POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES
Pol. J. Food Nutr. Sci. 2003, Vol. 12/53, SI 1, pp. 65–73

FUNCTIONAL PROPERTIES OF FOODS OF ANIMAL ORIGIN AND THE METHODS OF THEIR ASSESSMENT

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Key words: functional foods, PUFA, CLA, milk, meat, eggs

The purpose of this review is to address the present state of knowledge in the area of functional foods of animal origin. A large number of food products can be defined as functional foods, *i.e.* foods containing specific nutrients and(or) non-nutrients, and affecting beneficially human health, beyond what is traditionally known as nutritional effects. Nutritional strategies have become a major means to obtain functional foods of animal origin. Both n-3 PUFA-enriched and CLA-enriched milk, meat and eggs have been successfully obtained. However, their functional properties, were related practically to plasma lipid profile and(or) development of atherosclerosis. Moreover, they were demonstrated mainly in animal models and to a limited extent in humans. Therefore, further studies are required to determine other health-related benefits of these products, *e.g.* their anticarcinogenic and antinflamatory properties; preferably using human subjects. It is also becoming obvious, that in further studies on functional properties of foods, nutrient-gene interactions must be recognised.

INTRODUCTION

Generally, a large number of food products can be defined as functional foods, *i.e.* foods containing specific nutrients and(or) non-nutrients, and affecting beneficially human health, beyond what is traditionally known as nutritional effects. Thus, there is no precise and universally accepted definition of these foods. Consequently, it has been suggested to understand the term "a functional food" as a new idea, rather than a defined product [Bellisle *et al.*, 1998; Diplock *et al.*, 1999; Roberfroid, 2000, 2002]. Accordingly, an ideal functional food is considered to be: (1) a conventional or everyday food; (2) consumed as a part of the conventional diet; (3) composed of naturally occurring components; (4) enhancing target function(s) beyond its nutritive value; (5) reducing the risk of disease, and (6) having sound, scientifically-based and verified claims. As such, the above definition, covers all major features of functional foods and is meant to set guidelines for research and development in the field of modern human nutrition. In a more practical way, a functional food is defined as: (1) a natural food in which one of the components (nutrient or non-nutrient) has been naturally enhanced through special growing conditions; (2) a food to which a component has been added to provide benefits (*e.g.* the addition of selected probiotic bacteria to improve gut health); (3) a food from which a component has been removed (*e.g.* the reduction of saturated fatty acids); (4) a food in which the nature of one or more components has been modified (*e.g.* protein hydrolysates in infant formulas); (5) a food in which the bioavailability of one or more components has been increased, and (6) any combination of the above possibilities. As indicated in the European Consensus Document [Diplock *et al.*, 1999], the most pertinent aspect in communicating of health-related benefits of functional foods is that any claim of their functionality must be scientifically-based, *i.e.* it must be both objective and appropriate. Therefore the development of functional foods must rely on identification and validation of relevant biological markers of particular target functions and (or) the risk of a particular disease. More precisely, these markers can be classified according to whether they relate to: (1) exposure to the food component under study (*e.g.* the level of this component itself or its metabolites in the body fluids or tissues); (2) enhanced target function(s) or biological responses (*e.g.* changes in concentrations of relevant metabolites, specific proteins, enzymes or hormones as possible responses to a functional component); (3) an appropriate endpoint of the reduced disease risk (*e.g.* progression and regression of atherosclerotic lesions), and (4) individual susceptibility or genetic polymorphism controlling the effect of the functional component under study (*e.g.* nutrient-gene interactions).

The purpose of this review is to address the present state of knowledge in the area of functional foods of animal origin. First, the recent examples of functional properties of pure polyunsaturated fatty acids of *n*-3 series (*n*-3 PUFA) and those of conjugated linoleic acid (CLA) isomers, as identified and validated in laboratory animals and humans, will be presented. Second, the efficiency of current nutritional strategies to obtain PUFA(*n*-3)-enriched and CLA-enriched milk, meat and eggs and their functional

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effects, when fed to animal models and humans, will be described. Finally, the need to use nutritional genomics (*i.e.* nutrigenomics) tools [Trayhurn, 2003] to identify the mechanisms by which functional foods alter processes occurring within tissues in the human body, will be emphasised.

