**AN ALIMENTARY PRODUCT IN POWDER FORM CONTAINING EXCLUSIVELY ROASTED COFFEE, CITRUS AURANTIUM DRY EXTRACT, OFFICINAL HERBS AND SPICES: PATENT NO. 0001361629 21JUNE, 2009 AND OTHER PATENTS PENDING.**

**DOSSIER OF DOCUMENTATIONS AND SCIENTIFIC STUDIES RELATED TO THE HEALTHY PROPERTIES OF THE INGREDIENTS CONTAINED IN *FITNESS COFFEE ANTIOXIDANT FULLY ACTIVE BLEND*, PRODUCT EAN CODE 8033229470028 (THE PRODUCT *FITNESS COFFEE ORIGINAL,* PRODUCT EAN CODE 8033229470073, HAS THE SAME FORMULA).**

## Description and mode of use of the product

**Trade marks:** fitness coffee and antioxidant fully active blend are trade marks of GVM I.E. Italy

**Description:** the product, in powder form, is packed usually in 250g (8.8oz) bags made with a triple barrier foil (PT-ALU-PT). Other size of packages may be available.

**Preparation:** brewed or infused with all traditional methods of preparation (Stovetop moka, Drip filter, French press, Espresso machine, Turkish style, Infused in hot water, Boiled, etc.

**Benefits**: all the herbal components are bioavailable and go inside the cup due their micronized form.

**Serving size**: 7g. (0.25oz); Calories: 3.3g; Total Carbohydrate: 0.11g; Sugars: 0.05g.

**Composition**: roasted coffee: 6,3g (90%); bitter orange dry extract: 0.1g (4% Synephryne 0.004g*); proprietary micronized herbal blend: 0.56g.

**Suggested daily intake**: 2/3 cups (Warning: do not exceed the quantities suggested; do not take in case of hypertension, anxiety, pregnancy, nursing).

*as suggested by Health Minister the SUL (safe upper level) of Synephrine daily Synepryne is 30mg (corrisponding to about 8 servings/cups).

**Claims:** helps boost energy levels and burn fat, supports your metabolism, aids in weight loss: these claims reported on the product package are related to the healthy properties of the world known herbal ingredients and are fully supported by their century use all over the world and by many related documentations and scientific studies, some of that we reproduced below in this dossier. For UK

But, before to go to them, here we report **two reports**: one on the Citrus Aurantium healthy properties and one on the antioxidants found in Fitness Coffee Original (*as above mentioned it has the same formula of Fitness Coffee Antioxidant fully active blend*).

### First report: Synephrine weight loss effects

#### What is Synephrine

It is the main "active" compound found in the fruit of a plant called Citrus aurantium. The fruit is also known as zhi shi (in traditional Chinese medicine), and as green orange, sour orange and bitter orange in other parts of

the world. Synephrine is chemically very similar to the ephedrine and pseudo-ephedrine found in many OTC cold/allergy medications and in a number of weight loss and energy supplements which contain Ma Huang. But synephrine differs from ephedrine in that synephrine is considered a semi-selective sympathomimetic (because it targets some tissues such as fat, more than it targets others such as the heart) versus a non-selective sympathomimetic (like ephedra which targets many tissues equally and thus often causes side effects). For example, although some high-dose ephedra-containing supplements have been associated with certain cardiovascular side effects as elevated blood pressure and heart palpitations, researchers at Mercer University in Atlanta have shown that Citrus aurantium extract (because it targets fat tissue rather than heart tissue) has no effect on hemodynamics such as heart rate and blood pressure.

### Theory

Because synephrine is a mild stimulant, it is thought to have effects in terms of providing an energy boost, suppressing appetite, and increasing metabolic rate and caloric expenditure. In traditional Chinese medicine, zhi shi is used to help stimulate qi and defined as the body's vital energy or life force – but in order to maximize the metabolic benefits of these extracts, total synephrine intake should probably be kept to a range of 2-10 mg/day. Although synephrine and several other compounds found in zhi shi are structurally similar to ephedrine and are known to act as stimulants (via adrenergic activity), zhi shi does not appear to have the same negative central nervous effects of ma huang (ephedra). Through its stimulation of specific adrenergic receptors (beta-3, but not beta-1, beta-2 or alpha-1), zhi shi is theorized to stimulate fat metabolism without the negative cardiovascular side effects experienced by some people with Ma Huang (which stimulates all beta-adrenergic receptors).

### Scientific Support

The effects of synephrine alone or in combination with other ingredients such as coffee and guarana (both are caffeine sources) generally fall into the category of acting as a mild stimulant. The extract of citrus aurantium, in addition to synephrine, also contains tyramine and octopamine. Octopamine may be related in some way to appetite control. There are clinical studies showing that synephrine-containing supplements help promote weight loss, and at least three clinical studies showing an enhanced thermogenesis (calorie expenditure) from these supplements. There is a great deal of research currently underway into the weight loss benefits of synephrine and supplements with related thermogenic effects, leading researchers in the Department of Physiology at Georgetown University to conclude that "Citrus aurantium may be the best thermogenic substitute for ephedra."

### Safety and Value

Early animal studies using high doses of isolated (purified) synephrine have been shown to raise blood pressure - but more recent human studies of citrus aurantoum extracts (standardized for synephrine) have not demonstrated any cardiovascular side effects. When used as directed (see dosage below), synephrine-containing supplements appear to be quite safe and should be treated as a mild stimulant with appetite controlling and mild thermogenic properties. The most likely explanation for weight loss effects attributed to citrus aurantium supplements is the mild appetite reduction and thermogenisis. Users can expect variable effects including reduced appetite and heightened feelings of energy – both of which are likely to result in weight loss.

### Dosage

Because synephrine is one small component of the Citrus aurantium fruit, a standardized extract is recommended. A dose of 4-20 mg of synephrine per day is a typical dose found in products providing 200-600 mg of a standardized citrus aurantium extract (3-6% synephrine).


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## Background

Interest and knowledge about the medical importance of balance between prooxidanter and antioxidants is increasing every day. With prooxidanter meant oxidizing agents / substances, which consist mainly of so-called free radicals, most of which generated a "bi-product" when the body's cells use oxygen in their energy. Oxidants are negatively affecting our tissues and it is considered today to be an integral part in the onset of illness and disease. It is therefore essential to be able to counteract these oxidants and the body takes place in a complex interaction between specific enzymes, vitamins, certain minerals, peptides, carotenoids, flavonoids, etc., we are talking about an antioxidant defense. To meet the individual's need for good antioxidant defense requires that the intake of food and drink is rich in just such components. Great attention has been paid to the content of vegetables and fruit, especially where the color pigments in the berries are carotenoids and flavonoids. For drinks especially tea and red wine described as rich in flavonoids.
Naturally, the amount of antioxidants in a product is of great importance, but it is also important to examine how effectively they work as just an antioxidant. It is said that there is a certain antioxidant capacity in the sense of the ability to contrast oxidants. There are a few different methods for such measurements, and most will relate the effect to mmol / l . Trolox (a kind of artificial vitamin E). In addition to measurements of blood plasma and other body fluids used such measurements on individual food products, but above all on different foodstuff. A search of medical databases show up towards the 4000 research which antioxidant capacity of the components of the diet studied.

## Uploader

Against the above background it is therefore interesting to measure the antioxidant capacity of Fitness Caffé when this coffee also has added other ingredients with a potential anti-oxidative effect. For the measurement has been used a CR3000RC instrument and related reagents for antioxidant capacity, FORD (Oxygen Free Radical Defense). For each sample preparation is a dilution series analyzed to have an adjustment to the method sensitivity and reading in the linear area. Besides the two variants of Fitness Caffé has to compare some other beverages tested. The reported results are an average based on three determinations.

## Results

Tabell 1. BEARCO KB ! MN BIOCARE APRIL 6, 2009

| Dryck/ Bevanda | A-O kapacitet Capacità Antiossidanti mmol/l | Intag 1,5 dl Kopp Assunzione tazza 1,5 dl (mmol) |
|---|---|---|
| Eget bryggt Caffè tradizionale | 19,2 | 2,9 |
| Fitness Caffè Original Mediterraneo formula | 26,7 | 4,1 |
| Fitness Caffè Energins formula | 25,0 | 3,8 |
| Te –påse Tea bag (in bustina) | 7,6 | 1,1 |
| Te- kula Tea infusion (tisana) | 10,2 | 1,5 |
| Rödvin (Merlot-italien) Vino rosso Merlot | 17,2 | 2,6 |
| Rödvin (Cabernet-italien) Vino rosso Cabernet | 20,4 | 3,1 |

The table reports the measured values for each drink with the variety set to mmol / l referring to Trolox. Plain self-brewed coffee showing of 19.2 mmol / l. The corresponding value for Fitness Caffè Original 26.7 mmol / l. As a comparison, were analyzed two kinds of tea. A brewed from traditional tea bag and one with the tea ball from the loose mix (green tea). Red wines from different grape varieties were also tested.
For comparison got powder from Fitness variants also stand in cold water.
The water was pale brown color after 48 hours when the powder is filtered .
Subsequent analysis showed 2.6 mmol / l for Original

## Comments

The results indicate that Fitness Caffé has an antioxidant capacity which is about 40% higher than usual Drip Coffee.

The antioxidant capacity of the Fitness Caffè Original is about 350% higher than the tea bag and 250% higher than tea infusion, variants which also have been reported in published research.

Red wines are often cited as a good antioxidant source and these preliminary data show that coffee can match well with the tested red wines.

It is important to emphasize that the temperature is very important to extract the substances from coffee that have antioxidant effect thus requires a quality coffee makers in order to be optimal in this context.

Finally should be noted that the above results and comments are relating to the in vitro tests and therefore they provide some indication of the antioxidant effect relative to each other. In order to determine final effect in vivo tests need be carried out de facto on the persons with regular consumption of coffee / processing etc.

