung cancer, endometrial cancer, and ovarian cancer prevention.

The effect of dietary flavonoids on colorectal adenoma recurrence has been studied in a polyp prevention trial (Bobe et al., 2008). Two recent case-control studies suggested that some flavonoid sub-groups may play a role in preventing colorectal cancer. Previous prospective cohort studies generally reported no association; however, only a small subset of flavonoids was evaluated and partial flavonoid databases were used. The authors used the U.S. Department of Agriculture flavonoid database to examine the association between consumption of total flavonoids, 6 flavonoid subgroups, and 29 individual flavonoids with adenomatous polyp recurrence in the Polyp Prevention Trial. The Polyp Prevention Trial was a randomized dietary intervention trial, which examined the effectiveness of a low-fat, high-fibre, high-fruit, and high-vegetable diet on adenoma recurrence. Intakes of flavonoids were estimated from a food frequency questionnaire. Multivariate logistic regression models (adjusted for age, body mass index, sex, regular non-steroidal anti-inflammatory use, and dietary fibre intake) were used to estimate odds ratios and 95% confidence intervals for both any and advanced adenoma recurrence within quartiles of energy-adjusted flavonoid intake (baseline, during the trial, and change during the trial). Total flavonoid intake was not associated with any or advanced adenoma recurrence. However, high intake of flavonoids, which are at greater concentrations in beans, onions, apples, and tea, was associated with a decreased risk of advanced adenoma recurrence. Similar inverse associations were observed to a smaller extent for isoflavonoids, the flavonol kaempferol, and the isoflavonoids genistein and formononetin.

Studies in ileostomy subjects have shown colonic availability of apple polyphenols after oral intake of apple juice (Kahle et al., 2005, Kahle et al., 2007, Veeriah et al., 2008). Most of the orally administered apple polyphenols were absorbed from or metabolized in the small intestine. Between 0 and 33% of the oral dose was recovered in the ileostomy bags. Minor amounts of unmetabolized polyphenols were recovered in the ileostomy effluent, which would reach the colon under physiologic circumstances. More specific studies with phloretin-O-glycosides, phloretin-2'-O-glucoside and phloretin-2'-O-(2 "-O-xylosyl)glucoside, which are thought to be unique to apples and apple products, have also shown the absorption, metabolism, and excretion of these cider dihydrochalcones in healthy humans and subjects with an ileostomy (Marks et al., 2009).
However, phenols may exert direct effects within the gastrointestinal tract, because of the high concentrations present. These effects could include binding of prooxidant iron, scavenging of reactive nitrogen, chlorine, and oxygen species, and perhaps inhibition of cyclooxygenases and lipoxygenases (Halliwell et al., 2005). Furthermore as previously mentioned dietary derived phenolics may be degraded by the colonic microflora and absorbed via the colon in small concentrations which nevertheless exert modulatory effects in cells through intracellular signalling cascades such and as in growth, proliferation and apoptosis. Future studies on apple consumption should focus on whole apples rather than individual phytochemicals, as many polyphenols have additive or synergistic effects.

***Summary: Colorectal Cancer***

Plausable mechanisms for colorectal cancer protection includes possible direct effects within the gastrointestinal tract such as binding of pro-oxidant iron, scavenging of reactive nitrogen, chlorine, and oxygen species, and perhaps inhibition of cyclooxygenases and lipoxygenases. Small amounts of microbially degraded phenolics may also be absorbed via the colon and exert modulatory effects on cellular growth, proliferation and apoptosis. However, to date human studies of the relationship of common flavonoids to colorectal cancer prevention have not provided conclusive evidence and further research is warranted on whole apples in (animal) models of colon cancer. The metabolic fate of consumed apple phytonutrients using a metabolomics approach may yield new insights.

### 7.2.4 Endometrial and Ovarian Cancer

***Human Studies***

As mentioned earlier, The Women's Health Study (38,408 women, 11.5 year follow-up, 3,234 cancer cases) has reported that the results do not support a major role of 5 common flavonoids (quercetin, kaempferol, myricetin, apigenin and luteolin) or selected flavonoid-rich foods (tea, apple, broccoli, onion, and tofu) in breast cancer, colorectal cancer, lung cancer, endometrial cancer, and ovarian cancer prevention (Wang et al., 2009).

***Summary: Endometrial and Ovarian Cancer***

There is no evidence of reduced endometrial and ovarian cancer from consumption of apples by humans.

### 7.2.5 Lung Cancer

***Human Studies***

A positive association between the intake of antioxidant flavonoids and subsequent risk of lung cancer has been reported in a study of 9,959 Finnish men and women (Knekt et al., 1997). The sex- and age-adjusted relative risk of all sites of cancer combined between the highest and lowest quartiles of flavonoid intake was 0.80 (95% confidence interval 0.67-0.96). This association was mainly a result of lung cancer, which presented a corresponding relative risk of 0.54. The association between flavonoid intake and lung cancer incidence was not due to the intake of antioxidant vitamins or other potential confounding factors, as adjustment for factors such as smoking and intakes of energy, vitamin E, vitamin C, and beta-carotene did not materially alter the results. Of the major dietary sources of flavonoid intake, apples showed a significant inverse association with lung cancer incidence with a relative risk of 0.42 (95 percent confidence interval 0.23-0.76) between highest and lowest quartiles.

Marchand et al (2000) found that in a case control study of 582 patients with incident lung cancer and 582 age-, sex-, and ethnicity-matched control subjects a statistically significant inverse association between lung cancer risk and the main food sources of the flavonoids quercetin (onions and apples) and naringin (white grapefruit). The lung cancer OR for the highest compared with the lowest quartile of intake was 0.5 (95% CI = 0.3-0.9) for onions (P for trend =.001) and 0.6 (95% CI = 0.4-1.0) for apples.

In a prospective study lung cancer risk and fruit and vegetable consumption in 77 283 women in the Nurses' Health Study and 47 778 men in the Health Professionals' Follow-up Study was assessed (Feskanich et al., 2000). Significantly lower risks were observed among the women for increases of 1 serving/day of apples and pears (RR = 0.63; 95% CI = 0.43–0.91), oranges (RR = 0.58; 95% CI = 0.37–0.91), and cauliflower (RR = 0.39; 95% CI = 0.16–0.92).

While there is some evidence for effects of flavonoid intake on reduced risk of lung cancer in some populations, but not in other populations (Cui et al., 2008; Lagiou et al., 2004), these studies have not been intervention studies with apples. The Women's Health Study has reported that the results do not support a major role of selected flavonoid-rich foods including apple in breast cancer, colorectal cancer, lung cancer, endometrial cancer, and ovarian cancer prevention (Wang et al., 2009). In contrast, an inverse association between apple consumption and incidence of lung cancer has been reported in a Finnish epidemiological study with 10,054 participants (Knekt et al., 2002).

Quercetin is the most abundant naturally occurring flavonoid. Quercetin is high in certain fruits (apples and grapes) and vegetables (onions and broccoli). Frequent intake of quercetin-rich foods was inversely associated with lung cancer risk (OR = 0.49; 95%CI: 0.37–0.67) and did not differ by P450 or GST genotypes, gender or histological sub-types (Lam et al, 2010). The association was stronger in subjects who smoked >20 cigarettes per day (OR = 0.35; 95% CI: 0.19–0.66; P-trend 5 0.003). Notably, in a small subset of cases with dietary information and gene expression data, the authors observed a down-regulation of P450s genes and up-regulation of GST genes in subjects with high frequency of intake of quercetin-rich foods. This finding is consistent with an influence of dietary quercetin on mRNA expression of key metabolic genes in human lung tissues and suggests a possible mechanism for the protective effect of quercetin-rich food consumption against lung cancer risk. Importantly, the metabolic genes affected by quercetin intake are key regulators of the metabolism of tobacco carcinogens, suggesting an interplay between quercetin intake, tobacco smoking and risk of lung cancer.

Flavonoids from apples known as catechins have been reported to be related to lung cancer in animal models, however, in contrast, the Zutphen Elderly Study (Arts et al, 2001) reported a non-significant relationship between catechins from apple, the major source of non-tea catechins, to lung cancer incidence (RR and 95% CI for a 7.5-mg catechin increase: 0.67, 0.38-1.17).

***Summary: Lung Cancer***

Although, there is no conclusive causal evidence of reduced lung cancer from consumption of apples by humans, promising mechanistic data for the role of the apple flavonoid quercetin is emerging and further animal and human intervention studies are warranted.