FUNCTIONAL PROPERTIES OF PURE POLYUNSATURATED FATTY ACIDS OF *n*-3 SERIES (*n*-3 PUFA) AND CONJUGATED LINOLEIC ACID (CLA) ISOMERS

Functional properties of polyunsaturated fatty acids of *n*-3 series (*n*-3 PUFA)

As demonstrated in numerous studies, feeding PUFA (*n*-3 series) to laboratory animals and humans resulted in evident functional effects. Most of these studies used fish oils as sources of eicosapentaenoic (20:5; EPA) and docosahexaenoic (22:6; DHA) acids. There is no doubt that *n*-3 PUFA are important from a nutritional point of view as they function as essential structural components of the phospholipids in cellular membranes, especially in the retina and brain. Another important feature of *n*-3 PUFA is their role in the prevention or amelioration of several chronic diseases such as: obesity, cardiovascular disease (CVD), cancers of breast, colon and prostate [Rose, 1997; Hardman, 2002], and inflammation [Calder, 2002]. However, the strongest evidence exists for the inverse relation between consumption of *n*-3 PUFA and the occurrence of CVD and its complications [Simopoulos, 1999; Mantzioris *et al.*, 2000; Connor, 2000]. Recently, PUFA were implicated in the hypothesis that they may improve the metabolic syndrome, acting as energy partitioners, *i.e.* directing fatty acids away from triacylglycerol storage toward their oxidation; also enahancing glucose conversion to glycogen. Consequently, PUFA protect against adverse symptoms of the metabolic syndrome and reduce the risk of CVD [Clarke *et al.*, 2001]. Interestingly, the capacity of young men to convert α-linolenic acid (18:3) to DHA seems to be limited, when compared with that of young women [Burdge & Wootton, 2002]. Therefore, the dietary supply of preformed DHA may be essential to maintain its optimum level in cell membrane phospholipids in young men.

Functional properties of conjugated linoleic acid (CLA) isomers

Conjugated linoleic acid (CLA) isomers are fairly new non-plant components that have been the focus of research efforts in recent years. CLA is a collective name referring to the positional and geometric (*cis, trans*) conjugated dienoic isomers of linoleic acid (18:2*n*-6), present mainly in ruminant milk and meat [Fritche & Steinhart, 1998; Lawson *et al.*, 2001]. The double bonds in CLA are usually either in C positions 9 and 11 or 10 and 12. Moreover, each of the double bonds can be in the *cis* or *trans* configuration. The CLA isomers have been shown to have health-promoting properties as components of animal diets.

Indeed, CLA was found to act as a fat-to-lean repartitioning agent in murine models [Stangl *et al.*, 2000; Szymczyk *et al.*, 2000; Sisk *et al.*, 2001], broiler chickens [Simon *et al.*, 2000; Szymczyk *et al.*, 2001], pigs [Ostrowska *et al.*, 1999, 2003] and also in humans [Blankson *et al.*, 2000]. However, the anti-obesity potential of CLA in human is equivocal. In contrast to spectacular effects of CLA in mice and rats, its effects in humans were less apparent [Zambell *et al.*, 2000; Terpstra, 2001]. To offer an explanation, the above difference could result from relatively high and easily altered metabolic rate in murine models, compared to that in humans. More recently, isomer-specific effects of CLA in model animals were analysed and the anti-obesity potential of *trans*-10, *cis*-12 isomer was indicated [Evans *et al.*, 2002]. This potential was also reported in human subjects [Belury *et al.*, 2003].