Stockholm April 6, 2009

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## Fitness Coffee ingredients: their healthy properties and related scientific documentations and studies

### 1. Coffee

- **Proper Name:** Coffea arabica, Coffea robusta
- **Source Matherial:** green beans
- **Healthy properties:** coffee is described as source of antioxidants and of other healthy properties
- (***see at the bottom of this dossier for all related documentation on the roasted coffee***)
- **Some References (***other references are at the bottom of this dossier***)**


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**2. Bitter Orange dry extract**

- **Proper name**: Citrus Aurantium Amara dry extract
- **Source raw Matherial**: peels of fruit (4% Synephrine dry extract)
- **Dosage**: the daily safe upper intake of Synephrine reccomended by Italian Minister of Health is 30 mg pro die. It should be taken 8 daily cups of Fitness Coffee to overcome the amount of 30 mg so the product is safe for the normal use (*see report above*)
- **Uses or Purposes:** statements of Citrus Aurantium in Fitness Coffee (*see report above*)

**3. Peppermint**

- **Proper name**: Mentha piperita
- **Source Matherial**: leaves
- **Route of Administration**: oral
- **Uses or Purposes:**

Traditionally used in Herbal Medicine to aid digestion (stomachic)
Traditionally used in Herbal Medicine to help relieve flatulent dyspepsia (carminative)

- **Known Adverse Reaction(s)** : No statement is required
- **Preparation:** Infusion
- **Duration of use:** No statement is required
- **References:**


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## 4. Turmeric

- **Proper name:** Curcuma longa L. (USDA 2008)
- **Source matherial**: Powdered rhizome
- **Route of Administration**: oral
- **Uses or Purposes:**

  Traditionally used in Herbal Medicine to help relieve flatulent dyspepsia (carminative)
  Turmeric has a long history of use in folk medicine for the treatment of wounds, infections, and other health problems. It contains phenolic compounds that are thought to provide antioxidant and anti-inflammatory benefits, as well as support a healthy immune system.
  Recent studies have investigated its potential to lower cholesterol levels, improve cardiovascular health, reduce the risk of Alzheimer's and diabetes as well as cancer-fighting properties.
  Used in Herbal Medicine to aid digestion
- **Preparation:** Infusion
- **Duration of use:** No statement is required
- **Known Adverse Reaction(s)** : No statement is required
- **References:**

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## 5. Licorice

- **Proper name:** Glycyrrhiza glabra (USDA 2008)

- **Source matherial:** decorticated roots

- **Route of Administration**: oral

- **Uses or Purposes:**

Traditionally used in Herbal Medicine as an expectorant to help relieve chest complaints, such as catarrhs, coughs and bronchitis
Used in Herbal Medicine to help relieve inflammatory conditions of the gastrointestinal tract, such as gastritis in adults

- **Preparation:** Infusion
- **Known Adverse Reaction(s):** No statement is required

- **References:**


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## 6. Ginger

- **Proper name**: Zingiber officinale (USDA 2008)

- **Source Matherial**: rhizome

- **Route of Administation**: oral

- **Uses or Purposes:**

Traditionally used in Herbal Medicine to help relieve digestive upset/disturbances including lack of appetite, nausea, digestive spasms, indigestion, dyspepsia and flatulent colic (carminative).

Traditionally used in Herbal Medicine as an expectorant and anti-tussive to help relieve bronchitis as well as coughs and colds.

- **Duration of use:** No statement is required

- **Known Adverse Reaction(s):** No statement is required

- **References:**


- Bradley PR, editor. 1992. British Herbal Compendium: A Handbook of Scientific Information on Widely Used Plant Drugs, Volume 1. Bournemouth (GB): British Herbal Medicine Association.
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## 7. Green Tea

- **Proper name:** Thea sinensis L. (USDA 2007)

- **Source material**: Leaf

- **Route of administration:** Oral
- **Uses or Purposes:** Statement(s) to the effect of green tea.

  An accumulated number of population studies suggests that consumption of green and black tea beverages may bring positive health effects. One hypothesis explaining such effects is that the high levels of flavonoids in tea can protect cells and tissues from oxidative damage by scavenging oxygen-free radicals. Chemically, the flavonoids found in green and black tea are very effective radical scavengers. The tea flavonoids may therefore be active as antioxidants in the digestive tract or in other tissues after uptake. A substantial number of human intervention studies with green and black tea demonstrates a significant increase in plasma antioxidant capacity in humans ~1 h after consumption of moderate amounts of tea (1-2 cups). There are initial indications that the enhanced blood antioxidant potential leads to reduced oxidative damage to macromolecules such as DNA and lipids. In conclusion, tea flavonoids are potent antioxidants that are absorbed from the gut after consumption. Tea consumption consistently leads to a significant increase in the antioxidant capacity of the blood. Beneficial effects of increased antioxidant capacity in the body may be the reduction of oxidative damage to important biomolecules. The scientific support is strongest for the protection of DNA from oxidative damage after black or green tea consumption. However, the quality of the studies now available is insufficient to draw firm conclusions. Therefore, further evidence from human intervention studies is required.

  Source of antioxidants for the maintenance of good health (Camargo et al. 2006; Coimbra et al. 2006; Henning et al. 2004; Nakagawa et al. 1999; Van het Hof et al. 1997).

  To be used with a program of reduced intake of dietary calories and increased physical activity (if possible) to help in weight management (Nagao et al. 2005; Westerterp-Plantenga et al. 2005; Chantre and Lairon 2002; Dulloo et al. 1999)

- **Known adverse reaction(s):** No statement required.
- **References:**


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## 8. Cardamom

- **Proper Name:** Elettaria Cardamomum (Blumenthal 1998)
- **Source Matherial:** seeds
- **Route of Administration:** oral
- **Uses or Purposes:**

  By including cardamom and other curry herbs in the regular diet, one can experience both the culinary pleasure as well as the medicinal actions, which include appetite stimulation, carminative, stimulant and stomachic actions. Flatulent dyspepsia is avoided, foods are better digested and assimilated, the blood is thinned.

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## 9. Cinnamon

- **Proper name:** Cinnamomum Zeylanicum (European pharmacopoeia 1997)
- **Source Matherial:** dried micronized bark.

- **Route of Administration:** oral
- **Uses, Purposes, Claims**

  http://www.food.gov.uk/multimedia/pdfs/listofukhealthclaims05.pdf **(page 109)**

  Claims: Digestion ID 1969,
  Antioxidant ID 1975,
  Weight control/slimming/Inhibition of dietary fat absorption/Inhibition of dietary sugar absorption ID 1704.
  Governmental, AuthoritativeAV, O/SO mgraph, other txb, individual studies
  CINNAMOMUM ZEYLANICUM. BARK .
  DIGESTION Textbooks etc .
  The Ayurvedic Pharmacopoeia of India Pt. 1:1/114
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  A novel inhibitor of bacterial endotoxin derived from cinnamon bark. Biochem. Biophys. Res. Commun., Vol.234(2):506-510. See also Antimicrobial and antioxidant studies.
- It has a broad range of historical uses in different cultures, including the treatment of diarrhea, rheumatism, and certain menstrual disorders (Leung 1996). Antibacterial actions have also been demonstrated for cinnamon (Azumi 1997).
- It has been used as a digestive and as a fat reducer according to Ayurvedic (traditional Indian medicine) texts which date back to 1400 BCE (Ravindran 2002).
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## 10. Yerba Mate

- **Proper Name:** Ilex Paraguariensis (British Pharmacopoeia 1996)
- **Source Matherial**: leaves
- **Route of Administration:** oral
- **Uses or Purposes:** Statement(s) to the effect of:

  Yerba mate is used as a tonic, diuretic, and as a stimulant to reduce fatigue, suppress appetite, and aid gastric function in herbal medicine systems throughout South America. It also has been used as a depurative (to promote cleansing and excretion of waste). In Brazil, mate is said to stimulate the nervous and muscular systems and is used for digestive problems, renal colic, nerve pain, depression, fatigue, and obesity (Dickel 2007).

  Yerba mate also has a long history of use worldwide. In Europe it is used for weight loss, physical and mental fatigue, nervous depression, rheumatic pains, and psychogenic- and fatigue-related headaches (Alicaridis 1987, Anderson 2001). In Germany it has become popular as a weight-loss aid. Yerba mate is the subject of a German monograph which lists its approved uses for mental and physical fatigue. In France yerba mate is approved for the treatment of asthenia (weakness or lack of energy), as an aid in weight-loss programs, and as a diuretic. It also appears in the *British Herbal Phamacopoeia* (1996) and indicated for the treatment of fatigue, weight loss, and headaches.

- **References:**


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- Dickel, M. L., et al. "Plants popularly used for loosing weight purposes in Porto Alegre, South Brazil." *J. Ethnopharmacol.* 2007


## 11. Star Anis

- **Proper Name:** Illicium Verum
- **Source Matherial:** fruits
- **Route of Administration:** oral
- **Uses or Purposes:** Statement(s) to the effect of: The Chinese have used star anise for 1,300 years. Star anise has been known to be used as a stimulant, to prevent gas, and to enhance sexual desire. Furthermore, the anise oil is reported to have a weak antibacterial activity. Star anise is used as an expectorant in cough mixtures and lozenges. It has been used internally for dyspeptic (stomach upset) complaints (Leung 1996).
- Star anise is used orally to ease loss of appetite, stomach discomfort, gas, and bloating (Jellin 1996).
- The German Commission E has approved the use of star anise seeds to soothe gastrointestinal complaints (Blumenthal 1998).
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- Leung AY, Foster S. Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. 2nd ed. New York: Wiley-Interscience; 1996.


## 12. Anis

- **Proper Name:** Pimpinella anisum
- **Source Matherial:** fruits
- **Route of Administration:** oral
- **Uses or Purposes:** Statement(s) to the effect of:

  It is a particularly useful tonic to the whole digestive system and its antispasmodic and expectorant effects make it of value in the treatment of various respiratory problems (Chevallier 1996). The seed is antiseptic, antispasmodic, aromatic, carminative, digestive, expectorant, pectoral, stimulant, stomachic and tonic (Launert 1981, Holtom 1979)

- **References:**


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## 13. Rhodiola rosea

- **Proper Name:** Rhodiola rosea
- **Source Matherial:** Roots
- **Route of Administration:** oral
- **Uses or Purposes:** Statement(s) to the effect of:

- The traditional use of R. rosea as a tonic in Siberian and Russian medicine stimulated extensive research leading to identification of R. rosea as an adaptogen — a substance that nonspecifically increases the resistance of an organism and does not disturb normal biological parameters. Studies in cell cultures, animals, and humans have revealed antifatigue, anti-stress, antihypoxic (protection against damaging effects of oxygen deprivation), anticancer, antioxidant, immune enhancing and sexual stimulating effects (Germano 1999, Saratikov 1987).
- In Sweden, R. rosea was recognized as an Herbal Medicinal Product in 1985 and has been described as an antifatigue agent in the Textbook of Phytomedicine for Pharmacists (Sandberg 1993). In the textbook of pharmacology for dispenser training in Sweden, R. rosea is mentioned as a plant with a stimulant action. Also, the Pharmaceutical Book (Lakemedelsboken 97/98) mentions R. rosea as one of the most commonly used psychostimulants in the group of officially registered herbal medicinal

products (Sandberg 1998).