### 7.2.6 Prostate Cancer

***Human Studies***

A randomised trial of cranberry versus apple juice in the management of urinary symptoms during external beam radiation therapy for prostate cancer showed no significant difference in the urinary symptoms experienced during radiation therapy related to the consumption of cranberry juice compared with apple juice (Campbell et al., 2003).

***Summary: Prostate Cancer***

There is no evidence of reduced prostate cancer from consumption of apples by humans.

### 7.2.7 Overall Summary: Carcinogenesis Studies

In general, the evidence for the role of fruit and vegetables on reducing cancer risk is waning. However, this does not rule out the potential for specific fruits and/or fruit components. Although observational studies have shown an inverse association between the consumption of apples and the risk of several cancers, definitive studies are lacking. The peels of apple, which have been shown to possess exceptionally high concentrations of antioxidants, are often discarded. Laboratory cell-based studies have shown that apple peel extract possesses strong anti-proliferative effects against cancer cells, and apple peels should not be discarded from the diet. However, detailed mechanistic studies, especially in appropriate *in vivo* animal models, are needed to confirm the anti-proliferative and preventative effects of apple extracts against cancer. Lastly, eating fruit and vegetables may help maintain a healthy body weight, which can reduce the risk of cancer since obesity is only second to smoking as a preventable cause. Further research may be warranted on whole apples in animal models of colon cancer.

## *7.3 Cardiovascular Risk*

***Animal Studies***

A recent study has investigated the acute effects of apple juice intake on some of the biochemical atherosclerosis risk factors in high cholesterol fed rabbits. Rabbits were assigned to 4 groups: no cholesterol, a diet containing 1% cholesterol, a diet containing 1% cholesterol with 5 ml apple juice, a diet containing 1% cholesterol with 10 ml apple juice. Using 5 and 10 ml apple juice with cholesterolaemic diet caused a significant reduction in glucose and fibrinogen. Using 10 ml of apple juice with the cholesterolaemic diet caused a significant reduction in oxidised-LDL, malondialdehyde and nitrite in comparison with the hypercholesterolaemic diet. No significant difference was found between apple juice taking groups and the hypercholesterolaemic diet group in total cholesterol, HDL-cholesterol, triglycerides, LDL-cholesterol, apolipoprotein A and B, serum glutamic pyruvic transaminase, serum glutamic oxaloacetate transaminase, nitrate and C-Reactive Protein. The results suggested that there might be a protective acute effect in postprandial use of apple juice on some of the risk factors of atherosclerosis particularly as an antioxidant (Asgary et al., 2009). Feeding a high concentration of apple powder (20%) to rats has also recently been reported to significantly lower high density lipoprotein (HDL) cholesterol (by 11%) compared to a control group (Kristensen et al., 2009). Apple (Malus pumila) procyanidins have also been shown to have a potent vasorelaxation effect in rat thoracic aorta. The data showed that that apple procyanidins mediated this effect via cyclic guanosine monophosphate production pathways (Matsui et al., 2009).

In a very similar study by another group, Setorki and co-workers have determined the effect of apple juice on some risk factors of atherosclerosis and on the development of atherosclerosis in rabbits fed a high-cholesterol diet. Male rabbits were randomly divided into four groups: normal diet, high cholesterol diet (1% cholesterol), 1% cholesterol supplemented with 5 ml apple juice (low dose) and 1% cholesterol supplemented with 10 ml apple juice (high dose) for 2 months. Both doses of apple juice significantly decreased total cholesterol (TC), triglycerides, C-reactive protein (CRP), fibrinogen, factor VII levels, atherosclerotic lesion in right and left coronary arteries and increased nitrite and nitrate compared to the cholesterolaemic diet. The higher level of apple juice caused a significant reduction in LDL-C and increase HDL-C, but 5 ml of apple juice had no effect. Significant differences were observed between the 5 and 10 ml apple juice groups by LDL-C. No significant difference was found between 5 and 10 ml apple juice groups with regard to CRP, nitrite, nitrate, fibrinogen, factor VII, triglycerides, HDL-C and TC concentrations. The data suggest that apple juice can effectively prevent the progress of atherosclerosis in rabbits (Setorki et al., 2009).

Tourkostami et al. have investigated the protective effect of apple peels, Psyllium seeds and celery fibre supplement as hypolipidemic agents in rats fed on a high-fat diet. The fibre contents were 91.74%, 85.37% and 86.43% respectively. Study groups included both standard and high fat diets that were supplemented with 5% apple peels, 5% psyllium seeds or with 5% celery for 3 months. The data showed that the effects of apple peels where greater than psyllium seeds and celery respectively in lowering plasma total cholesterol and triacylglycerol levels, with the authors suggesting that the beneficial effects may be due to the high fibre content of the apple peels (Tourkostami et al., 2009).

The absorption of dietary cholesterol oxidation products and their downstream metabolic effects in rats have also been reported to be reduced by procyanidin-rich dietary apple polyphenol (APP) from unripe apples. However, serum total cholesterol, high-density lipoprotein cholesterol, and triglyceride levels increased following the intake of dietary APP. Dietary APP also promoted the excretion of exogenous cholesterol oxidation products, cholesterol, and acidic steroids in faeces (Ogino et al., 2007). The implications of such effects on lipid metabolism in the rat remain unclear.

The chemical composition of Gala apples has recently been evaluated and the effect of their consumption on weight gain, food intake, serum levels of total cholesterol, high density lipoprotein (HDL)-C, low density lipoprotein (LDL)-C, triglycerides, hepatic cholesterol and faecal cholesterol have been studied in male albino Wistar rats fed a hypercholesterolemic diet. Six animals were utilized for each treatment (control, 5, 15 and 25% apple diet), during 30 and 60 days. This study showed that one apple (200 g) can provide 14.5% of recommended total fibre and 55% of recommended vitamin C, besides supplying considerable quantities of phenolic compounds and tannins. The animals did not show a significant reduction in their weight gain and food intake with an increase in the concentration of apple in the diets. At the end of 30 days, all of the diets provided a significant reduction in the levels of triglycerides compared to the control group. The 15 and 25% apple diets showed significant reductions in the serum levels of total cholesterol and LDL-C and an increase in the level of faecal cholesterol in relation to the control group. The 25% apple diet provided a significant reduction in the hepatic cholesterol levels compared to the control group. After 60 days, the serum levels of total cholesterol, LDL-C, HDL-C and triglycerides in rats fed with 5, 15 and 25% apple diets were similar to the control group. The results suggest consumption of Gala apples assists in the control of hyperlipidemia in rats (Salgado et al., 2008).

A crude apple polyphenol extract and low-viscosity apple fibre isolated from cider apples administered  to apo E-deficient mice for 4 months did not affect total plasma cholesterol and triacylgycerol levels, and hepatic cholesterol level was lower in the group supplemented with both fibre and polyphenols (Auclair et al., 2008). Uric acid concentrations and antioxidant capacity (FRAP) in plasma were reduced in all groups supplemented with polyphenols or fibre. Apple constituents supplied at nutritional doses limited the development of atherosclerotic lesions in the aorta of apo E-deficient mice suggesting that apple fibre and polyphenols may play a role in preventing atherosclerosis in rats by decreasing uric acid plasma level. A preventative effect of (mashed) apple consumption on markers of atherosclerosis development has also been reported in hamsters (Decorde et al., 2008), and by apple juice in male rabbits fed a high cholesterol diet (Asgary et al., 2009).

***Human Studies***

Flavonoid food composition data from 3 recently available US Department of Agriculture databases have been used to try to improve estimates of dietary flavonoid intake and to evaluate the association between flavonoid intake and cardiovascular disease (CVD) mortality. The study participants were 34,489 post-menopausal women in the Iowa Women's Health Study, USA who were free of CVD and had complete food-frequency questionnaire information at baseline. After multivariate adjustment, significant inverse associations were observed between anthocyanidins and coronary heart disease (CHD), CVD, and total mortality for any versus no intake; between flavanones and CHD; and between flavonoids and total mortality. No association was found between flavonoid intake and stroke mortality. Individual flavonoid-rich foods associated with significant mortality reduction included bran (added to foods; associated with stroke and CVD); apples or pears or both and red wine (associated with CHD and CVD); grapefruit (associated with CHD); strawberries (associated with CVD); and chocolate (associated with CVD). The study concluded that dietary intakes of flavanones, anthocyanidins, and certain foods rich in flavonoids were associated with reduced risk of death due to CHD, CVD, and all causes (Mink et al., 2007). In addition, an inverse association between apple consumption and incidence of ischemic heart disease mortality has been reported in a Finnish epidemiological study with 10,054 participants (Knekt et al., 2002)

However, in contrast, a prospective evaluation of intakes of flavonoids in relation to risk of nonfatal myocardial infarction and fatal CHD in the Nurses' Health Study (66,360 women) did not support an inverse association between flavonoid intake and CHD risk (Lin et al., 2007). The study assessed dietary information from the study's 1990, 1994, and 1998 food frequency questionnaires and computed cumulative average intakes of flavonols and flavones. During 12 years of follow-up (1990-2002), the authors documented 938 non-fatal myocardial infarctions and 324 CHD deaths among the 66,360 women. They observed no association between flavonoid intake and risk of non-fatal myocardial infarction or fatal CHD.