CLA was also shown to have hypocholesterolemic and(or) antiatherogenic properties. In early studies on rabbits, fed an atherogenic diet, CLA decreased blood total cholesterol, LDL-cholesterol and cholesterol deposition in the aorta wall [Lee *et al.*, 1994]. Similarly, CLA decreased cholesterol fractions and atherosclerotic lesions in the aortas of hamsters fed an atherogenic diet [Nicolosi *et al.*, 1997]. In experiments on rats [Szymczyk & Pisulewski, 2002], feeding the mixture of CLA isomers increased favourably the ratio HDL-cholesterol:total cholesterol. However, no such effect was noted in a similar experiment using broiler chickens [Szymczyk *et al.*, 2001]. The most convincing effects of CLA were reported by Kritchevsky *et al.* [2000]. In their studies, dietary CLA (0, 5 and 10 g/kg) reduced progression of athereosclerosis (-34, -64, -58%, respectively) in rabbits fed atherogenic diet. Moreover, in the same studies, CLA induced significant (-35%) regression of established atherosclerotic lesions. Recently, isomer-specific effects of CLA on plasma lipid profile and CVD risk in humans have bee studied [Noone *et al.*, 2002]. Both isomers under study (*cis*-9, *trans*-11 and *trans*-10, *cis*-12) exerted similar effects. However, the obtained results clearly indicated that CLA had positive functional effects by conserving cardio-protective HDL-cholesterol concentrations and reducing those of the potentially atherogenic VLDL-cholesterol. Several mechanism underlying the observed changes were identified: (1) an increase in plamitoyl-transferase activity, (2) inhibition of proliferation and differentiation of adipocytes, (3) inhibition of lipoprotein lipase activity, (4) a reduction of circulating cholesterol, and (5) enhanced peroxisomal β-oxidation of fatty acids *via* peroxisome proliferation [Stangl, 2000]. The decrease in circulating cholesterol, resulting from CLA feeding, was attributed to preferential CLA accumulation in the core of LDL particles, (2) inhibition of hepatic synthesis of apolipoprotein β-containing lipoproteins by *trans*-10, *cis*-12 isomer, (3) increased LDL receptor activity and thus enhanced cholesterol catabolism in the liver, and (4) decreased activity of intestinal acyl CoA:cholesterol tranferase (ACAT) [Yeung *et al.*, 2000].

Anticarcinogenic properties of CLA were discovered first in hamburger extracts [Pariza *et al.*, 1979; Pariza & Hargraves, 1985]. In following experiments, feeding CLA isomers inhibited the development of chemically-induced mouse epidermal [Ha *et al.*, 1987; Belury *et al.*, 1996; Liu & Belury, 1997] and esophagal [Ha *et al.*, 1990] tumors. CLA isomers were also reported to inhibit chemically--induced formation of DNA adducts in mice [Zu & Schut, 1992] and reduced the number of chemically-induced aberrant crypt foci per colon (a marker of colon carcino-

genesis), in rats [Liew *et al.*, 1995]. The above effect of dietary CLA could be explained by mechanisms probably involving enhanced apoptosis of colon mucosa cells [Park *et al.*, 2001]. It is also worth emphasising that in rat mammary tumor models, CLA showed particularly strong anticarcinogenic properties, in a dose-dependent manner only up to 1% of dietary CLA [Ip *et al.*, 1991; 1994; 1996]. In studies of Schulz *et al.* [1992], CLA isomers were found cytostatic and cytotoxic to human malignant melanoma, colecteral, and breast cancer cells lines, *in vitro*. Similarly, CLA inhibited development of lung cancer cells [Schonberg & Krokan, 1995]. Moreover, severe combined immunodeficient (SCID) mice were used as models for growing human breast cancer cells. It was found that in the mice fed CLA, before inoculation with the tumor cells, the development of tumors was largely inhibited [Visonneau *et al.*, 1997]. In a similar experiment, inoculation of SCID mice with human prostatic cancer cells was prevented by CLA isomers [Cesano *et al.*, 1998]. Several mechanisms underlying the reported anticarcinogenic effects of CLA isomers have been recently discussed. These may include: (1) reduction of cell proliferation, (2) alterations in the components of the cell cycle, and (3) induction of apoptosis. Also, CLA modulate markers of immunity, eicosanoid synthesis in numerous species, lipid metabolism and gene expression. All the above pathways may be involved in the reduction of carcinogenesis by CLA isomers [Belury, 2002].