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# Addendum

## Scientific documentations and studies on healthy properties of roasted coffee

1) **ANTIOXIDANTS IN COFFEE**

Plant phenols are a large and diverse group of compounds including cinnamic acids, benzoic acids, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans and lignins. It has been shown that plant phenols have strong antioxidant activity in vitro (1). As a result it has been hypothesised that plant phenols might protect cellular DNA, lipids and proteins from free radical- mediated damage in vivo. Since free radicals are believed to play a role in the development of chronic diseases such as cardiovascular disease and cancer then the consumption of plant phenols may protect against these diseases. As reviewed recently, five out of seven published observational epidemiological studies have shown that flavonols protect against cardiovascular disease but only one out of four studies showed that they protect against cancer (2). Hence the available evidence for a protective effect of flavonols against cardiovascular disease and cancer is far from conclusive and other categories of plant phenols have yet to be investigated.

2) **Chlorogenic acids** are a family of esters formed between trans-cinnamic acids and quinic acid. The commonest individual chlorogenic acid is formed between caffeic acid and quinic acid. It has been shown that both chlorogenic acid and caffeic acid are strong antioxidants in vitro (1). Coffee beans are one of the richest dietary sources of chlorogenic acid and for many consumers this will be their major dietary source (3). It has been reported that a 200 ml cup of arabica coffee contains between 70 and 200 mg chlorogenic acid whereas a cup of robusta coffee contains between 70 and 350 mg (3). It has been estimated that coffee drinkers might ingest as much as 1 g per day cinnamate esters (mostly chlorogenic acid) and 500 mg per day cinnamates (mostly caffeic acid). Coffee could supply as much as 70% of the total making it far and away the most important dietary source of this group of antioxidants (3). The amount of chlorogenic acid or caffeic acid available to act as an antioxidant in vivo will depend on absorption from the gut which may be incomplete and any subsequent metabolism which may be extensive. It has recently been demonstrated that humans absorb about 33% of ingested chlorogenic acid and about 95% of ingested caffeic acid (4). A study of human chlorogenic acid metabolism showed that the unabsorbed chlorogenic acid which reaches the colon is hydrolysed to caffeic acid and quinic acid by the colonic microflora (5). Following dehydroxylation by the colonic microflora, absorption and further metabolism in the liver and kidney, benzoic acid is formed and

conjugated to glycine to form hippuric acid. About half the ingested chlorogenic acid appears as urinary hippuric acid (5). This metabolism can be expected to considerably diminish the antioxidant activity of chlorogenic acid in vivo as hippuric acid has no antioxidant activity.

3) **The roasting of coffee beans dramatically increases their total antioxidant activity**. A roasting time of 10 minutes (medium-dark roast) was found to produce coffee with optimal oxygen scavenging and chain breaking activities in vitro (6). A study of robusta and arabica coffees from six different countries showed that robusta samples contained significantly more reducing substances than arabica samples and that protective activity measured ex vivo was significantly greater in roasted samples than in green coffee (7). Using the ABTS•+ method (the gold standard), it was confirmed that light roast or medium roast coffee has a significantly higher antioxidant activity in vitro than green coffee (8). This difference was observed despite a 19% and 45% decrease in the chlorogenic acid content of light and medium roast coffee respectively implying that other compounds make significant contributions to the total antioxidant activity of roasted coffee. Melanoidins are brown polymers formed by the Maillard reaction during the roasting of coffee beans and account for up to 25% of the dry matter. It has recently been shown by the ABTS•+ method that coffee melanoidins have significant antioxidant activity in vitro (9). The total antioxidant activities of different plant phenol- containing beverages have been compared. Using a method based on the ex vivo oxidation of low density lipoprotein (LDL), it has been shown that coffee has significantly more total antioxidant activity than either cocoa, green tea, black tea or herbal tea (10). Using the ABTS•+ method, it has been confirmed that coffee has a significantly greater total antioxidant activity in vitro than cola, beer, a variety of fruit juices, lemon ice tea or black tea (11). A study conducted in 2004 looked at dietary sources of antioxidants and found that the single greatest contributor to total antioxidant intake was coffee (12). A further study in 2006 (13) set out to determine the content of phenolic acids in the most consumed fruits and beverages. Coffee, as wel as black and green teas were the best source among beverages with coffee containing 97mg/100 g whilst teas contained 30-36 mg/100 g

4) **It can be concluded** that coffee possesses greater in-vitro antioxidant activity than other beverages, due in part to intrinsic compounds such as chlorogenic acid, in part to compounds formed during roasting such as melanoidins and in part to as yet unidentified compounds. Authors of a study published in 2002 (14) suggested that uric acid was the main component responsible for plasma antioxidant capacity increase after tea drinking, whereas molecules other than uric acid (probably phenolic compounds) are likely to be responsible for the increase in plasma antioxidant capacity after coffee drinking. Whether the antioxidants characteristic of coffee are protective against chronic diseases such as cardiovascular disease and cancer remains to be determined. Research continues, and the conclusion of a study published in 2006 (15) consisting of a cohort of 41,836 postmenopausal women, was that 'Consumption of coffee, a major source of dietary antioxidants, may inhibit inflammation and thereby reduce the risk of cardiovascular disease and other inflammatory diseases in postmenopausal women'.

   It should be noted that these results of course refer to a specific sub group and it would not, at this stage, be appropriate to extrapolate them across to the general population before further research clarifies these conclusions.

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**1.1) "Americans get more of their antioxidants** from coffee than any other dietary source," said Joe Vinson, lead author of a study on antioxidants conducted by the University of Scranton, in Pennsylvania. "Nothing else comes close."

**1.2) While antioxidants help to reduce cell damage** and aging, scientists have yet to determine if they are the compounds responsible for coffee's weird -but wonderful- health benefits. There are many organic and inorganic compounds in a regular cup of coffee, including chemicals called phenolic compounds, melanoidins, and diterpenes. Some of these chemicals are believed to be beneficial, such as chlorogenic acid, which is a natural compound found in coffee beans and other plants in Fitness Coffee® formula, that is an antioxidant and believed to aid in digestion, according to the American Journal of Clinical Nutrition.

**1.3) "Coffee contains** more than a thousand chemicals, some of which have antioxidant and antimutagenic activities," Mia Hashibe, assistant professor of medicine at the University of Utah (USA).

## OTHER COFFEE HEALTH PROPERTIES

**Regular coffee drinkers have a 39 percent decreased risk of head and neck cancer**, according to a new study published in the journal Cancer Epidemiology, Biomarkers & Prevention. Those who drank an estimated four or more cups a day had significantly fewer cancers of the mouth and throat than non coffee drinkers, the study found.

**Coffee may be good for the brain**, too. A study earlier this year by neuroscientists at the University of Lisbon showed that drinking coffee can help to prevent the neural degeneration associated with brain disorders and aging. The scientists found that drinking up to four cups of coffee a day over a long period of time actually prevented the deterioration of memory.

**Other research** has shown that coffee is good for the cardiovascular system. Women who drank one to three cups of java a day reduced their risk of cardiovascular disease by 24 percent, according to the Iowa Women's Health Study that tracked 27,000 women for 15 years, although it was noted that this benefit diminished as the quantity of coffee rose above three cups. And while coffee has been given a bad rap for supposedly upping the risk of stroke and coronary heart disease, scientific studies have revealed the contrary. Drinking coffee lowers the risk of stroke by 19 percent among women, according to a 2009 Harvard Medical School study that tracked the coffee habits and stroke occurrences among 83,000 American women for nearly a quarter century.

**The risk of some cancers may be cut** by drinking coffee. Research presented at the 2009 American Association for Cancer Research Frontiers in Cancer Prevention Research Conference showed that coffee cut male coffee drinkers' risk of aggressive prostate cancer by 60 percent, based on a 20-year study of 50,000 men. And people who drink coffee reduce their risk of developing liver cancer by 41 percent, compared to people who never drink coffee, according to a study in the journal Hepatology. The researchers theorized that compounds found in coffee may block the action of enzymes involved in detoxifying carcinogenic compounds that may lead to liver cancer, the third largest cause of cancer deaths around the world, after lung and stomach cancer. Other recent studies have shown that coffee is protective against certain brain tumors, endometrial cancer and advanced prostate cancer.

**Scientists have actually identified** a chemical in coffee, called N-methylpyridinium, which inhibits acid production. The compound is more common in dark roasts like espresso as used in Fitness Coffee® and French roast blends, according to the Research Platform of Molecular Food Science at the University of

Vienna.