Quercetin, a flavonoid found in apples, berries, and onions, has been shown to reduce blood pressure in hypertensive rodents. The efficacy of quercetin supplementation to lower blood pressure in hypertensive humans has been evaluated in men and women with pre-hypertension (n = 19) and stage 1 hypertension (n = 22) in a randomized, double-blind, placebo-controlled, crossover study to test the efficacy of 730 mg quercetin/d for 28 d vs. placebo. Blood pressure (mm Hg, systolic/diastolic) at enrolment was 137/86 in pre-hypertensives and 148/96 in stage 1 hypertensive subjects. Blood pressure was not altered in pre-hypertensive patients after quercetin supplementation. In contrast, reductions in systolic (-7 mm Hg), diastolic (-5 mm Hg), and mean arterial pressures (-5 mm Hg) were observed in stage 1 hypertensive patients after quercetin treatment. However, indices of oxidant stress measured in the plasma and urine were not affected by quercetin. These data indicate that quercetin supplementation reduces blood pressure in hypertensive subjects, but in contrast to animal-based studies, there was no quercetin-evoked reduction in systemic markers of oxidative stress (Edwards et al., 2007).

Flavonoid intake and the risk of cardiovascular disease has been studied in 38,445 women in a prospective study with a mean follow-up of 6.9 years (Sesso et al., 2003). On the basis of a food-frequency questionnaire, flavonoids were categorized into quintiles, and food sources were categorized into 4 groups. Relative risks were computed for important vascular events (519 events; excluding revascularizations) and CVD (729 events), including myocardial infarction, stroke, revascularization, and CVD death. The mean flavonoid intake was 24.6 mg/d, primarily as quercetin (70.2%). For both CVD and important vascular events, no significant trend was observed across quintiles of flavonoid intake. No individual flavonoid was associated with CVD. Broccoli and apple consumption were not associated with a reduction in CVD risk.

It should be noted that Boyer and Liu (Boyer and Liu, 2004) in evaluating the Sesso et al (2003) study concluded that the study showed that apple intake was associated with a reduction in risk of cardiovascular disease. This is incorrect as the reduction was not statistically significant, and therefore there was no valid association of apple consumption with cardiovascular risk in the Sesso et al (2003) study. Similarly, Boyer and Liu (2004) also concluded that apple intake was associated with a reduced risk of death from coronary heart disease in men when evaluating the Hertog et al study (Hertog et al., 1993), however, again the relationship was not statistically significant meaning that there was no valid relationship between apple consumption and reduced risk of of death from coronary heart disease.

Intervention studies have shown that several biomarkers of cardiovascular risk are influenced by the consumption of polyphenol-rich foods (reviewed by (Manach et al., 2005a).

A clinical trial titled "Effect of apple flavonols on risk of cardiovascular disease" (Trial identifier - NCT00568152) has been registrered. This randomised three period cross over trial is being carried out in adults (19-64 years) to assess the acute and chronic effects of a large dose of apple procyanidin (PA) compared with a low dose of apple PA (negative control) and aspirin (positive control), on platelet function and other risk factors of cardiovascular disease. Platelet function is being assessed prior to a run in diet and at the start and end of each intervention. Volunteers have been assigned at random to consume 230g of low PA apple puree or high PA apple puree or aspirin (75mg) each day for 2 weeks followed by a minimum 14 day wash out. The hypothesis is that consuming apple PA reduces platelet function consistent with reduced risk of cardiovascular disease. The clinical trial is being carried out by the Norfolk, Institute of Food Research, Norwich, Norfolk, United Kingdom. ClinicalTrials.gov processed this record on January 20, 2010, but no study results have been posted.

A mini-review of the effects of apples on plasma cholesterol levels and cardiovascular risk has recently been published (Jensen et al., 2009). The authors evaluated nine human studies of which four studied the effects of whole apples, two the effects of dried apples, and three the effects of filtered apple juice. In general, there was a cholesterol-lowering effect, in the range of 5 - 8%, after the intake of approximately three whole apples, whereas the consumption of apple juice (375 - 720 ml) had no effect on plasma cholesterol levels and may result in adverse effects on plasma triglyceride levels. Limitations in the study designs did not allow the authors to draw conclusions on the effect of the intake of whole, dried apples (15 - 52 g). In nine experimental studies in animal models, feeding apple products resulted in decreased levels of plasma (11 - 43%) and liver (23 - 67%) cholesterol in the majority of studies. There was an increased excretion of bile acids (3 - 56%) and cholesterol (5 - 41%) in rats fed with apple products. The authors concluded that it appears likely that a reduction in plasma total and LDL cholesterol occurs after a dietary intake of apples, and that the major mechanism behind the cholesterol-lowering effect of apples involves an increased clearance of plasma cholesterol due to enhanced faecal excretion of bile acids and cholesterol.

A randomized double-blind, placebo-controlled study on moderately obese male and female subjects (71 subjects) with a body mass index ranging from 23 to 30 has been conducted to evaluate the efficacy of 12-week intake of polyphenols extracted from apples and hop bract (600 mg/day) (Nagasako-Akazome et al., 2007). This level of polyphenols would be approximately available in 3 apples. The study showed that 12-week ingestion of polyphenol-containing capsules significantly decreased total cholesterol and LDL-cholesterol levels. The effects of the apple polyphenol-containing capsules were more marked than those of the hop bract polyphenol-containing capsules. The visceral fat area and the level of adiponectin in the group administered apple polyphenols improved in comparison with the control group. Blood and physical examinations revealed no clinical problems, and no adverse reactions were observed during the ingestion period. These results suggest that apple polyphenols regulate fat metabolism in healthy subjects with relatively high body mass index, although it should be noted the levels ingested via the capsules were greater than levels of polyphenols found in an apple.

Apple fibre and gum arabic has been reported to lower total and low-density lipoprotein cholesterol levels in men with mild hypercholesterolemia (Mee and Gee, 1997), however these data have been contradicted by another study which did not support the hypothesized hypocholesterolaemic effect of apple juice containing pectin/gum arabic in 110 hypocholesterolaemic men and women (Davidson et al., 1998).

The mechanism by which apple phytochemicals could lower plasma LDL cholesterol is thought to involve the uptake of LDL particles in the liver and increase subsequent cholesterol excretion as bile acids. Apple phytochemicals may facilitate a decrease in intracellular fat and cholesterol synthesis, similar to the effects observed through the cholesterol lowering drug class called statins. It has been suggested that apple phytonutrients pectin and the polyphenol-rich fraction are more effective when fed combined together than when fed separately on cholesterol metabolism. This implies interactions between fibres and polyphenols of apple and account for the biological effects of the whole fruit, which are less effective when components are assessed separately.

***Summary: Cardiovascular Risk***

The evidence for a role of fruit and vegetables, specifically apples, on protection from cardiovascular diseases is strengthening. There have been a range of animal and human studies investigating the potential effects of apples and their nutritional components on blood cholesterol levels, lipid metabolism, atherosclerosis, blood pressure and various forms of heart and cardiovascular disease.

Previous reports demonstrated that hypocholesterolaemic activity of apple was associated with its pectin and fibre. The results of animal studies on cholesterol lowering effects are consistent with double blind placebo human studies, indicating that apple polyphenol supplements (in capsules, approximating polyphenol content in three apples) can reduce total cholesterol and LDL-cholesterol levels by 5% to 8%. A clinical intervention trial using whole apples in comparison to a kilojoule matched control on LDL cholesterol would provide definitive evidence of the hypolipidaemic effect of whole apples.