Antiinflammatory effects of CLA feeding to animal models have been the subject of several studies [*e.g.* Miller *et al.*, 1994; Wong *et al.*, 1997; Turek *et al.*, 1998; Hayek *et al.*, 1999], reviewed recently by Roche *et al.* [2001]. These studies indicated that CLA may affect T-cells and thus cell-mediated immune response in model animals. However, CLA affected also humoral immune response in rats [Sugano *et al.*, 1998]. In studies on human subjects, CLA isomers (*cis*-9, *trans*-11 and *trans*-10, *cis*-12) were shown to affect cell-mediated response in a isomer-specific manner. Indeed, while the former isomer promoted the immune response, the latter attenuated this response. In contrast to the reports with animal models and the above report, CLA did not affect the immune status in young healthy women [Kelly *et al.*, 2000]. Several hypotheses were put forward to explain the effects of CLA on immune responses. In line with mechanisms described for effects of *n*-3 PUFA [Calder, 2002], the most probable explanation of these responses may refer to partial replacement of arachidonic acid (20:4), in inflammatory cell membranes by CLA isomers. Arachidonate acts as a precursor of highly-active mediators of inflammation and its replacement leads inevitably to decreased production of arachidonic acid-derived mediators (eicosanoids: PG, TX, LT). Although CLA isomers may act mainly as arachidonate antagonists, they may also exert a number of different effects such as suppressed production of proinflammatory cytokines and down-regulate inflammatory gene expression, as indicated described for *n*-3 PUFA.

In addition to the above described functional properties of CLA isomers, they were also indicated as stimulators of bone formation in chickens [Li & Watkins, 1998] and rats [Kelly *et al.*, 2001]. CLA isomers (*trans*-10, *cis*-12, in particular) were also implicated in modulation of type 2 diabetes and other related conditions of insulin resistance [Houseknecht *et al.*, 1998; Belury *et al.*, 2003]. These recent properties were related to alterations in the molecular mechanisms underlying the metabolic syndrome [Clarke, 2001].

NUTRITIONAL STRATEGIES TO OBTAIN PUFA (*n*-3)-ENRICHED AND CLA-ENRICHED MILK, MEAT AND EGGS

PUFA (*n*-3)-enriched milk, meat and eggs

The use of nutritional strategies to improve the composition and quality of food products of animal origin has emerged recently at the interface of animal science, food science and human nutrition. This new approach has been effectively used to alter the product composition to be more consistent with human dietary guidelines. Thus, feeding animals with oil seeds, plant and fish oils, as rich sources of monounsaturated and polyunsaturated fatty acids (*n*-3 series), resulted in their subsequent incorporation into milk lipids [Kowalski *et al.*, 1999; Goodridge *et al.*, 2001]. Equally, dietary pattern of fatty acids was reproduced in carcass fat of beef cattle [Scollan *et al.*, 2001], sheep [Bolte *et al.*, 2002; Wachira *et al.*, 2002], pigs [Wiseman & Agunbiade 1998; Matthews *et al.*, 2000], and poultry [Leskanich & Noble, 1997]. Also egg yolk lipids were easily enriched with dietary monounsaturated and polyunsaturated fatty acids [van Elswyk, 1997; Botsoglou *et al.*, 1998; Ayerza & Coates *et al.*, 2001; Galobart *et al.*, 2002].