## Scientific studies on coffee and other vegetals ingredients

### Effects of coffee on the total plasma antioxidant capacity in humans and bioavailability of coffee polyphenols

Mirella Nardini, Fausta Natella and Cristina Scaccini

**Background**

Polyphenols have been reported to exert a variety of biological actions, such as free radical scavenging, metal chelation, modulation of enzymatic activity and, more recently, to affect signal transduction, activation of transcription factors and gene expression (1-4).
Epidemiological studies have suggested associations between the consumption of polyphenols-rich foods and beverages and the prevention of many human diseases.
Despite extensive literature describing the effects of polyphenols, our knowledge about their absorption from diet is scarce, one major question arising on the absorption of bound forms of phenolic compounds:
A number of beverages derived from vegetables have been tested for their in vitro and in vivo antioxidant activity (white and red wine, green and black tea, beer) (5-7). In particular, in the last years, a number of studies focused on the capacity of tea to elicit in vivo antioxidant protection in humans, giving pictures both contrasting or of largely different extent. However, a recent paper clearly demonstrated that consumption of a single dose of black or green tea induces a significant rise in plasma antioxidant activity *in vivo* (8). Black tea contains catechins, thearubigins and theaflavines, which are oxidation products of catechins formed during enzymatic oxidation by polyphenol oxidase in fresh tea leaves.
Although coffee is as rich as tea in phenolic antioxidants and is equally consumed in the world, its antioxidant activity in vivo has been never studied.
Coffee contains several phenolic components, other than tocopherols, endowed with antioxidant capacity, and the total polyphenols amount ranges from 200 to 550 mg per cup. Among the phenolic compounds identified are chlorogenic acids, a family of esters formed between quinic acid and several cinnamic acids such as caffeic, ferulic and p-coumaric acid, caffeoylquinic acid being by far the most abundant. Based on 10 g coffee per cup of brew, a cup content of chlorogenic acid (5'-caffeoyl quinic acid, the most abundant isomer) can range from 15 to 325 mg. A value of 200 mg/cup has been reported for coffee, brewed by drip filtering.
The aims of our studies were:
• To assess the capacity of coffee in affecting the plasma redox homeostasis in humans in fasting conditions, using tea as control. Total antioxidant capacity and the concentration of the main antioxidants were measured on plasma before and after the supplementation of a standard cup of coffee or black tea. Metabolic parameters in plasma were also measured to control the eventual effect of acute coffee and tea consumption on lipid metabolism.
• To determine the bioavailability of phenolic acids, with particular concern on the conjugated forms. The preliminary set up of a hydrolysis method was necessary to avoid the d+egradation of phenolic acids in the alkaline conditions commonly used.

**Design of the study and brief description of methods utilized**

*In vitro study on detection of bound phenolic acids in coffee and compared analysis of antioxidant capacity and phenolic concentration in coffee and tea*

Coffee brew was prepared using a commercial automatic brewing machine (60 g of roasted and ground coffee from an Italian brand per liter water) and used within 10 min from preparation. Coffee (non-hydrolyzed or subjected to alkaline hydrolysis in the presence of EDTA and ascorbic acid) was analyzed using an HPLC system, consisting of a Perkin-Elmer Series 4 Liquid Chromatograph with gradient pump, column thermoregulatory, auto sampling injector equipped with electrochemical coulometric detector
The total antioxidant capacity of coffee and tea (prepared by 5 min infusion of 20 g in 1 liter of water at 100°C) was measured using two different systems, the loss of fluorescence of rphycoerithryn

(TRAP test) and the competition kinetic with the bleaching of a carotenoid, the crocin (Crocin test), triggered by the peroxyl radicals generated by thermal decomposition of 2,2'-azobis(2-amidinopropane) HCl (AAPH).
Total phenols were measured by the Folin Ciocalteau method after deproteinization of samples with ammonium sulfate (9). Caffeine, theobromine and theophylline were detected by HPLC (10).

*In vivo study on modulation by coffee and tea drinking of plasma antioxidant capacity in humans*

A standard amount (200 ml) of brewed coffee was administered in fasting conditions to 10 healthy non-smoker moderate-coffee drinkers. In a different session (2 weeks apart) black tea was administered as control. Beverages were ingested within 10 min from brewing.
The total antioxidant capacity of plasma was measured using the same methods employed for the analysis of beverages. Single molecules with antioxidant capacity were individually measured (SH groups, ascorbic and uric acid, alpha tocopherol).
Plasma total cholesterol and triacylglycerols, HDL-cholesterol and LDL-cholesterol, total homocysteine were also measured to control any metabolic effect.

*Bioavailability of phenolic acids from coffee in humans*

Aliquots of plasma samples (0.5 ml) from each subject were thawed and treated according to one of the three following procedures: no treatment, to detect free phenolic acids; β-glucuronidase treatment (used to selectively hydrolyze glucuronidated forms of hydroxycinnamic acids) and alkaline hydrolysis treatment (used to liberate phenolic acids from bound complexes) to detect total (free + bound) phenolic acids. *o*-Coumaric acid was selected as internal standard due to the absence of detectable amounts of this compound in human plasma samples before and after coffee administration, with or without β-glucuronidase or alkaline hydrolysis treatments.
The presence of phenolic acids in treated and untreated samples was assessed by HPLC-ECD.

## Results

*In vitro study on detection of bound phenolic acids in coffee (11) and compared analysis of antioxidant capacity and total phenols concentration in coffee and tea (12)*

Coffee brew was analyzed for phenolic acids composition, before and after hydrolytic treatment. Chlorogenic acid (5'-caffeoyl quinic acid) was present in non-hydrolyzed coffee at high concentration, while free caffeic acid, *p*-coumaric acid and ferulic acid were undetectable. After hydrolysis, ferulic acid, *p*-coumaric acid and high levels of caffeic acid were detected. The amount of caffeic acid released upon hydrolysis was higher than the



amount expected from hydrolysis of chlorogenic acid based on 1 to 1 stoichiometry. This result is explained by the fact that coffee also contains dicaffeoylquinic acid derivatives and different isomer of caffeoylquinic acids besides 5'-caffeoylquinic acid, the one detected in our experiments. From our data, we calculated that a cup of coffee (200 ml) contained 95.8±4.6 mg chlorogenic acid (5'-caffeoylquinic acid). After hydrolytic treatment, the total phenolic acids content of a cup of coffee was: caffeic acid, 166.0 ± 14.0 mg, *p*-coumaric acid 2.8 ± 0.2 mg, ferulic acid 28.6 ± 2.5 mg.
The measure of the antioxidant capacity of the two beverages, using both TRAP and Crocin test methods indicates that coffee is more powerful in scavenging peroxyl radicals than tea, at least in an hydrophilic environment. Total phenols, expressed as gallic acid equivalents, are still higher in coffee than in tea, but the difference is not as dramatic (+ 40%) as for the antioxidant capacity. Thus, the antioxidant capacity of the beverages cannot be explained by the mere measure of total phenols.
To further characterize the two beverages, we measured the concentration of 1,3,7-trimethyl xanthine (caffeine), 3,7-dimethyl xanthine (theobromine) and 1,3-dimethyl xanthine (theophylline). Caffeine in a cup of coffee (200 ml), as administered in the in vivo study, corresponded to 181 mg, while 200 ml of tea contained 130 mg of caffeine. The figures for theobromine were 28.9 and 5.9 mg/200 ml, respectively for coffee and tea. In both samples, theophylline was under the detection limit of our method.
As trimethyl xanthines don't have antioxidant activity against peroxyl radicals, we can

postulate that the higher antioxidant activity of coffee in respect to tea is probably linked to its different pattern in antioxidant compounds. Alpha-tocopherol was present in negligible amount in coffee and it was absent in tea (data not shown). Thus, we can exclude a participation of α-tocopherol to the beverage's AC. Finally, the contribution of other compounds with antioxidant activity present in roasted coffee, namely Maillard products or melanoidins, can not be excluded.

*In vivo study on modulation by coffee and tea drinking of plasma antioxidant capacity in humans (12)*

The ingestion of 200 ml of coffee in bolus produced a statistically significant increase at t = 1 (5.5%, $P<0.05$) in the plasma antioxidant capacity, measured by the TRAP method, maintaining a 4% increase after two hours. The 4.7 % increase of TRAP 1 hour after tea administration did not reach statistical significance.

In the case of coffee, the Crocin test gave a similar trend in the modulation of antioxidant activity, even if the differences were not statistically significant. In the case of tea, the AC measured by the Crocin test, decreased significantly ($P<0.005$) after 2 hours, paralleling the decline of the reduced form of ascorbic acid.

The apparent lack of statistical significance in the increases of AC by TRAP test for tea and by Crocin test for coffee disappears when inter-individual differences are taken into account. In fact, analyzing individual data, we found that subjects did not reach the maximum value at the same time. Prevalently the peak time was 1 hour. However, in the case of the measurement of AC by Crocin test, 4 subjects reached the maximum value 2 hours after coffee drinking and in the case of the measurement of AC by TRAP, 3 subject reached the peak 2 hour after tea drinking. This event can be linked to differences in the efficiency of absorption and/or metabolism of antioxidant compounds. Comparing the individual AC at time 0 with the AC at the peak time (1 hour or 2 hours depending on the subjects), we observed a significant increase in plasma AC using both methods after coffee drinking. The increase in plasma AC after tea drinking reached statistical significance only when measured by TRAP method.

The two methods employed to measure AC differ for their capacity to be affected by uric acid: in fact plasma uric acid contribution to TRAP is about 60%, while its contribution to the Crocin test is equal to zero. Because coffee and tea drinking induced a significant increase of plasma uric acid, we can speculate that the increase in plasma AC measured by the TRAP method was largely affected by the increase of plasma uric acid concentration. After coffee drinking, we observed a significant increase of AC also using the Crocin test. As uric acid do not contribute to the Crocin test, we can speculate that molecules other than uric acid (probably phenolic acids) are responsible for the observed increase of antioxidant capacity. Caffeic acid is the most abundant phenolic compound in coffee brew and it is endowed with strong antioxidant activity in vitro and in vivo. As caffeic acid is present in human plasma at µmolar concentration after coffee drinking (*13*), we can assume that it is at least in part directly responsible for the increase in plasma antioxidant capacity observed in this study. Therefore, whilst the contribution of phenolic compounds from tea to the AC is essentially indirect, 'influencing' the plasma uric acid level (even if a slight direct contribution can not be ruled out) phenolic compounds from coffee could act both directly and indirectly.

*Bioavailability of caffeic acid from coffee in humans (13)*

In order to study the absorption of coffee phenolic acids, plasma samples collected before and after coffee administration were analyzed for content of both free and total (free + bound) phenolic acids, using two different procedures of hydrolysis to release phenolic acids from bound forms. In the first procedure, β-glucuronidase was used to selectively hydrolyze glucuronidated forms of hydroxycinnamic acids. In the second procedure, an alkaline hydrolytic treatment was used to liberate phenolic acids from bound complexes.