## *7.4 Diabetes Mellitus*

***Animal Studies***

Phloridzin is a dihydrochalcone typically contained in apples. A recent study has shown that a diet containing 0.5% phloridzin significantly reduced the blood glucose levels in streptozotocin (STZ)-induced diabetic mice after 14 days. Phloridzin was detected in the plasma of STZ-induced diabetic mice fed the phloridzin diet for 14 days, although its concentration was much lower than that of the phloridzin metabolites (Masumoto et al., 2009).

Mueller et al, have evaluated whether infant food components affect diabetes development in the non-obese autoimmune diabetes (NOD) mouse. A basal low-diabetogenic diet was identified by feeding litter-matched female NOD mice standardized diets with and without casein and wheat proteins after weaning. In subsequent trials, basal diet with supplements of wheat (5, 10 and 30%), gluten, wheat globulin/albumin, corn (5%), potato (5%), apple (5%) or carrot (5%) was fed to litter-matched female NOD mice after weaning. Mice were followed for diabetes development and insulin autoantibodies. The data showed that early supplementation of a basal low-diabetogenic diet with low concentrations of the cereals wheat or corn is associated with a moderate increase in the rate of diabetes. The other food supplements tested had minimal or no effect on diabetes development (Mueller et al., 2009).

***Human Studies***

A prospective study and cross-sectional analysis has examined the association of dietary flavonoid intake with type 2 diabetes, and biomarkers of insulin resistance and systemic inflammation. In 38,018 women aged $\geq$ 45 y and free of cardiovascular disease, cancer and diabetes with an average 8.8 years of follow-up, the relative risks of incident type 2 diabetes (1,614 events) was calculated according to dietary intake flavonoids. During 332,905 person-years of follow-up, none of total flavonoids, quercetin, kaempferol, myricetin, apigenin and luteolin was significantly associated with risk of type 2 diabetes. Women consuming, 1 apple/day showed a significant 28% reduced risk of type 2 diabetes compared with those who consumed no apples. Tea consumption was also inversely associated with diabetes risk but with a borderline significant trend for 4 cups/day. The results did not support the hypothesis that high intake of flavonoids reduces the development of type 2 diabetes, although a modest inverse association with intake of apples and tea was observed (Song et al., 2005). An inverse association between apple consumption and incidence of type 2 diabetes has also been reported in a Finnish epidemiological study with 10,054 participants (Knekt et al., 2002).

In another large epidemiological study of 35,816 postmenopausal women, with an 18 year follow-up, dietary flavonoids and flavonoid-rich foods (apples, pears, berries, broccoli, bran, citrus, tea) were not associated with risk of type 2 diabetes (Nettleton et al., 2006). The data do not support a diabetes-protective effect of flavonoids.

A small study of 10 normal subjects has evaluated the effects on plasma-glucose, and serum-insulin of test meals based on apples (juice, puree, whole apples), each containing 60 g available carbohydrate. Plasma-glucose rose to similar levels after all three meals. However, there was a rebound fall after juice, and to a lesser extent after puree, which was not seen after apples. Serum-insulin rose to higher levels after juice and puree than after apples. The removal of fibre from food, and also its physical disruption, can result in faster and easier ingestion, and disturbed glucose homoeostasis which is possibly due to inappropriate insulin release. If repeated frequently, these effects have implications towards the development of diabetes mellitus (Haber et al., 1977).

The postprandial blood glucose responses to pure glucose, apple and banana in insulin-dependent diabetics have been reported to be almost identical, and while apples contain considerable amounts of fructose, they represent an important source of rapidly absorbable carbohydrate (Vaaler et al., 1982).

The role of fat on carbohydrate absorption has been investigated in 14 type-I-diabetics who were connected to a glucose-monitored insulin infusion pump. The patients received test meals in the form of potatoes, rice and apples with equal carbohydrate content, in each case with and without added fat. Comparison of carbohydrate carriers showed an increase of blood sugar and insulin consumption which was highest after the potato meal and significantly lower after rice and apple ingestion. Addition of fat caused lowering of blood sugar and insulin consumption in the potato meal, but no major differences with rice and apple. Addition of fat may delay absorption of rapidly split carbohydrates more than absorption of slowly split carbohydrates such as rice. The lack of influence of fat on postprandial blood sugar and insulin consumption after an apple meal was related to the slight increase of blood sugar caused by the high content of fructose (Wakhloo et al., 1984).

Chewing of diced apples, and other foods such as sweetcorn, white rice and potato, has been shown to increase postprandial blood glucose levels, compared to consumption without chewing. Swallowing without chewing reduced the glycaemic response to each food (Read et al., 1986).

No important differences in glycaemic responses in type 2 diabetic patients was reported for apples, pears, oranges, grapes, plums, peaches, apricots and bananas (Lunetta et al., 1995).

A large portion of the calories from apples comes from sugars which are primarily made up of fructose (~60% of total sugars), glucose (~20%) and sucrose (~20%). As a result, the Glycaemic Index (GI) of apples is between 28 - 44 and is in the low GI category (<55). http://www.glycemicindex.com

***Summary: Diabetes Mellitus***

The incidence of diabetes has been shown in a number of large epidemiological studies to be lower in people who consume apples. Of particular interest are the results of two other large epidemiological studies which both concluded that flavonoids did not have a diabetes-protective effect, suggesting that the beneficial effect observed with apple consumption may be related to a mechanism other than the action of phytonutrients such as quercetin.

Although a diabetes protective relationship is not definitive, it should also be noted that the Glycaemic Index (GI) of apples is low (28 – 44) and this could contribute to an apparent diabetes prevention effect. Furthermore as whole apples are low in energy density and may impact on appetite control, diabetes prevention mediated by potential weight control may also be a possible mechanism.

## *7.5 DNA Damage*

***Human Studies***

If the rate of DNA damage exceeds the capacity of the cell to repair it, the accumulation of errors can overwhelm the cell and result in early senescence, apoptosis or cancer. Inherited diseases associated with faulty DNA repair functioning result in premature aging, increased sensitivity to carcinogens, and correspondingly increased cancer risk. The effect of consumption of organically and conventionally produced apples on antioxidant activity and DNA damage in humans has been evaluated in peripheral blood lymphocytes from six healthy volunteers who consumed either organically or conventionally grown apples (Golden Delicious, 1000 g) from two neighbouring commercial farms in a double-blinded, randomized, cross-over study. The average content of total identified and quantified polyphenols in the organically and conventionally produced apples was 308 and 321 pg/g fresh weight, respectively. No statistically significant differences in the sum of phenolic compounds or in either of the polyphenol classes were found between the agricultural methods. Consumption of neither organically nor conventionally grown apples caused any changes in antioxidant capacity of low-density lipoproteins (lag time test), enclogenous

DNA strand breaks, Fpg protein-sensitive sites, or capacity to protect DNA against damage caused by hydrogen peroxide. However, a statistically significant decrease in the levels of endonuclease III sensitive sites and an increased capacity to protect DNA against damage induced by iron chloride were determined 24 h after consumption in both groups of either organic or conventionally grown apples, indicating the similar antigenotoxic potential of both organically and conventionally grown apples (Briviba et al., 2007).

***Summary: DNA Damage***

The principle that DNA damage is a fundamental cause of disease that can be diagnosed and nutritionally prevented on an individual, genetic subgroup, or population basis is gaining momentum. The effects of apple consumption on reducing biomarkers of DNA damage has had limited investigation. This is an area which could be further explored using new emerging biomarkers of DNA damage in humans.

## *7.6 Duodenal Ulceration*

***Human Studies***

Eighty three patients with recently healed duodenal ulcers diagnosed by endoscopy were used to study the effect of dietary supplementation with apple pectin in the maintenance treatment of duodenal ulcers. Recurrences occurred in 85% of patients taking pectin and in 71% of patients taking placebo. The average amount of pectin taken was 12.7 g/day in patients who relapsed and 12.4 g/day in those who did not. At the doses taken, dietary supplementation with apple pectin did not reduce the incidence of duodenal ulcer relapse (Kang et al., 1988).

***Summary: Duodenal Ulceration***

There is no evidence of reduced duodenal ulceration from consumption of apples by humans.