CLA-enriched milk, meat and eggs

In line with the above strategies, similar efforts were made to obtain CLA-enriched milk [Kelly *et al.*, 1998; Chouinard *et al.*, 1999; Dhiman *et al.*, 1999; Baumgard *et al.*, 2001], beef [Madron *et al.*, 2002], lamb [Wachira *et al.*, 2002], pork [Ramsay *et al.*, 2001; Wiegand *et al.*, 2002], poultry meat [Simon *et al.*, 2000; Szymczyk *et al.*, 2001; Du & Ahn, 2002; Badinga *et al.*, 2003], and eggs [Du *et al.*, 2000; Jones *et al.*, 2000; Raes *et al.*, 2002; Cherian *et al.*, 2002]. The results of the above studies are promising, generally consistent and confirm the ability of farm animals to incorporate efficiently dietary CLA isomers into milk, meat and egg lipids.. At the same time, the negative feature of these studies is an adverse effect of CLA isomers on fatty acid patterns of the modified products. Namely, CLA isomers consistently increase the level of saturated fatty acids (SFA) and decrease that of monounsaturated fatty acids (MUFA), whereas the level of polyunsaturated fatty acids (PUFA) in these products seems to be less affected. Consequently, efforts are made to prevent the above adverse changes. For example, feeding olive oil to laying hens largely prevented CLA-induced increases in SFA (16:0 and 18:0) and a decrease in oleic acid (18:1) in egg yolk lipids [Aydin *et al.*, 2001].

FUNCTIONAL PROPERTIES OF PUFA (*n*-3)-ENRICHED AND CLA-ENRICHED MILK, MEAT AND EGGS IN ANIMAL MODELS AND HUMANS

Functional properties of PUFA (*n*-3)-enriched milk, meat and eggs

Although direct extrapolation of the functional properties of PUFA (*n*-3) and conjugated linoleic acid

(CLA) isomers from animal studies to humans may be premature, it seems desirable to obtain and to evaluate PUFA (*n*-3)-enriched and CLA-enriched animal products as functional foods for human consumption.

Milk fat consumption is considered to have adverse health-related effects in humans. Dairy fat that is rich in saturated fatty acids which have been shown to elevate total, LDL-, and HDL-cholesterol and apolipoprotein B and A concentrations, compared with polyunasturated fats (oils and soft margarines). The presence of cholesterol and saturated fatty acids in milk fat have been identified as major factors that raise total- and LDL-cholesterol and apolipoprotein B concentrations, thus resulting in increased risk of cardiovascular disease [Schaefer, 2002; Muller *et al.*, 2003]. Therefore several strategies to reduce adverse effects of milk fat consumption were proposed, involving milk fractionation technology [Jacques *et al.*, 1999] and feeding dairy cows with a protected (from ruminal biohydrogenation) lipid supplement [Noakes *et al.*, 1996].

In the studies of Jaques *et al.* [1999], fractionation technology was used to obtain modified milk fat (with reduced cholesterol). Human subjects were offered three experimental diets containing: regular milk fat, modified milk fat (as above) and non-hydrogenated margarine. The composition of dietary fats is given in Table 1. Surprisingly, regular and modified mik fat had no effect on plasma total and LDL-cholesterol, whereas margarine decreased these markers (Table 1). To offer an explanation, it is frequently indicated that the consumption of SFA, rather than cholesterol, is the major factor of hypercholesterolemia and(or) and development of atherosclerosis. Indeed, cholesterol consumption exerts fairly week hypercholesterolemic effects in humans [Howell *et al.*, 1997]. In the experiment of Noakes *et al.* [1996], modified milk fat was derived from cows fed a protected lipid supplement. Human subjects in this study were offered two experimental diets containing either regular dairy products or modified dairy products. Composition of fat (regular and modified) is shown in Table 2. The obtained results clearly indicated that favourable changes in the fatty acid profile of modified milk fat (decreased SFA and increased MUFA and PUFA proportions), induced lower total plasma cholesterol and LDL-cholesterol concentrations in the subjects (Table 2), thus indicating a functional effect of the modified milk fat.

TABLE 1. Total cholesterol and its VLDL, LDL and HDL fractions in human blood plasma as affected by dietary fat [Jacques *et al*., 1999].