Less than 12.6±7.4 ng/ml of free caffeic acid (corresponding to 0.07 µM) was detected in the untreated control plasma samples taken immediately prior to coffee brew administration (time 0). A significant increase in free caffeic acid plasma levels was found in untreated

plasma samples 1 h after coffee brew consumption in respect to time 0.
After β-glucuronidase treatment, total caffeic acid in plasma was significantly higher at both 1 h and 2 h after coffee administration than at time 0 with a maximum absorption peak at 1 h for all subjects. Alkaline hydrolysis treatment of plasma samples gave similar results, with significantly higher levels of total caffeic acid at 1 h and 2 h in respect to time 0 and maximum absorption peak at 1 h. Both β-glucuronidase and alkaline hydrolysis treatment released a considerable amount of caffeic acid at 1 h and 2 h after coffee consumption. Interestingly, the plasma levels of caffeic acid measured after both hydrolysis procedures were very similar and no significantly different by ANOVA.

## Conclusions

1. Following our experimental conditions, coffee drinking increases plasma antioxidant capacity, probably due to bioavailability and antioxidant activity of its peculiar group of phenolic compounds (chlorogenic acids).
2. Coffee administration resulted in increased total plasma caffeic acid concentration, with an absorption peak at 1h. Caffeic acid was the only phenolic acid found in plasma samples after coffee administration, while chlorogenic acid was undetectable. Most of caffeic acid was present in plasma in bound form, mainly in the glucuronate/sulfate forms. Due to the absence of free caffeic acid in coffee, plasma caffeic acid is likely to be derived from hydrolysis of chlorogenic acid in the gastrointestinal tract.

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# Effects of Coffee Consumption on Oxidative Susceptibility of Low Density Lipoproteins and Serum Lipid Levels in Humans

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## Abstract

Since little is known about how coffee intake affects low density lipoprotein (LDL) oxidative susceptibility and serum lipid levels, we conducted an *in vivo* study in 11 healthy male students of Wakayama Medical University aged between 20 and 31 years fed an average Japanese diet. On days 1-7 of the study, the subjects drank mineral water. On day 7, the subjects began drinking coffee, 24 g total per day, for one week. This was followed by a one week “washout period” during which mineral water was consumed. Fasting peripheral venous blood samples were taken at the end of each one week period. LDL oxidation lag time was approximately 8% greater ($p < 0.01$) after the coffee drinking period than the other periods. Serum levels of total cholesterol and LDL cholesterol (LDLC) and malondialdehyde (MDA) as thiobarbituric acid reactive substances (TBARS) were significantly decreased after the coffee drinking period. Finally, regular coffee ingestion may favorably affect cardiovascular risk status by modestly reducing LDL oxidation susceptibility and decreasing LDL cholesterol and MDA levels.

Coffee is one of the most widely consumed psychoactive beverages in our society. It is a well known and extensively utilized psychotropic agent with effects on mood, cognitive performance, and motor activity. In fact, many investigators have shown that caffeine, one of the main constituents of coffee, has a variety of pharmaco- logical and cellular responses in a wide spectrum of biological systems [1]. These include stimulation of the central nervous system and cardiac muscle, increased urinary output, and relaxation of smooth muscle. In addition to caffeine, coffee also contains a relatively large amount of chlorogenic acid, a tea catechin analog, showing biologi- cal effects such as antioxidation, antimutation, anticarcinogenesis, antibiotic, antihypercholesterolemia, anti- hypertension, and antiinflammatory actions [2]. Accordingly, coffee must be a useful beverage for health. In contrast, some investigators have reported that caffeine and/or heavy coffee intake may serve as risk factors for lifestyle related diseases including heart disease and osteoporosis as well as periodontal diseases [3, 4], which are intimately associated with nutrition, exercise, alcohol, smoking, and several other lifestyle factors. It is currently believed that oxidative modification of low density lipoproteins (LDL) by free radicals is a key early event in the pathogenesis of atherosclerosis [5]. The rapid uptake of oxidatively modified LDL via a scavenger receptor leads to the formation of foam cells, and oxidized LDL also has a number of other atherogenic properties [5]. Several dietary antioxidants have been shown to inhibit the oxidative modification of LDL [6–8] and, hence, have the potential to decrease LDL oxidation and atherogenesis *in vivo* [9, 10]. However, the efficiency of the protection of LDL *in vivo* will depend on several factors, including the absorption of the antioxidants and how they interact with lipoproteins. A number of mechanisms are likely to contribute to inhibition of LDL oxidation by antioxidants. However, little information is available with regard to the absorption or metabolism of coffee [11–13]. In view of this, the aim of the present study was to investigate the *in vivo* effects of coffee on the oxidative modification of LDL and lipid metabolism.

**Table 1.** Serum lipid levels at baseline (B), after one week (TC), LDL cholesterol (LDLC), and MDA decreased coffee consumption (A) and one week washout period significantly while triglycerides (TG), HDL cholesterol after coffee consumption (W) (HDLC), and lipoprotein a (Lp(a)) did not change sig

| Substance | B | A | W |
|---|---|---|---|
| TC, mg/dl | 185.2 ± 18.3[a] | 178.7 ± 16.4[b] | 157.9 ± 18.6 |
| TG, mg/dl | 93.3 ± 30.7 | 67.5 ± 21.7 | 73.4 ± 25.7 |
| LDLC, | 122.1 ± 24.9[c,d] | 112.1 ± 20.2[d] | 99.0 ± 17.8 |
| mg/dl | | | |
| HDLC, | 56.5 ± 13.2 | 55.8 ± 12.3 | 50.5 ± 13.2 |
| mg/dl | | | |
| MDA, | 2.9 ± 0.4[e,f] | 2.4 ± 0.3 | 2.5 ± 0.4 |
| nmol/ml | | | |
| LP(a), mg/dl | 25.1 ± 16.2 | 23.2 ± 11.4 | 23.7 ± 13.9 |

Note: Values are means ± SD. TC, total cholesterol; TG, triglycerides; LDLC, low density lipoprotein cholesterol; HDLC, high density lipoprotein cholesterol; MDA, malondialdehyde; Lp(a), lipoprotein a.

a

$p < 0.0005$ compared with W.

b

$p < 0.002$ compared with W.
$p < 0.005$ compared with W.

d

$p < 0.05$ compared with B and W.

e

$p < 0.005$ compared with A.

f

$p < 0.005$ compared with W.

**Table 2.** Laboratory data at baseline (B), after one week coffee consumption (A), and one week washout period after coffee consumption (W)

| Substance | B | A | W |
|---|---|---|---|
| GOT, U/liter | 21.5 ± 5.3 | 21.7 ± 5.0 | 20.3 ± 6.5 |
| GPT, U/liter | 26.3 ± 20.3 | 21.8 ± 14.0 | 23.7 ± 13.5 |
| GTP, U/liter | 27.9 ± 14.1 | 25.2 ± 12.8 | 25.6 ± 11.7 |
| $Ca^{2+}$, mg/dl | 9.4 ± 0.4 | 9.3 ± 0.4 | 9.0 ± 0.3 |
| $P_i$, mg/dl | 3.6 ± 0.5 | 3.5 ± 0.4 | 3.3 ± 0.5 |
| $Ca^{2+}$ in urine, | 11.3 ± 7.1 | 11.6 ± 7.0 | 14.8 ± 10.8 |
| mg/dl | | | |

Note: Values are means ± SD. GOT, aspartate aminotransferase; GPT, alanine amino transpeptidase; GTP, glutamyl transpeptidase.

(101.0 ± 8.4 min in B, 130.0 ± 12.9 min in A, and 113.3 ±

7.8 min in W, respectively) (Fig. 1).

**Serum lipid levels.** Throughout the study after coffee consumption, serum lipids, including total cholesterol nificantly (Table 1). These changes continued at one week after coffee consumption.

**Other laboratory data.** Throughout the study, there were no significant changes regarding other blood and urine chemistries (Table 2).

**Caffeine and chlorogenic acid concentrations.** As shown in Fig. 2, coffee consumption led to an increase in serum caffeine concentration from 0.31 ± 0.03 μg/ml in B, 1.68 ± 0.03 μg/ml in A, and 0.51 ± 0.03 μg/ml in W, respectively. Chlorogenic acid was not detected in serum (data not shown). On the other hand, urine chlorogenic acid concentrations did not show significant changes

(1.01 ± 0.76μg/ml in B, 2.18 ± 2.74 μg/ml in A, and 1.30 ± 0.23 μg/ml in W, respectively

## DISCUSSION

The effects of coffee on health have been debated throughout the last four centuries since the Arab Abdelkader first wrote about the drink in 1587. Recently, many investigators have suggested that polyphenols as antioxidants in Japanese tea appear to protect the human body against cardiovascular disease, liver disease, and malignancies [2]. Regarding antioxidants, coffee contains chlorogenic acid and caffeic acid, common constituents of coffee. Therefore, coffee should also be expected to be a useful beverage for human health. According to the report of Cooper et al. [17], the median daily caffeine consumption was estimated to be 210 mg (range 0849.6 mg) from intakes of coffee, tea, and other caffeinated beverages. According to the report of McAnlis et al. [18], coffee did not affect serum lipid levels and susceptibility of LDL to oxidation in humans. The object of this study was, therefore, to assess whether consumption of relatively large amounts of coffee and the control of the beverage over a week affected serum lipid levels and/or protected the LDL from oxidation. According to the data from serum caffeine concentrations of the present study, this study design is appropriate for investigating the effect of coffee consumption on serum lipids and *in vitro* LDL susceptibility. In this study, coffee ingestion resulted in a significant decrease in serum levels of cholesterol, LDL cholesterol, and MDA, and a significant decrease in susceptibility of LDL to oxidation, indicating that coffee consumption might protect against atherosclerosis due to lowering serum lipid levels and improving LDL susceptibility. Although the reasons for discrepancies compared with a few previous reports [18, 19] are not yet clear, it may be due to the difference of the study design, especially relatively large amounts of coffee consumption and the strictly controlled beverage intake in our study. Recently, however, a compounds in coffee are absorbed into the serum at high enough quantities to protect the LDL from oxidative modification *in vivo*.

There is, however, very little known about coffee absorption and metabolism *in vivo* [11□13], and the extent of absorption of coffee remains an important unsolved problem in judging its potential health effects. In this study, chlorogenic acid, one of the major antioxidants of coffee, was not detected in serum and did not change in parallel with coffee consumption in urine, demonstrating that plasma chlorogenic acid might be present as a conjugated form with glutathione [16]. This study also showed urinary chlorogenic acid concentrations did not change after coffee consumption, indicating that chlorogenic acid is present in the diet as part of fruits and vegetables [16].