## *7.7 Energy Intake / Satiety*

***Human Studies***

Consumption of whole fruit has been reported to reduce ratings of satiety more than fruit juice, but little is known about the effects of different forms of fruit on subsequent energy intake. A recent study (Flood-Obbagy and Rolls, 2009) has tested how consuming preloads of apples in different forms (apple, applesauce, and apple juice with and without added fibre) prior to a meal influenced satiety and energy intake at meal. Preloads were matched for weight, energy content, energy density, and ingestion rate. Once per week for 5 weeks, 58 adults consumed one of four preloads (266 g; 125 kcal [523 kJ]), or no preload (control), followed by a test meal consumed *ad libitum* 15 min later. Results showed that eating apple reduced lunch energy intake (preload + test meal) by 15% (187 ± 36 kcal [782 ± 151 kJ]) compared to control and decreased energy intake compared to applesauce and both juices. Fullness ratings differed significantly after preload consumption (apple >

applesauce > both juices > control). Overall, whole apple increased satiety more than applesauce or apple juice. Adding naturally occurring levels of fibre to juice did not enhance satiety. These results suggest that solid fruit affects satiety more than pureed fruit or juice, and that eating fruit at the start of a meal can reduce energy intake.

A crossover, clinical intervention with 20 normal and 20 obese men and women has been undertaken where participants consumed 300-kcal of a solid (apple), semi-solid (apple sauce), and beverage (apple juice) at a meal or 2 hours later (snack) with the study reporting effects on appetite / satiety. Whether consumed with a meal or alone as a snack, the beverage elicited the weakest appetitive response, the solid food form elicited the strongest appetitive response and the semi-solid response was intermediate. The appetite shift was greatest for the solid food when consumed as a snack. The interval between test food consumption and the first spontaneous eating event >100 kcal was shortest for the beverage. No significant treatment effects were observed for test day energy intake or between lean individuals and individuals with obesity. The authors concluded that consumption of an energy-yielding beverage either with a meal or as a snack poses a greater risk for promoting positive energy than macronutrient-matched semi-solid or solid foods consumed (Mattes and Campbell, 2009).

A small study of 10 normal subjects has evaluated the effects on satiety, plasma-glucose, and serum-insulin of test meals based on apples, each containing 60 g of available carbohydrate. Fibre-free juice could be consumed 11 times faster than intact apples and 4 times faster than fibre-disrupted puree. With the rate of ingestion equalised, juice was significantly less satisfying than puree, and puree than apples (Haber et al., 1977).

The effect of apple juice (3 ml/kg) given 2.6 h pre-operatively has been investigated in 80 healthy children of ages five to ten years in a prospective, randomized, single-blind study. The children who drank apple juice pre-operatively had decreased gastric volume, thirst, and hunger, without any change in the gastric pH (Splinter et al., 1989), although another study by the same group using higher amounts of apple juice (6 and 10 ml/kg) reported no effect of apple juice on gastric volume (Splinter et al., 1990).

Doubling the portion size of fruit and vegetable side dishes (broccoli, carrots, and applesauce) has been reported to increase (5-6 year old) children's intake of fruit and vegetables, and decrease the amount of pasta they consumed (as the main dish). The difference in meal energy intake between portion sizes was not significant and more studies are needed to understand whether increases in portion size can influence fruit and vegetable intake. The children ate more in response to a large quantity of a preferred low energy-dense fruit side dish at meals with the authors reporting that variations in portion size can be used strategically to help children achieve the recommended intake of fruits (Kral et al., 2009).

The effect of adding fruit or oats to the diet of free-living women on energy consumption and body weight has been evaluated. Fruit and oat cookies had the same amount of fibre and total calories (similar to 200 kcal), but differed in energy density. The clinical trial was conducted in a primary care unit in Rio de Janeiro, Brazil. Forty-nine women, ages ranging from 30 to 50 years, with body mass index (BMI) > 25 kg/m$^2$, were randomly chosen to add three apples (0.63 kcal/g energy density) or three pears (0.64 kcal/g energy density) or three oat cookies (3.7 kcal/g energy density) to their usual diet for 10 weeks. Fibre composition was similar (~6 g). The results showed a significant decrease in the energy density during the follow-up (-1.23 kcal/g and -1.29 kcal/g) for apples and pears, respectively, compared to the oat group. The energy intake also decreased significantly (-25.05 and -19.66 kcal/day) for the apple and pear group, respectively, but showed a small increase (+0.93) for the oat group. Apples and pears were also associated with weight reduction (-0.93 kg for the apple and -0.84 for the pear group), whereas weight was unchanged (+0.21) in the oat group. These results suggest that energy density of fruit, independent of the fibre amount may reduce energy consumption and body weight over time (de Oliveira et al., 2008).

***Summary: Energy Intake / Satiety***

Consumption of whole fruit has been reported to reduce ratings of satiety more than fruit juice, but little is known about the effects of different forms of fruit on subsequent energy intake. A recent study tested this and found that eating apple reduced lunch energy intake by 15% compared to apple sauce or apple juice.

A crossover clinical intervention trial where participants consumed 300-kcal of solid (apple) semi solid (apple sauce) or beverage (apple juice) at a meal or 2 hours later (snack) reported appetite shift was greatest for the apple consumed as a snack.

It can be concluded, that the consumption of solid fruit such as apples affects satiety more than pureed fruit or juice, and eating fruit at the start of a meal can reduce energy intake and consuming fruit as a snack has valuable satiety benefits which may have a possible subsequent effect on weight reduction. These effects appear to be related to the structure and energy density of apples rather than their fibre content per se.

## *7.8 Gastrointestinal Conditions*

### *Animal Studies*

Although diet is regarded as an important factor influencing inflammatory bowel diseases (IBD), there are no accepted dietary recommendations presently available. Lyophilised apples (7.6%) obtained from two cultivars (Golden Delicious and Marie Menard, low and high in polyphenols, respectively) were administered to HLA-B27 transgenic rats which develop spontaneous IBD. After 3 months feeding, rats fed Marie Menard apples had reduced myeloperoxidase activity and reduced cyclo-oxygenase-2 and inducible nitric oxide (NO) synthase gene expression in the colon mucosa and significantly less diarrhoea, compared with control rats. Cell proliferation in the colon mucosa was reduced significantly by feeding Golden Delicious apples, with a borderline effect of Marie Menard apples. Gene expression profiling of the colon mucosa, analysed using the Whole Rat Genome 4 x 44 K Agilent Arrays, revealed a down-regulation of the pathways of PG synthesis, mitogen-activated protein kinase (MAPK) signalling and TNFalpha-NF-kappaB in Marie Menard-fed rats. In the stools of the animals of this group a significant reduction of bacteria of the Bacteriodes fragilis group was observed. The administration of Marie Menard apples, rich in polyphenols and used only in the manufacturing of cider, ameliorated colon inflammation in transgenic rats developing spontaneous intestinal inflammation, suggesting the possible use of these and other apple varieties to control inflammation in IBD (Castagnini et al., 2009).

Some preliminary data have suggested that chronic ingestion of apple pectin may enhance the intestinal absorption of quercetin in a rat model (Nishijima et al., 2009). Quercetin is a flavanol that has been reported to have anti-inflammatory, antioxidant and anti-carcinogenic properties (particularly in pancreatic cancer) properties, although these effects have not been adequately substantiated in humans.

A recent study has reported that orally administered apple procyanidins had a protective effect against chemically-induced colitis and on intestinal epithelial cells in mice (Yoshioka et al., 2008). While this model is questionable for the determination of human health outcomes, it is an area that lends itself to human clinical trials in patients with inflammatory bowel disease.

***Human Studies***

In a prospective, double-blind, randomised, multicentre, parallel group study, children (6 months to 5.5 years of age) with acute, non-complicated diarrhoea received either a preparation containing apple pectin and chamomile extract (n = 39) or placebo (n = 40) in addition to the usual rehydration and realimentation diet. At the end of three days of treatment, the diarrhoea had ended significantly more frequently in the pectin/chamomile (33/39) than in the placebo group (23/40). Pectin/chamomile reduced the duration of diarrhoea significantly by at least 5.2 h. There were no further differences between the treatment groups (de la Motte et al., 1997). A very similar double-blind, randomized evaluation of clinical efficacy and tolerability of an apple pectin-chamomile extract in children with unspecific diarrhoea by a different group (Becker et al., 2006) supported the earlier findings of de la Motte and colleagues.

***Summary Gastrointestinal Conditions***

Apple pectin/camomile extracts may reduce the duration of diarrhoea in children, although the effects need to be confirmed in larger study populations.