Specification	Milk fat[1]	Modified milk fat[2]	Margarine[3]
Total cholesterol [mmol/L]	4.01^{A}	3.93^{A}	3.56^{B}
VLDL [mmol/L]	0.22^{a}	0.17^{b}	0.19^{a}
LDL [mmol/L]	2.74^{A}	2.67^{A}	2.33^{B}
HDL [mmol/L]	1.06	1.09	1.03
Triacylglycerols [mmol/L]	0.89^{a}	0.78^{b}	0.86^{a}

A,B - $p<0.01$; a,b - $p<0.05$; [1]Milk fat (%): SFA 71.9; MUFA 25.2; PUFA 3.1; Cholesterol 4.15 mg/g; [2]Modified milk fat (%): SFA 70.1; MUFA 26.8; PUFA 4.3; Cholesterol 0.31 mg/g; [3]Margarine (%): SFA 14.9; MUFA 39.3; PUFA 45.9; Cholesterol 0.00 mg/g

In line with the above experiments, several studies attempted to alter fatty acid composition of regular pork (by decreasing the level of SFA and increasing that of PUFA in tissue lipids) and to determine its functional properties in human subjects [Sandstrõm *et al.*, 2000; Stewart *et al.*, 2001]. In the experiment of Sandstrõm *et al.* [2000], growing pigs were fed three diets, *i.e.* basal diet, basal diet supplemented with rapeseed oil, and basal diet supplemented with rapeseed oil and vitamin E. Meat and meat products from the three groups were then incorporated in three diets offered to human subjects. Generally, the diets prepared with products derived from pigs fed rapeseed oil had a lower content of saturated fatty acids and a higher content of polyunsaturated fatty acids (see Table 3, for fatty acid composition). As indicated in Table 3, the diets based on modified meat and meat products decreased significantly total plasma cholesterol in human subjects, thus showing functional properties of the modified pork. At the same time, no differences were observed in LDL-, HDL- or VLDL-cholesterol, or in triacylglycerol concentrations. In a similar experiment [Stewart *et al.*, 2001], growing pigs were fed basal and modified diet, the latter providing 40% of total energy in the form of soybean oil. Regular and modified pork (with high content of polyunsaturated fatty acids), were then incorporated into two experimental diets offered to women subjects (see Table 4, for fatty acid composition). As shown in Table 4, the subjects consuming modified pork in their diets had significantly lower total plasma cholesterol and LDL-cholesterol. In addition, the modified diet resulted in an increase in the PUFA and a decrease in the SFA and MUFA contents in blood plasma, again indicating functional properties of modified pork.

TABLE 2. Total cholesterol, its LDL and HDL fractions and triacylglycerols in human blood plasma as affected by dietary fat [Noakes *et al.*, 1996].

Specification	Standard fat[1]	Modified fat[2]
Total cholesterol [mmol/L]	6.50^{A}	6.22^{B}
LDL [mmol/L]	4.49^{A}	4.25^{B}
HDL [mmol/L]	1.30	1.28
Triacylglycerols [mmol/L]	1.57	1.54

A,B - $p<0.01$; [1]Standard fat (%): SFA 65.0; MUFA 22.8; PUFA 2.2; [2]Modified fat (%): SFA 50.4; MUFA: 35.3; PUFA: 9.1

TABLE 3. Plasma total cholesterol, its LDL and HDL fractions, triacylglycerols, and α-tocopherol in human blood plasma as affected by dietary fat [Sandstrõm *et al.*, 2000].

Specification	Standard fat (Standard diet)[1]	Modified fat (Standard diet + rapeseed oil)[2]	Modified fat (Standard diet + rapeseed oil + vit. E)[3]
Total cholesterol [mmol/L]	3.62^{a}	3.47^{b}	3.44^{b}
VLDL [mmol/L]	0.18	0.16	0.18
LDL [mmol/L]	2.25	2.20	2.19
HDL [mmol/L]	1.19	1.18	1.15
Triacylglycerols [Mmol/L]	0.69	0.63	0.69
α-Tocopherol [mol/L]	18.2^{a}	16.9^{b}	17.8^{a}

a,b - $p<0.05$; [1]Standard diet (%): SFA 38; MUFA 45; PUFA 14; [2]Standard diet + rapeseed oil (%): SFA 30; MUFA 47; PUFA 20; [3]Standard diet + rapeseed oil + vit. E (%): SFA 28; MUFA 47; PUFA 21