In summary, it therefore seems likely that any cardioprotective effects of coffee are a reduction of serum lipid and MDA levels, and a decrease in the susceptibility of LDL to oxidation. However, large scale controlled studies, including age, sex, race, and lifestyle will be required to resolve whether coffee is a useful beverage for human health.

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## Intakes of Antioxidants in Coffee, Wine, and Vegetables Are Correlated with Plasma Carotenoids in Humans

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ABSTRACT

The consumption of fruits and vegetables reduces the risk of major chronic degenerative diseases. The active compounds and the mechanisms involved in this protective effect have not been well defined. The objective of this study was to determine the contribution of various food groups to total antioxidant intake, and to assess the correlations of the total antioxidant intake from various food groups with plasma antioxidants. We collected 7-d weighed dietary records in a group of 61 adults with corresponding plasma samples, and used data from a nationwide survey of 2672 Norwegian adults based on an extensive FFQ. The total intake of antioxidants was 17 mmol/d with β-carotene, α-tocopherol, and vitamin C contributing <10%. The intake of coffee contributed 11.1 mmol, followed by fruits (1.8 mmol), tea (1.4 mmol), wine (0.8 mmol), cereals (i.e., all grain containing foods; 0.8 mmol), and vegetables (0.4 mmol). The intake of total antioxidants was significantly correlated with plasma lutein, zeaxanthin, and

lycopene. Among individual food groups, coffee, wine, and vegetables were significantly correlated with dietary zeaxanthin, β-carotene, and α-carotene. These data agree with the hypothesis that dietary antioxidants other than the well-known antioxidants contribute to our antioxidant defense. Surprisingly, the single greatest contributor to the total antioxidant intake was coffee. J. Nutr. 134: 562–567, 2004.

A diet rich in fruits and vegetables reduces the incidence of major diseases such as cancer, cardiovascular disease, diabetes, cataracts, and inflammatory disease (1–9). Recommendations to increase fruit and vegetable consumption have therefore been implemented in most countries (1– 4). Because the active compounds and the mechanisms involved in this protective effect have not been well defined, the recommendations suggest that eating a variety of fruits and vegetables will provide the best protection (1–9). A possible mechanism mediating the protective effect is related to bioactive compounds in fruits and vegetables that reduce oxidative stress, a condition that arises when the formation of reactive oxidants (most importantly reactive oxygen species, reactive nitrogen species, and reactive iron species) outstrips the antioxidant defense, and oxidative damage occurs (10 –14). Fruits and vegetables contain at least several hundred different types of antioxidants (i.e., electron-or hydrogen-donating reductants) that may react directly with such reactive oxidants, forming products with much lower reactivity and therefore potentially protecting against oxidative damage (10 – 14). Another mechanism involves activation of genes of encoding proteins in the antioxidant defense system and/or silencing of genes that may contribute to the oxidative stress (15–17). Analysys of promoter regions have suggested that several response elements may be involved in such transcriptional regulation, including activator protein-1 sites, nuclear this protective factor k-B sites, and antioxidant/electrophilic response elements. Among several different bioactive compounds, antioxidants are suggested to play a role in transcriptional dietaryeffect have not been well defined, the recommendations sug-ments. Among several different bioactive compounds, dietary gest that eating a variety of fruits and vegetables will provide antioxidants are suggested to play a role in transcriptional the best protection (1–9). regulation via these response elements.

Recent studies suggest that the well-known antioxidants (e.g., -tocopherol, vitamin C, and -carotene) contribute a relatively small part of the total antioxidants in most dietary plants, whereas the majority is contributed by antioxidants such as other carotenoids, phenolic acids, sulfides, flavonoids, and lignans (27–29). If many of these antioxidants work in a network (25–26), the total amount of antioxidants derived from the combinations of individual antioxidants that occur naturally in foods may be a better concept than individual dietary antioxidants.

The ferric reducing ability of plasma (FRAP)[4] assay (30) quantifies the total concentration of water-and fat-soluble antioxidants as the concentration of electrons or hydrogen atoms released in a redox reaction above a certain redox potential. The FRAP assay is based on the reduction of $Fe^{3+}$ (ferric iron) to $Fe^{2+}$ (ferrous iron) in the presence of antioxidants. Because the ferric-to-ferrous iron reduction occurs rapidly with all reductants with half-reaction reduction potentials above that of $Fe^{3+}/Fe^{2+}$, the values in the FRAP assay will express the corresponding concentration of electron-donating antioxidants. By using this method, we observed recently that there is a >1000-fold difference between the total concentrations of antioxidants in various dietary plants (29). Interestingly, most berries, walnuts, sunflower seeds, ginger, and pomegranates are among the high antioxidant dietary plants, whereas many dietary plants contribute little to the total antioxidant intake (29).

We determined the contribution of different food groups in the Norwegian diet for the total antioxidant intake in a group of 61 adults (based on a 7-d dietary weighed record) and a nationwide survey of 2672 Norwegian adults (based on an extensive FFQ). In addition, to study the concordance of the concept of “total antioxidants” with plasma biomarkers, we tested whether plasma antioxidant vitamins and thiols were correlated with intake of total antioxidants.

**SUBJECTS AND METHODS**

***The 7-d weighed dietary record study.*** The subjects (*n* = 61), all native Norwegians, were recruited consecutively in a general clinical practice at the time of their regular check-ups. Patients at high risk of cardiovascular disease dominate this practice. Eligible subjects were men and women ≥ 18 y old who had not previously undergone dietary intervention. A research assistant met the participants in groups of 2–6. At these group meetings, the participants filled in a short food questionnaire (31). A blood sample was taken from non-fasting subjects, and body weight and height were measured. The project was approved by the regional ethics committee, and written informed consent was obtained from all of the participants.

The individuals were between 30 and 82 y old with BMI between 18.9 and 49.5 kg/m$^2$ (**Table 1**). The men (*n* = 28) had a mean age of 57 y and the women (*n* = 33) had a mean age of 59 y. Most of the participants were nonsmokers and 48% used lipid-lowering agents (mainly statins).

The 7-d weighed dietary record was collected on consecutive days. The participants were provided with a record notebook and a digital scale with an accuracy of 1 g and a maximum capacity of 3000 g. They were given thorough practical and written instructions on how to weigh and describe in detail the consumption of foods and beverage. We stressed that the purpose of the study was to record their normal food intake and that any temptation to change the diet so as to lose weight or simplify the recording, should be resisted. After recording, the diet record was checked for completeness and then coded by a nutritionist and a research assistant. The data were double-checked for consistency and coding errors.

Estimates of basal metabolic rate (BMR) were calculated from standard formulas for men and women aged 30–59, 60–74 and >74 y old based on height, weight, age, and sex. A comparison of reported

[4] Abbreviations used: BMR, basal metabolic rate; EI, energy intake; FRAP, ferric reducing ability of plasma.

**TABLE 1**

*Characteristics of the subjects in the 7-d weighed-record study*[1]

| | |
|---|---|
| Age, *y* | 57.8 +/- 9.4 |
| Height, *cm* | 169 +/- 9 |
| Weight, *kg* | 80.9 +/- 16.0 |
| BMI, *kg/m2* | 28.2 +/- 4.7 |
| EI:BMR | 1.12 +/- 0.24 |
| | *n (%)* |
| User of lipid lowering agent | 29 (48) |
| Men | 28 (46) |
| User of dietary supplements | 24 (39) |
| Current smokers | 4 (7) |

1 Values are means +/- SD, *n* = 61 or *n* (%).

energy intake with estimates of BMR was used to calculate the number of respondents who underreported their energy intake. Based on estimates of BMR with 95% confidence limits and a diet recording period of 7 d, a ratio between measured energy intake (EI) and BMR (EI:BMR) < 1.10 for individual records may indicate underreporting (32); in 28 subjects this ratio was ≤1.10.

Blood samples from nonfasting subjects were centrifuged at 1000 X *g* for 15 min and plasma was kept at 18°C for a maximum of 2 d before being stored at - 70°C. Serum values from fasting subjects for cholesterol and triglycerides were obtained from the patient's recent medical records. FRAP assay was performed as described (29,30). Cholesterol and triglycerides were analyzed by Capio Laboratoriemedisin.

***Nationwide survey.*** Total antioxidant intake was also assessed in the NORKOST2 study, a nationwide probability sample of 2672 Norwegian adults whose diets were characterized using an extensive, self-administered FFQ (33). No blood samples were obtained in NORKOST2.

***Calculation of intakes of nutrients and total antioxidants.*** Daily intake of nutrients and total antioxidants was computed using a food database and software systems developed at the Institute for Nutrition Research, University of Oslo. The food database is based mainly on the official food composition table (34), and is continuously updated. The food database was supplemented with data on total antioxidant concentrations in foods measured by the FRAP assay [(29) and R. Blomhoff, unpublished results]. Cod liver oil and vitamin-mineral supplements were included in the calculations. In this study, we used the term FRAP total to describe the total intake of antioxidants, whereas the term FRAP without coffee expresses the total antioxidant intake minus antioxidants derived from coffee. The terms FRAPcoffee, FRAPtea, FRAPwine, FRAPcereal, FRAPfruit, and FRAPvegetable express total antioxidant intakes from the respective food groups. Cereals include all grain-containing foods.

***Reagents for HPLC analysis.*** Lutein, α-carotene, β-carotene, lycopene, astaxanthin, BHT, tocol, glutathione, cysteine, homocysteine, and cysteinyl-glycine were supplied by Sigma. Tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol) were supplied by Calbiochem. Zeaxanthin was obtained from Carl Roth. The homocysteine HPLC kit (cat. no. 195-4075) was provided by Bio Rad. The water used was Milli-Q water with resistivity of 18.2 MΩ.cm.