## *7.9 Inflammation*

***Human Studies***

Serum C-reactive protein (CRP) is a biomarker for chronic inflammation and a sensitive risk factor for cardiovascular diseases. The associations between dietary flavonoid intake and serum CRP concentrations among U.S. adults after adjusting for dietary, socio-demographic, and lifestyle factors have been reported. Subjects were $\geq$ 19 year old adults (n = 8335), and did not include pregnant and/or lactating women. Flavonoid intake of U.S. adults was estimated by the USDA flavonoid databases matched with a 24 h dietary recall. Intakes of apples and vegetables were inversely associated with serum CRP concentrations after adjusting for co-variates. Total and individual flavonoid intakes were inversely associated with serum CRP concentration after adjusting for the co-variates. Among the flavonoid compounds investigated, quercetin, kaempferol, malvidin, peonidin, daidzein, and genistein had inverse associations with serum CRP concentration, and these associations did not change after the additional adjustment for fruit and vegetable consumption. Intake of dietary flavonoids was inversely associated with serum CRP concentrations in U.S. adults in this study and therefore intake of flavonoid-rich foods may have an effect on reducing inflammation-mediated conditions (Chun et al., 2008).

Some cardioprotective effects associated with fruit and vegetable consumption may be mediated via the anti-thrombotic effects of compounds such as flavonoids. A randomised, controlled parallel human dietary intervention with healthy female and male volunteers (n = 77, 19-52 years) was designed to study the effects of high and low intake of vegetables, berries and apple on platelet function and inflammatory markers. The volunteers consumed one of four strictly controlled isocaloric 6-week diets containing either 810 or 196 g/10 MJ of vegetables, berries and apple and rich either in linoleic acid (11% of energy) or oleic acid (12% of energy). No differences between the treatment groups were seen in platelet count or volume, markers of platelet activation (*ex vivo* aggregation to ADP and thrombin receptor activating peptide, protein kinase C activity, urinary 2,3-dinor-thromboxane B2 excretion, plasma P-selectin), plasma intercellular adhesion molecule-1, sensitive C-reactive protein, or antiphospholipid antibodies. The results showed that in healthy volunteers 6-week diets differing markedly in the amounts of vegetables, berries and apple did not differ in their effects on platelets or inflammation (Freese et al., 2004).

The effects on biomarkers of oxidative stress, lipemia and inflammation appear to be inconclusive, with more consistent effects observed on endothelial function and haemostasis and support a reduction of risk by polyphenols in agreement with the few epidemiological studies already published. Clinical studies have generally used foods or beverages containing a mixture of different polyphenols and therefore the exact nature of the most active compounds remains largely unknown. Bioavailability also differs greatly from one polyphenol to another, so that the most abundant polyphenols in the diet are not necessarily those leading to the highest concentrations of active metabolites in target tissues (Manach et al., 2005b).

***Summary: Inflammation***

There is no substantial evidence to support a role of apple consumption on chronic inflammation, as studies to date have provided contradictory results.

## *7.10 Muscular Injury*

### *Animal Studies*

Polyphenols from apples have been reported to have a protective effect against exercise-induced muscle strain injury in Wistar rats. Apple polyphenol (APP) and control (CON) groups were fed diets with and without 5% APP, respectively. After a 3-wk feeding period, the gastrocnemii of the animals were subject to lengthening contractions with electrical stimulation and forced ankle dorsiflexion. Isometric torques were measured before and after the lengthening contractions and on days 1, 2, 3, 5, and 7 after the contractions. The APP group had significantly lower torque deficits than the CON group on days 3, 5, and 7 after the eccentric contractions suggesting that dietary APPs may have protective effects against lengthening contraction-induced muscle injury in rats (Nakazato et al., 2009).

## *7.11 Respiratory Conditions*

### 7.11.1 Allergy

***Human Studies***

The clinical effects of apple polyphenols on persistent allergic rhinitis (commonly due to house dust mites) have been studied in a randomized double-blind placebo-controlled parallel arm study. Persistent allergic rhinitis is usually treated with antihistamines and local steroids, but they often cause adverse effects such as sedation and drowsiness. Polyphenols derived from apples have been reported to suppress histamine release from rat cells, reduce auricular swelling in allergic mice, and alleviate skin inflammation in atopic patients. Thirty-three patients aged 15 to 65 years with moderate or severe persistent allergic rhinitis in whom the symptoms persisted for 3 years or longer were treated without apple polyphenols (control group), with a low dose of apple polyphenols (50mg), or with a high dose of apple polyphenols (200mg), and changes in the clinical symptoms were examined. Note that one apple may contain 200mg polyphenols. Significant improvements were observed in sneezing attacks and nasal discharge in the high-dose group and in sneezing attacks in the low-dose group suggesting that apple polyphenols are effective in alleviating symptoms of persistent allergic rhinitis (Enomoto et al., 2006).

In contrast to apple polyphenols potentially offering protection from allergy, apple allergy has also been described. However the incidence is very low. In food allergic patients it is generally below 2 %. Apple allergy is most frequently associated with birch pollinosis in Northern Europe and North America with 40 to 90 % of birch pollen allergic patients sensitized to this fruit. However, it should be noted that birch trees are not native to Australia.
Apple is known as one of the major foods involved in so-called "Oral Allergy Syndrome", which presents IgE-mediated symptoms occurring mainly at the mucosa of lips, tongue and throat after ingestion of apples and other fruits.

The allergenicity of a putatively hypoallergenic apple cultivar, the Santana apple, on apple allergic individuals has been evaluated using a method of challenge in which loss of allergenicity is minimized. The study population consisted of 15 apple allergic individuals, who underwent an open oral challenge with 3 different apple cultivars: Santana, Golden Delicious, and Topaz. Food challenges were performed during the birch tree pollen season. After challenge with the Santana apple, 8 of 15 study participants (53%) developed no symptoms, which was a significantly greater proportion than after challenge with the Topaz apple (1 participant) and Golden Delicious apple (1 participant) suggesting that the Santana apple may be an option for consideration for some apple allergic individuals (Kootstra et al., 2007).

Clinical efficacy of apple polyphenol for treating cedar pollinosis has been reported. A double-blind comparative study was conducted on cedar pollinosis patients in order to evaluate the treatment efficacy of apple polyphenol (500mg/day). The results showed that the sneezing score was significantly lower for the apple polyphenol group than with the placebo group during the early period of pollen dispersion and during the main dispersion period. In addition, no adverse reactions were induced by apple polyphenol during the study. These results suggest that apple polyphenol may alleviate the symptoms of cedar pollinosis (Kishi et al., 2005).

The mechanisms of allergic sensitization, the types of clinical manifestations, the influence of allergen content variability, and the diagnostic and therapeutic issues connected with plant food allergy, using apple as a model have recently been reviewed (Pevec et al., 2008). Mechanistic studies suggested reduction of allergenicity by protein-polyphenol interaction as potential mechanisms responsible for protection allergy protection by apples.

***Summary: Allergy***

The immune system may be modulated with nutrition to prevent the development or to treat the symptoms of allergy. Among other foods, consumption of apples has been linked to reduced incidence of atopic dermatitis and respiratory allergy. Apple polyphenols may help alleviate some of the symptoms such as sneezing. This effect has been noted with apple polyphenol amounts able to be consumed in 1 apple. This is an important area for further research in humans to confirm this effect.

### 7.11.2 Asthma

***Human Studies***

Food and nutrient intakes and asthma risk has been studied in young adults to determine whether the food and nutrient intakes of adults with asthma differ from those of adults without asthma. A community-based, cross-sectional study of 1601 young adults was conducted where the subjects completed a respiratory questionnaire, a validated semi-quantitative food-frequency questionnaire, skin-prick testing, and lung function tests, including a methacholine challenge test for bronchial hyperreactivity. Apples and pears appeared to be related to lower asthma and bronchial hyperreactivity, but intervention studies using whole foods are required to ascertain whether such modifications of food intake could be beneficial in the prevention or amelioration of asthma (Woods et al., 2003).

An inverse association between apple consumption and incidence of asthma has also been reported in a Finnish epidemiological study with 10,054 participants (Knekt et al., 2002), and in a smaller study of 16-50 year old participants in 40 general practices in the UK (Shaheen et al., 2001).

An epidemiological study with 2,512 middle-aged men has also reported an association between good lung function and high intake of apples (five or more apples per week). An association between high average apple consumption and slow decline in lung function lost significance after adjustment for confounders including body mass index, smoking history, social class, exercise, and total energy intake (Butland et al., 2000).