Current nutritional strategies of hen egg modification focus mainly on egg yolk lipids. Farrell [1998], fed laying hens with basal diet and three experimental diets supplemented with different combinations of plant and fish

oils, to obtain eggs enriched with *n*-3 PUFA. The resulting composition of egg yolk lipids (individual *n*-3 PUFA) was significantly altered and largely reflected that of the lipid supplement (Table 5a). The most spectacular effect was the reduced the ratio of *n*-6 to *n*-3 PUFA in egg yolk lipids. The eggs were then offered (7 eggs per week, for 24 weeks) to four respective groups of human subjects. There were no significant differences (Table 5b) in plasma lipid components among treatment groups consuming four different types of eggs. At the same time, significant increases in plasma EPA, DHA and total *n*-3 PUFA concentrations were seen in the subjects consuming PUFA-enriched eggs, compared with controls. Moreover, the enriched eggs favourably reduced the ratio of *n*-6 to *n*-3 PUFA (Table 5c).

TABLE 4. Total cholesterol and its LDL and HDL fractions in human blood plasma as affected by dietary fat [Stewart *et al.*, 2001].

Specification	Standard fat (Standard diet)[1]	Modified fat (Standard diet + soybean oil)[2]
Total cholesterol [mmol/L]	4.01[a]	3.39[b]
LDL [mmol/L]	2.33[a]	1.78[b]
HDL [mmol/L]	1.38	1.19

a,b - $p<0.05$; [1]Standard diet (%): SFA 39.9; MUFA 44.8; PUFA 15.3; [2]Standard diet + soybean oil (%): SFA 25.0; MUFA 34.0; PUFA 41.4

TABLE 5a. Polyunsaturated (*n*-3) fatty acid content of egg yolk lipids as affected by fat sources in hen feed mixtures (% of total fatty acids) [Farrell, 1998].

Fatty acid	Standard mixture	Standard mixture + fish oil	Standard mixture + fish oil + linseed oil	Standard mixture + fish oil + linseed oil + rapeseed oil
C18:3	0.20	0.36	2.26	2.32
C20:5	0.20	1.00	0.58	0.45
C22:5	0.06	0.63	0.52	0.42
C22:6	0.44	5.27	3.80	3.38
Total	0.94	7.34	7.24	6.60
n-6/*n*-3	25.75	1.25	1.52	1.80

TABLE 5b. Total cholesterol, its LDL and HDL fractions and triacylglycerols in blood plasma of subjects consuming 7 eggs/week as affected by the source of fat used to modify egg lipid composition [Farrell, 1998].

Specification	Control	Fish oil	Fish oil Linseed oil	Fish oil Linseed oil Rapeseed oil
Total cholesterol [mmol/L]	4.4	5.3	4.6	4.5
LDL [mmol/L]	3.6	4.5	3.8	3.8
HDL [mmol/L]	0.84	0.84	0.87	0.70
Triacylglycerols [mmol/L]	0.84	1.30	1.05	0.97

Functional properties of CLA-enriched milk, meat and eggs

There is no information available on functional effect of CLA-enriched dairy products in humans. However it is worth indicating that rats consuming the CLA-enriched butter [Ip *et al.*, 1999], consistently accumulated more total CLA in the mammary gland and other tissues (four- to six-fold more) compared with those fed pure CLA (threefold increase), at the same level of dietary intake. Moreover, CLA isomers (both free and incorporated into butter fat) exerted strong anticarcinogenic effect by reducing the development of chemically-induced ammary tumors by 53%.

Table 5c. Polyunsaturated fatty acid (*n*-3) content in blood plasma of subjects consuming 7 eggs/week as affected by the source of fat used to modify egg lipid composition (% of total fatty acids) [Farrell, 1998].