***HPLC analysis of carotenoids.*** The precipitating solution was prepared by mixing 20 µL internal standard (1 mg of astaxanthin in 4 mL benzene) in 10 mL of 2-propanol containing 10 mg/L BHT. A 200-µL aliquot of plasma sample was transferred to a 2-mL amber glass vial and 900 µL of the precipitating solution was added. The mixture was mixed on a vortex for 5 min and centrifuged for 15 min at 3000 X *g* at 4°C; 100 µL of the supernatant was injected into the HPLC system. A Waters pump was used to deliver the mobile phase to the analytical column (YMC Carotenoid S5µm, 4.6 X 250 mm from Waters) and detection was done by a Waters Tunable Absorbance Detector at 453 nm. The mobile phases used were A (100% water),

**TABLE 2 RESULTS**

*Daily dietary intake estimated from the 7-d weighed-record study*[1]

| | | Median |
|---|---|---|
| | Mean +/- SD | (P25, P75)[2] |
| Intake of energy, *MJ* | 7.5 +/- 1.9 | 7.6 (6.2, 8.8) |
| Energy from protein, *%* | 16.2 +/- 2.4 | 16.0 (14.5, 17.4) |
| Energy from fat, *%* | 30.8 +/- 6.0 | 30.6 (26.7, 35.1) |
| Energy from carbohydrates, *%* | 50.8 +/- 6.7 | 51.2 (45.9, 55.1) |
| Energy from added sugar, *%* | 7.0 +/- 4.3 | 6.4 (4.4, 9.3) |
| Fiber, *g* | 21.3 +/- 7.7 | 20.0 (15.7, 24.9) |
| -Carotene, *g* | 3198 +/- 2161 | 2769 (1603, 4218) |
| -Tocopherol, *mg* | 11.2 +/- 5.8 | 9.2 (6.3, 15.3) |
| Vitamin C, *mg* | 124 +/- 59 | 109 (79, 162) |
| | *mmol* | |
| FRAPtotal | 17.3 +/- 9.4 | 16.5 (10.5, 22.2) |
| FRAPwithout coffee | 6.2 +/- 3.2 | 5.5 (3.9, 7.7) |
| FRAPcoffee | 11.1 +/- 9.3 | 10.5 (4.7, 15.7) |
| FRAPtea | 1.4 +/- 2.4 | 0.7 (0.0, 1.6) |

| | | |
|---|---|---|
| FRAPwine | 0.8 +/- 1.3 | 0 (0, 1.3) |
| FRAPcereal | 0.8 +/- 0.3 | 0.8 (0.6, 1.0) |
| FRAPfruit | 1.8 +/- 1.2 | 1.8 (0.8, 2.7) |
| FRAPvegetable | 0.4 +/- 0.3 | 0.4 (0.2, 0.6) |
| | | |

1 Dietary intake values include supplements.
2 P25, 25th percentile; P75, 75th percentile.

B (30% acetone in absolute ethanol), and C (100% acetone). A gradient elution was used with initial conditions of 10% A and 90%Bata flow rate of 2 mL/min. This was immediately followed by a linear increase to 100% B in 5 min. After elution with 100% B for 5 min, there was a linear increase to 100% solvent C in 1.5 min. After an additional 11 min, the system was returned to the initial conditions in 1 min and was equilibrated for 7 min before the next injection.

***HPLC analysis of total thiols.*** Total thiols were determined using the homocysteine kit from BioRad with further validation of the method for total glutathione, total cysteine, and total cysteinylglycine. The internal standard solution, the sample preparation, and HPLC method used were as described by the manufacturer.

***HPLC analysis of tocopherols and tocotrienols.*** The precipitating solution was prepared by mixing 125 µL of internal standard (5.8 mg tocol dissolved in 10 mL absolute ethanol) with 20 mL of 2-propanol containing 10 mg/L BHT. To a 200-µL aliquot of the plasma sample, 600 µL of the precipitating solution was added. The mixture was mixed on a vortex and centrifuged for 15 min at 3000 x *g* at 4°C; 20 µL of the supernatant was injected into the HPLC system. A Waters pump was used to deliver the mobile phase (acetonitrile:methanol:dichloromethane:water, 60:30:5:5 by vol) at 1.0 mL/min to the analytical column (Suplex pKb-100, 5 µm, 250 mm x 4.6 mm id). Detection was done using a Shimadzu fluorescence detector with excitation at 294 nm and emission at 330 nm.

***Statistical methods.*** Because the intake data and plasma metabolite concentrations were not normally distributed, these values are presented both as sample means with SD and medians with percentiles. Furthermore, the intake data and the plasma concentrations of carotenoids and tocopherols were natural log transformed to normalize their distribution before calculation of the Pearson correlation coefficients. The correlations are presented both unadjusted and adjusted for age, BMI, serum triglycerides, and serum cholesterol (Pearson's partial correlation coefficients). The difference in plasma concentration of triglycerides, cholesterol, carotenoids, thiols, and tocopherols between men and women was tested with an unpaired *t* test. All of the statistical analyses were performed using SPSS 11.0. A significance level of 5% was used.

***Contribution of different food groups to intake of antioxidants.*** Daily intakes of total antioxidants, β-carotene, α-to-copherol, vitamin C, and energy in the 7-d weighed dietary records are presented in **Table 2**. The estimated intake of total antioxidants was 17.3 +/- 9.4 mmol/d. The mean intakes of β-carotene, α-tocopherol, and vitamin C corresponded to 0.01, 0.05, and 1.41 mmol antioxidants (i.e., electron-or hydrogen-donating reductants), respectively, because these molecules can donate 1, 2, and 2 electrons or hydrogens, respectively, in a redox reaction. Thus, in total they contributed 1.47 mmol compared with a total intake of 17.3 mmol.

The intake of coffee contributed 11.1 mmol or 64% of the total antioxidant intake, followed by fruits and berries (1.8 mmol), tea (1.4 mmol), wine (0.8 mmol), cereals (0.8 mmol), and vegetables (0.4 mmol). Small amounts were also contributed by edible fat (0.3 mmol), cakes (0.2 mmol), and potatoes

(0.1 mmol); 0.1 mmol total antioxidants was contributed by each of fish, milk, meat, sweets, and beer. Of the noncoffee antioxidants, on average, β-carotene, α-tocopherol, and vitamin C intake contributed 23.7%.

Similar results were obtained when the contribution of different food groups to total antioxidant intake was

estimated using a representative nationwide dietary survey in Norway that is based on an extensive, self-administered FFQ (**Table 3**). Thus, these data sets demonstrated that coffee was a major contributor to total antioxidant intake. Furthermore, of the noncoffee antioxidants, fruits (including berries), tea, cereals, wine, and vegetables contributed 30, 24, 12, 10, and 6% (mean of the two studies) of total antioxidants, respectively.

***Correlations between total antioxidant intake and intakes of β-carotene, α-tocopherol and vitamin C.*** In the 7-d weighed dietary records, the intake of vitamin C was significantly correlated with the intake of total antioxidants from fruits and vegetables ($r$ = 0.82 and $r$ = 0.46, respectively) (**Table 4**). Furthermore, intake of β-carotene was also significantly correlated with total intake antioxidants in vegetables ($r$ = 0.55).

Due to increased statistical power, a more precise description of the correlations was possible in the nationwide dietary survey (Table 4). β-Carotene intake estimated from NORKOST2 was correlated with total antioxidant intake from

**TABLE 3**

*Contribution of different food groups to antioxidant intake in the 7-d weighed-record study and NORKOST2 study*

| Total intake of antioxidants,2 *mmol* | | |
|---|---|---|
| | 7-d weighed-record study | NORKOST21 |
| | 17.3 +/- 9.4 | 17.6 +/- 10.6 |
| | *% of total antioxidant intake* | |
| Cereals | 5 | 4 |
| Fruits and berries | 11 | 7 |
| Fruit juices | 2 | 2 |
| Vegetables | 2 | 2 |
| Coffee | 64 | 68 |
| Tea | 8 | 9 |
| Wine | 5 | 2 |
| Other foods | 5 | 8 |

1 The NORKOST2 used an extensive, self-administered FFQ developed by the National Nutrition Council (33).

2 Values are means +/- SD, $n$ = 61 (weighed-record study) or 2672 (NORKOST2).



**TOTAL ANTIOXIDANTS IN COFFEE, TEA, WINE, FRUITS, AND VEGETABLES**

**TABLE 4**

*Pearson correlation coefficients between total antioxidant intakes and intakes of β-carotene, α-tocopherol and vitamin C*1

| | 7-d weighed-record study | | | NORKOST2 | | |
|---|---|---|---|---|---|---|
| | β-Carotene | α-Tocopherol | Vitamin C | β-Carotene | α-Tocopherol | Vitamin C |
| $FRAP_{total}$ | 0.08 | 0.33* | 0.15 | 0.11** | 0.03 | 0.11** |
| $FRAP_{without\ coffee}$ | 0.16 | 0.25 | 0.60** | 0.22** | 0.16** | 0.47** |
| $FRAP_{coffee}$ | 0.00 | 0.24 | -0,07 | 0.04 | -0,03 | -0,04 |
| $FRAP_{tea}$ | -0,05 | -0,02 | 0.19 | 0.15 | 0.09** | 0.11** |
| $FRAP_{wine}$ | 0.02 | 0.14 | 0.09 | 0.00 | 0.06* | 0.05* |
| $FRAP_{cereal}$ | 0.14 | 0.25 | 0.00 | 0.11** | 0.12** | 0.20** |
| $FRAP_{fruit}$ | 0.15 | 0.10 | 0.82** | 0.17** | 0.15** | 0.73** |

| | | | | | | |
|---|---|---|---|---|---|---|
| FRAPvegetable | 0.55** | 0.16 | 0.46** | 0.57** | 0.16** | 0.52** |

1 In the 7-d weighed-record study, $n$ = 61; for the nationwide dietary survey in Norway (NORKOST2), $n$ = 2672. All variables were natural log transformed before computing correlation coefficients. * $P < 0.01$, ** $P < 0.001$.

cereals, fruits, and vegetables whereas α-tocopherol and vitamin C intake was correlated with total antioxidant intakes from tea, wine, cereals, fruits, and vegetables. Intake of total antioxidants from coffee was not correlated with intake of any of the single antioxidants.

***Correlations between total antioxidant intakes and plasma antioxidants.*** The plasma concentrations of carotenoids, thiols, and tocopherols in the 7-d weighed dietary record study (**Table 5**) were all within normal ranges. There were no differences between men and women except for a significantly higher cholesterol concentration in women compared with men (data not shown).