Maternal food consumption during pregnancy and asthma, respiratory and atopic symptoms has been evaluated in 5-year-old children. The study reported no evidence for associations between maternal intake of most foods (maternal diet during pregnancy was assessed by food frequency questionnaire) during pregnancy and asthma, respiratory and allergic outcomes in 5-year-old children, except for apples and fish. Consumption of apples and fish during pregnancy may have a protective effect against the development of childhood asthma and allergic disease (Willers et al., 2007), although further confirmatory studies are required.

***Summary: Asthma***

Based on food frequency questionnaire protocols, apples have been reported to be associated with lower incidence of asthma and bronchial hyperreactivity. However, to establish causation, human clinical trials are needed.

## *7.12 Rheumatoid Arthritis*

***Human Studies***

A suggestive elevated risk of apple consumption and rheumatoid arthritis has been reported in a Finnish epidemiological study with 10,054 participants (Knekt et al., 2002).

***Summary:***

There is no conclusive evidence of increased rheumatoid arthritis from consumption of apples by humans.

## *7.13 Recommendations for Future Research*

- Future research which may be of benefit for the apple industry to pursue includes:
    - Comprehensive compositional and nutritional analysis of specific named varieties of Australian apple varieties is needed as is information on effects of storage and cooking.
    - Further research on the role of whole apples and apple extracts for the prevention and treatment of stress-induced oxidative damage in the brain in animal studies and cognitive function studies in humans.
    - Detailed mechanistic studies, especially in appropriate *in vivo* animal models, are needed to confirm the anti-proliferative and preventative effects of apple extracts (including apple skin) against cancer. The metabolic fate of consumed apple phytonutrients using a metabolomics approach may yield new insights.
        - Further animal studies with apple cultivars with higher phytochemical content should be evaluated in models of mammary carcinogenesis.
        - Further research is warranted on whole apples and apple extracts in animal models of colon cancer.
        - Animal and human intervention studies of flavonoids from whole apples on lung cancer.
    - The role of apple polyphenols in regulating fat metabolism in healthy subjects with relatively high body mass index needs further exploration and confirmation.
    - A clinical intervention trial using whole apples in comparison to a kilojoule matched control on LDL cholesterol would provide definitive evidence of the hypolipidaemic effect of whole apples.
    - The effects of apple consumption on reducing biomarkers of DNA damage has had limited investigation. This is an area which could be further explored using new emerging biomarkers of DNA damage in humans.
    - The role of apple polyphenols in alleviating or preventing allergic symptoms and asthma warrants exploration. This effect has been noted with apple polyphenol amounts present in 1 apple. This is a particularly important area for further research in humans as well as mechanistic studies to confirm this effect. .

## 8. Accreditation of reviewers

The authors of this review are accredited reviewers for the Joanna Briggs Institute (JBI) and have undertaken "*Comprehensive Systematic Review*" training on "*Evidence Based Health Care and the Systematic Review of Evidence*", including a module on "*Systematic Review of Experimental and Non-Experimental Study*". The JBI is an initiative of the Royal Adelaide Hospital and the University of Adelaide and is an international collaboration involving medical and allied health researchers and academics across 40 countries in every continent.

## 9. Acknowledgement

The authors would like to acknowledge Dr Peter Fagan of CSIRO Food and Nutritional Sciences for contributing the section on the determination of antioxidant capacity, and for valuable discussions on the issues and challenges in interpretation of data on antioxidant capacity of foods.

## 10. About the authors

### Dr Peter Roupas

Dr Roupas obtained his PhD from the Department of Medicine at Monash University, Melbourne, Australia in 1988 and completed his postdoctoral research at the University of Michigan Medical School, USA. During his 3 years at the University of Michigan, he was awarded fellowships from the American Diabetes Association (Michigan) and the Juvenile Diabetes Foundation International (New York). On his return to Australia, to the Department of Clinical Biochemistry at the Royal Children's Hospital, Melbourne, he was awarded the 1991 Eli Lilly Diabetes Fellowship and a 4-year fellowship from the National Health and Medical Research Council (NHMRC) of Australia. For the past 14 years, Dr Roupas has been a Research Team Leader at CSIRO and is currently a Project Leader of projects for the CSIRO Food Futures Flagship and the Preventative Health Flagship in functional foods relating to the scientific substantiation of health messages for dietary guidelines and health claims for food standards / regulatory applications. He is currently the Team Leader of the Knowledge Management team within the Pre-Clinical and Clinical Health Substantiation group within CSIRO Food and Nutritional Sciences. Dr Roupas has been an editorial reviewer for 8 scientific journals, an author of 44 papers in peer-reviewed scientific journals, 30 conference papers and 7 book chapters. Dr Roupas is also a Scientific Editor for Elsevier Science UK, a member of the Editorial Board of the *Journal of Functional Foods*, and a member of the *Society of Editors*. Dr Roupas is also accredited as a Systematic Scientific Reviewer by the Joanna Briggs Institute, an initiative of the Royal Adelaide Hospital and University of Adelaide.

### Associate Professor Manny Noakes

Dr Noakes is currently the Senior Research Dietitian and Research Scientist at CSIRO Food Sciences and Nutrition where she is the Stream Leader for Diet and Lifestyle Programs. This stream is a multidisciplinary team aimed at developing substantiated lifestyle intervention strategies for overweight groups with obesity related morbidities. It relies on the integration of CSIRO's capabilities in nutrition, clinical science, physical activity, behavioural science and nutritional genomics. Dr Noakes has managed clinical nutritional trials for the CSIRO Clinical Research Unit since 1990 and has been involved in the conduct of many published clinical studies to establish the health potential of diets, food products, nutrients, supplements and pharmaceuticals. Further to this, CSIRO capabilities in analytical techniques related to food composition and the limitation of food composition databases is critical in understanding the usefulness and limitations of nutrient intake assessment. These skills are highly relevant to the assessment and interpretation of nutrient composition derived from dietary studies.

Dr Noakes was actively involved in the research, development and communication of the CSIRO Total Wellbeing Diet Books 1 and 2 which are aimed at minimising health risks through better nutrition and weight management. The books have collectively sold over 1 million copies in Australia as well as gaining international recognition with translations into 13 languages. The books' success has sparked considerable community activity in healthy lifestyle behaviours. In recognition of both the commercial success and the science which underpins the CSIRO Total Wellbeing Diet, Dr Noakes was awarded 2 CSIRO medals for both Business Excellence and Research Excellence in 2005. In addition, she was awarded an Outstanding Achievement Alumni Award by Flinders University, Australia in 2006 in recognition of her achievements in nutritional science. Dr Noakes is a former Chair of the Heart Foundation's Nutrition and Metabolism Advisory Committee, Australia and has been involved in the development and oversight of several comprehensive evidence-based position papers on key nutrition issues that relate to diet and cardiovascular disease. She is also on the advisory panel for the Food Information Program Criteria Working Group which has provided an excellent framework for developing nutrition benchmarks for food categories based on knowledge of current population intakes of these foods, food composition profiles and technological barriers to changing nutrient profiles. Dr Noakes is the author of over 100 scientific papers, including several scientific book chapters, and contributes to peer review of papers for many nutrition journals. Dr Noakes is also accredited as a Systematic Scientific Reviewer by the Joanna Briggs Institute, an initiative of the Royal Adelaide Hospital and University of Adelaide.

# 11. Appendix I. Methodology

***Capturing Medical and Scientific Information***

The information was sourced via detailed and thorough strategic electronic searches of medical, scientific and technical literature. The literature searches were carried out using the following databases:

**PubMed –** a service of the US National Library of Medicine that includes over 16 million citations from MEDLINE and other life science journals.

**SCOPUS -** an abstract database covering 25 million abstracts from over 14,000 journals across 4,000 publishers.

**CSIRO Electronic Journals Collection** (4,000 e-journals).

**AGRICOLA** - includes bibliographic citations for journal articles, monographs, proceedings, theses, patents, translations, audiovisual materials, computer software, and technical reports covering all aspects of primary international information sources in agriculture and related fields. The literature cited is mainly in English, but over one-third of the database comprises citations in Western European, Slavic, Asian, and African languages.

**FSTA –** Food Science and Technology abstracts – FSTA is the internationally recognised world's leading Food Science and Technology Abstracts database.

**Cambridge Scientific Abstracts -** CSA Internet Database Service (IDS) has over 40 databases providing bibliographic data in Agricultural Sciences, Aquatic Sciences, Biological and Medical Sciences, Computer Technology, Engineering Specialties, Environmental Sciences and Materials Science.