Specification	Control	Fish oil	Fish oil Linseed oil	Fish oil Linseed oil Rapeseed oil
C18:3	0.83	1.22	1.27	1.36
C20:5	0.60[b]	0.91[a]	0.78[a,b]	0.93[a]
C22:5	0.42[b]	0.57[a,b]	0.71[a]	0.58[a,b]
C22:6	1.26[b]	2.15[a]	2.14[a]	1.85[a,b]
Total	3.28[b]	5.03[a]	5.11[a]	4.93[a]
n-6/*n*-3	12.20[b]	6.51[a]	7.06[a]	7.70[a]

a,b - $p<0.05$

There is no information available on the functional effect of CLA-enriched meat and meat products in humans.

As indicated above, a large number of trials were conducted to obtain CLA-enriched eggs. However, no attempts were made to verify their functional properties in human subjects. Instead, the potential plasma cholesterol-lowering properties of CLA-enriched egg yolks were studied in rats [Szymczyk & Pisulewski, 2002]. The CLA-enriched egg yolks were obtained from hens fed the CLA-supplemented (1.5% dietary CLA) commercial feed. Two experimental diets were then fed rats: regular yolk egg diet (10% casein+10% freeze-dried egg yolk), and modified egg yolk diet (10% casein+10% CLA-enriched freeze-dried egg yolk). In spite of adverse effects of CLA isomers on fatty acid composition of egg yolk lipids (Table 6), feeding CLA-enriched egg yolks to rats resulted in favourable changes in plasma lipids: plasma total cholesterol and LDL-cholesterol tended to decrease, whereas concentrations of HDL-cholesterol were increased (Table 6). Also, liver cholesterol was significantly decreased in rats fed the CLA-enriched egg yolks. The above changes can be considered to be reliable indices of functional (hypocholesterolemic) effects of CLA isomers, at least in rats.

TABLE 6. Total cholesterol, its LDL and HDL fractions and triacylglycerols in blood plasma of rats fed sunflower oil, standard and CLA-enriched egg yolks [Szymczyk *et al.*, 2002].

Specification	Standard egg yolks[1]	CLA-enriched egg yolks[2]
Total cholesterol [mg/dL]	78.3	74.7
LDL [mg/dL]	31.7	24.3
HDL [mg/dL]	46.6	50.6
HDL/total cholesterol	0.59	0.67
Triacylglcerols [mg/dL]	170.3	171.4

[1]Standard egg yolks (%): SFA 31.0; MUFA 49.1; PUFA 19.3; [2]CLA-enriched egg yolks (%): SFA 53.1; MUFA 26.0; PUFA 20.3

CONCLUSIONS AND PERSPECTIVES OF FUNCTIONAL FOODS OF ANIMAL ORIGIN

In conclusion, nutritional strategies have become a major means to obtain functional foods of animal origin. Both *n*-3 PUFA-enriched and CLA-enriched milk, meat and eggs have been successfully obtained. However, their functional properties, were related practically to plasma

lipid profile and(or) development of atherosclerosis. Moreover, they were demonstrated mainly in animal models and to a limited extent in humans. Therefore, further studies are required to determine other health--related benefits of these products, *e.g.* their anticarcinogenic and antinflamatory properties; preferably using human subjects.

Without any doubt, further development in this field relies on the availability of valid and highly predictive markers of exposure to functional food components, markers of enhanced target function and those of the reduced disease risk. The appropriate markers are becoming available, as indicated by several recent papers [Branca *et al.* 2001; Hill & Peters, 2002; Rafter, 2002; Weaver & Liebman, 2002].

It is also becoming obvious, that in further studies on functional properties of foods, nutrient-gene interactions must be recognised [van Ommen & Stierum, 2002]. Nutrients are considered now dietary signals that are detected by cellular sensor system that in turn influences gene (transcriptomics) and protein (proteomics) expression and, finally metabolite (metabolomics) synthesis. Using these modern tools should allow the analysis of the response of the whole system (organism) to nutrients and(or) non-nutrients, including functional components of foods.

The above nutrigenomics approach [Trayhurn, 2003] is considered absolutely vital for the development of functional science. As indicated by Roberfroid [2001], if the scientific challenge of functional foods is ignored, it will become a marketing challenge overruling the science base. Consequently, the food industry and the consumers will certainly loose an opportunity to improve their nutritional knowledge.

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