The total intake of antioxidants was significantly correlated with plasma lutein, zeaxanthin, and lycopene (adjusted correlation coefficients 0.33, 0.40, and 0.31, respectively), whereas the noncoffee antioxidant intake was significantly correlated with plasma lutein and β-carotene (adjusted correlation coefficients 0.27 and 0.26, respectively). Intakes of coffee, wine, and vegetables were significantly correlated with zeaxanthin, β-carotene, and α-carotene (adjusted correlation coefficients 0.27, 0.31, and 0.28, respectively; **Table 6**).

**TABLE 5**

*Plasma metabolite concentrations in 61 men and women in the 7-d weighed-record study*

| | Mean +/- SD | | Median (P25, P75)1 |
|---|---|---|---|
| | | *mmol/L* | |
| Cholesterol | 5.3 | +/-1.1 | 5.2 (4.4, 6.1) |
| Triglycerides | 1.46 | +/-0.84 | 1.30 (0.85, 1.80) |
| | | *μmol/L* | |
| Lutein | 0.209 | +/-0.091 | 0.197 (0.148, 0.267) |
| Zeaxanthin | 0.058 | +/-0.039 | 0.063 (0.026, 0.084) |
| α-Carotene | 0.125 | +/-0.075 | 0.120 (0.066, 0.168) |
| β-Carotene | 0.517 | +/-0.312 | 0.425 (0.289, 0.704) |
| Lycopene | 0.898 | +/-0.856 | 0.563 (0.261, 1.324) |
| Glutathione | 5.07 | +/-1.41 | 5.00 (4.10, 6.00) |
| Cysteine | 322.3 | +/-44.8 | 315.0 (294.0, 343.0) |
| Homocysteine | 11.0 | +/-2.4 | 11.1 (9.3, 12.2) |
| Cysteinyl-glycine | 31.2 | +/-5.1 | 30.5 (27.4, 34.7) |
| Total antioxidants | 891 | +/- 147 | 908 (764, 956) |
| β-Tocopherol | 0.184 | +/-0.296 | 0 (0, 0.250) |
| γ-Tocopherol | 2.48 | +/-1.82 | 2.20 (2.10, 3.10) |
| α-Tocopherol | 32.5 | +/-9.0 | 31.1 (25.8, 36.4) |

1 P25, 25th percentile; P75, 75th percentile.

There were no differences between gender-specific adjusted correlations (data not shown). Furthermore, when we divided the plasma concentration of carotenoids into tertiles of total antioxidant intake, there was an increase in the concentration of zeaxanthin ($P < 0.05$) and a trend for an increase in the concentration of lutein ($P = 0.09$) across tertiles. The use of lipid-lowering agents by 29 of the 61 participants did not substantially

influence the correlations (data not shown).

We then evaluated the relations between antioxidant intake from various food groups and plasma α-, β-, γ-, and δ-tocopherols and thiols (glutathione, cysteine, homocysteine, cysteinyl-glycine). Correlations between total antioxidant intake from various food groups and plasma tocopherols and thiols were much lower than those with carotenoids.

## DISCUSSION

In the present study, we determined the contribution of different food groups to the total antioxidant intake in a group of 61 adults (based on 7-d weighed dietary records) and a nationwide survey of 2672 Norwegian adults (based on an extensive FFQ). We were surprised to observe that coffee (mean daily intake 480 mL) was the major contributor to the total intake of antioxidants ( 66%). High antioxidant levels in coffee were reported recently in several studies (35,36), but this high contribution to the total dietary intake of antioxidants was not noted before. Chlorogenic acid (the ester of caffeic acid with quinic acid), the most abundant polyphenol in coffee, is likely responsible for a substantial part of coffee antioxidants.

When we analyzed antioxidants in foods to be included in our food composition table (R. Blomhoff, unpublished data) we observed that green and black coffee beans contain 15.9 and 22.6 mmol total antioxidants/100 g, respectively. This difference between green and black coffee beans agrees with previous data showing that although some antioxidants tend to be damaged during the roasting process, other are formed in so-called Maillard reactions (the browning reaction) (37,38).

Of the noncoffee total antioxidant intake, fruits (including berries), tea, cereals, wine, and vegetables contributed 26, 25, 13, 10, and 6% (mean of the two studies) of total antioxidants, respectively. Furthermore, dietary β-carotene, A-to-copherol, and vitamin C contributed only 0.1, 0.3, and 8.5%, respectively, of the total intake of antioxidants. Of the non-coffee total antioxidant intake, β-carotene, A-tocopherol, and vitamin C together contributed 24%.

A major issue is whether the antioxidants from coffee are

**TABLE 6**

*Pearson correlation coefficients (r) and partial correlation coefficients ($r_{par}$) between antioxidant intakes and plasma concentrations of carotenoids in the 7-d weighed-record study*[1]

| Lutein | | Zeaxanthin | | α-Carotene | | β-Carotene | | Lycopene | |
|---|---|---|---|---|---|---|---|---|---|
| *r* | *r*par | *r* | *r*par | *r* | *r*par | *r* | *r*par | *r* | *r*par |
| 0.25 | 0.33* | 0.34** | 0.40** | 0.07 | 0.12 | 0.20 | 0.23 | 0.28* | 0.31* |
| 0.27* | 0.27* | 0.18 | 0.19 | 0.15 | 0.22 | 0.24 | 0.26* | 0.08 | 0.13 |
| 0.00 | 0.06 | 0.22 | 0.27* | −0.05 | −0.07 | 0.07 | 0.08 | 0.22 | 0.22 |
| 0.15 | 0.15 | 0.03 | 0.04 | 0.10 | 0.12 | 0.14 | 0.14 | 0.05 | 0.07 |
| 0.07 | 0.08 | 0.06 | 0.05 | 0.11 | 0.09 | 0.31* | 0.31* | 0.07 | 0.04 |
| 0.00 | 0.00 | −0.13 | −0.13 | −0.09 | 0.03 | −0.18 | −0.14 | −0.23 | −0.20 |
| 0.16 | 0.15 | 0.20 | 0.24 | 0.17 | 0.17 | 0.10 | 0.10 | −0.05 | 0.06 |
| 0.21 | 0.21 | 0.06 | 0.03 | 0.16 | 0.28* | 0.20 | 0.24 | 0.11 | 0.05 |

FRAPtotal FRAPwithout coffee FRAPcoffee FRAPtea FRAPwine FRAPcereal FRAPfruit FRAPvegetable

1 *r*par values were adjusted for triglycerides, cholesterol, age and BMI. * $P < 0.05$, ** $P < 0.01$.

bioavailable and bioactive. Several studies demonstrated bioactivity of coffee that support coffee's contribution to antioxidant defense. Many epidemiologic studies found that coffee is associated with reduced plasma γ-glutamyl transpeptidase, a suggested biomarker for early oxidative stress (39). Furthermore, coffee is protective in models of experimental carcinogenesis (40) and is associated with reduced incidence of human bladder and colorectal cancer (41), gallstone (42), Parkinson's disease (43), liver cirrhosis (44), and type 2 diabetes (45) in epidemiologic studies. Our observation of a significant contribution of coffee to the total intake of antioxidants suggests a possible mechanism behind these potentially beneficial effects of coffee. Coffee consumption has been shown to increase plasma homocysteine, and likely is also associated with a small increase in blood pressure after many years of consumption (46). Furthermore, coffee lipids contained in boiled coffee, but less so in filtered coffee, increase serum lipids (47). Thus, coffee may contain several bioactive compounds, some of which may be beneficial, whereas others may increase the risk of disease. We observed in the present study that total intake of antioxidants was significantly correlated with plasma lutein, zeaxanthin, and lycopene. Because lutein, zeaxanthin, and lycopene are only minor contributors to the total intake of antioxidants, our data agree with the hypothesis that many antioxidants may interact in a network, i.e., that dietary antioxidants other than lutein, zeaxanthin, and lycopene may save, recharge, or salvage these carotenoids when they have been used in a redox reaction.

The various food groups contain overlapping but also significantly different varieties of antioxidants. Thus, it should be expected that antioxidants from different food groups and within food groups may have different bioavailability and bioactivity. Therefore, we analyzed antioxidants in different food groups separately. The antioxidant intake in individual food groups, coffee, wine, and vegetables was significantly correlated with zeaxanthin, β-carotene, and α-carotene, respectively. Thus, our study demonstrating correlations between intakes of total antioxidants in individual food groups and plasma carotenoids agrees with the hypothesis that dietary antioxidants other than the well-known antioxidants contribute to our antioxidant defense. We did not observe any association between total intake of antioxidants, or food group antioxidants, and the level of plasma tocopherols or thiols. These observations suggest that the plasma tocopherols and thiols are not in complete equilibrium with plasma carotenoids. This could either be due to preferential chemical reactivities or to compartmentalization of the plasma pools (e.g., selective binding to the α-tocopherol transport protein). The individuals in our study were generally healthy with presumably low systemic oxidative stress. In clinical situations with prolonged systemic oxidative stress, more correlations or antioxidant networking might be expected.

The FRAP assay does not quantify dietary sulfur compounds because the redox potential for thiols is below the threshold used in the FRAP assay (26). Because thiols appear not to be well absorbed (48,49), we elected to use the FRAP assay in the assessment of dietary antioxidants to avoid the otherwise large "noise" of thiol antioxidants in foods. However, thiols have essential roles in many biochemical reactions in the body by virtue of their ability to be oxidized reversibly. Glutathione is the most important thiol-based redox buffer (49,50). In the response of a cell to oxidative stress, glutathione is first consumed in reactions that protect the cell (13–16,49,50). To counteract glutathione depletion, most cells increase their synthesis of glutathione. During persistent oxidative stress, cellular counteraction is not sufficient to replenish the consumption of glutathione, and total cell and plasma glutathione may be reduced. Plasma glutathione has therefore been used as a biomarker for systemic oxidative stress (13– 16,49,50). It would be interesting to assess the role of total dietary antioxidants on the thiols in clinical situations with profound systemic oxidative stress.

Further studies are warranted to explore whether the concept of "total antioxidants" is valuable in the search for the protective compounds and mechanism behind the beneficial effects of dietary plants. The present results are encouraging and substantiate the hypothesis that antioxidants work as least in part in an integrated network in vivo.

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***We also apreciate any contribution that could help us to improve our documentation.***

***GVM I.E. via Pilotti, 15 64100 Teramo (Italy)***
***07 February, 2011***