**Web of Science -** 5,700 major journals across 164 scientific disciplines.

**CABI -** Contains over 3.8 million records from over 10,000 journals, books, conferences, reports, and other kinds of literature published internationally. Subjects covered include animal and crop husbandry, animal and plant breeding, plant protection, genetics, forestry, economics, veterinary medicine, human nutrition, and rural development.

**ISI Proceedings** - ISI Proceedings is an index to the published literature of the most significant conferences, symposia, seminars, colloquia, workshops, and conventions in a wide range of disciplines, from anthropology to zoology.

**ClinicalTrials.gov** – contains details of registered Clinical trials and is provided by the U.S. National Institutes of Health

Following a detailed search and evaluation of the above databases for studies on apples, over 1700 papers were downloaded into a fully-searchable electronic (EndNote®) database for analysis.

# 12. Appendix II. Evaluation of the levels of evidence

Published human studies selected for retrieval were assessed for methodological validity. The levels of evidence used by the National Health and Medical Research Council for the assessment and application of scientific evidence appear in the following table:

| Level of evidence | Study design[1] |
|---|---|
| I | Evidence obtained from a systematic review of all relevant randomised controlled trials. |
| II | Evidence obtained from at least 1 properly-designed randomised controlled trial. |
| III-1 | Evidence obtained from well-designed pseudo-randomised controlled trials. |
| III-2 | Evidence obtained from comparative (non-randomised and observational) studies, including systematic reviews of such studies, with concurrent controls and allocation not randomised, cohort studies, case control studies, or interrupted time series with a control group. |
| III-3 | Evidence obtained from comparative studies with historical control, two or more single arm studies, or interrupted time series without a parallel control group. |
| IV | Evidence obtained from case series, either post-test or pre-test/post-test. |

[1] NHMRC (2000) How to use the evidence: assessment and application of scientific evidence, Commonwealth of Australia.

## 13. Appendix III: Health Claims - Food Standards Australia New Zealand (FSANZ)

At the present time, health claims for foods are not permitted under the FSANZ Food Standards Code, apart from a pre-approved health claim for folate and a reduction of risk of foetal neural tube defects and a recommendation for women to consume a minimum of 400 μg of folate per day in at least the month before and at least the first 3 months following conception.

FSANZ "Standard 1.1A.2 – Transitional Standard – Health Claims" currently governs the use of health messages, both on food labels and in advertising (all media). This Food Standards Code covers both packaged and non-packaged food, including apples.

FSANZ Standard 1.1A.2 states that:

- The label on or attached to a package containing or an advertisement for food shall not contain a claim or statement that the food is a slimming food or has intrinsic weight-reducing properties.

- Save where otherwise expressly prescribed by this Code, any label on or attached to a package containing or any advertisement for food shall not include a claim for therapeutic or prophylactic action or a claim described by words of similar import.

- Any label on or attached to a package containing or an advertisement for a food shall not include the word 'health' or any word or words of similar import as a part of or in conjunction with the name of the food.

- Save where otherwise expressly prescribed by this Code, any label on or attached to a package containing or any advertisement for food shall not contain any word, statement, claim, express or implied, or design that directly or by implication could be interpreted as advice of a medical nature from any person.

- Save where otherwise expressly prescribed by this Code, the label on or attached to a package containing or any advertisement for food shall not contain the name of or a reference to any disease or physiological condition.

Furthermore, the Australian Consumer and Competition Commission (ACCC) regulates food labelling and advertising and provides assistance to the food and beverage industry to understand their legal obligations to consumers when labelling, packaging and promoting their products. The main purpose of the ACCC guidelines is to educate businesses in the food and beverage industry about their obligation to ensure that their product labelling, packaging and advertising is accurate and is not likely to mislead consumers. Inaccurate or misleading labelling or advertising (in all media) for food products can result in serious litigation.

## 14. APPENDIX IV: NUTRITIONAL /COMPOSITIONAL TABLES

| **Apples - Macronutrient Content Value per 100g** | | | | | | |
|---|---|---|---|---|---|---|
| **Nutrient** | **Protein(g)** | **Carbohydrate (g)** | **Fat (g)** | **Fibre (g)** | **Energy (kcal)** | **Sugars, total** |
| Apple Granny Smith raw unpeeled (NUTTTAB) | 0.3 | 10.7 | 0 | 2.3 | 206kJ | 10.5 |
| Apple Pink Lady raw unpeeled (NUTTAB) | 0.3 | 11.9 | 0.4 | 2.4 | 247kJ | 11.9 |

| **Apples - Vitamin Content Value per 100g** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Nutrient** | **Thiamin (B1) (mg)** | **Riboflavin (B2) (mg)** | **Niacin equivalents (B3) (mg)** | **Folate (mcg)** | **Vitamin B6 (mg)** | **Pantothenic acid (mg)** | **Biotin (mcg)** | **Vitamin C (mg)** |
| Apple Granny Smith Raw Unpeeled (NUTTAB) | 0.03 | 0.01 | 0.1 | 0 | 0.06 | 0 | 0.9 | 5 |
| Apple Pink lady Raw Unpeeled (NUTTAB) | 0.02 | 0.01 | 0.1 | 0 | 0.04 | | | 5 |

| Apples - Vitamin Content Value per 100g | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Nutrient** | **Beta-carotene equiv (mcg)** | **Cryptoxanthin (mcg)** | **Lycopene** | **Lutein & Zeaxanthin (mcg)** | **Vitamin D** | **Vitamin E (mg)** | **Vitamin A Retinol equiv (mcg)** | **Vitamin K (mcg)** |
| Apple Granny Smith Raw Unpeeled (NUTTAB) | 5 | 10 | | | | 0.2 | 1 | |
| Apple Pink lady Raw Unpeeled (NUTTAB) | 9 | 0 | | | | 0.3 | 2 | |

| Apples - Mineral Content Value per 100g | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Nutrient** | **Copper (mg)** | **Phosphorus (mg)** | **Magnesium (mg)** | **Manganese (mg)** | **Molybdenum (mcg)** | **Sodium (mg)** | **Potassium (mg)** | **Selenium (mcg)** | **Zinc (mg)** | **Iron (mg)** | **Calcium (mg)** | **Iodine (mcg)** |
| Apple Granny Smith Raw Unpeeled (NUTTAB) | 0.021 | 9 | 4 | 0.048 | | 2 | 115 | 0 | 0.1 | 0.2 | 5 | |
| Apple Pink lady Raw Unpeeled (NUTTAB) | 0.052 | 7 | 4 | 0.07 | | 0 | 88 | 0 | 0.1 | 0.2 | 5 | |

| **Comparative Mean Flavonoid Content mg/100g edible portion** | | | | | | |
|---|---|---|---|---|---|---|
| **Anthocyanidins** | **Cyanidin** | **Delphinidin** | **Malvidin** | **Pelargonadin** | **Peonidin** | **Petunidin** |
| Apple Granny Smith with peel raw (USDA) | 1.33 | 0 | 0 | 0 | 0 | 0 |

| **Comparative Mean Flavonoid Content mg/100g edible portion** | | | | | | |
|---|---|---|---|---|---|---|
| **Flavan03-ols** | **(-)Epicatechin** | **(-)Epicatechin 3gallate** | **(-) Epigallocatechin** | **(-)Epigallocatechin-3 gallate** | **(+)-Catechin** | **(+)-Gallocatechin** |
| Apple Granny Smith with peel raw (USDA) | 3.6 | 0.01 | 0.71 | 0.24 | 0.62 | 0 |

| Comparative Mean Flavonoid Content mg/100g edible portion | | | | |
|---|---|---|---|---|
| **Flavanones and Flavanols** | **Hesperitin** | **Naringenin** | **Apigenin** | **Luteolin** |
| Apple Granny Smith with peel raw (USDA) | 0 | 0 | 0 | 0 |

| Comparative Mean Flavonoid Content mg/100g edible portion | | | | |
|---|---|---|---|---|
| **Flavonols** | **Isorhamnetin** | **Kaempferol** | **Myricetin** | **Quercetin** |
| Apple Granny Smith with peel raw (USDA) | | 0 | 0 | 3.25 |

| Comparative Mean Flavonoid Content mg/100g edible portion | | | | | | |
|---|---|---|---|---|---|---|
| **Proanthocyanidins** | **Monomers** | **Dimers** | **Trimers** | **4-6mers** | **7-10ers** | **Polymers** |
| Apple Granny Smith with peel raw (USDA) | 4.02 | 6.62 | 5.79 | 21.16 | 17.54 | 22.4 |

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