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The French paradox: Was it attributed to cheese consumption?

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Abstract

The low rates of cardiovascular mortality which have existed in France despite high saturated fat consumption constitute an epidemiological phenomenon called the French paradox. Although this phenomenon was originally attributed to consumption of red wine and its major constituent resveratrol, we hypothesized that cheese consumption may contribute to the occurrence of the French paradox. In the animal experiment, we demonstrated the effects of Gouda type cheese produced using *Lactobacillus (L.) helveticus* on some biological markers of the metabolic syndrome when given a 20 % higher caloric intake than an ordinary diet. The cheese intake reduced visceral fat and maintained a good balanced between serum cholesterol and adiponectin, suggesting that regular cheese consumption might be an effective prophylaxis against the metabolic syndrome.

Introduction

In 1819, Dr. Samuel Black revealed the geographic gradient in the occurrence of cardiovascular disease, translating into a 4-fold difference in the frequency of coronary vascular disease between Toulouse in France and Belfast in Ireland. Serge Renaud introduced the term "French paradox" in 1993 and revitalized epidemiological studies which tried to explain the illogically low rates of cardiovascular mortality in France. Although this phenomenon was originally attributed to consumption of red wine, cheese and cheese-based products are essential and indispensable ingredients of the typical French and Mediterranean diets. Among the dairy products, cheese has remained a part of dietary culture since the dawn of history, and is produced using unique food processing procedures, such as fermentation and ripening. A wide variety of cheeses have been produced around the world to satisfy different taste and health requirements, depending on differences in the lactic acid bacteria used as the starter and manufacturing process. Lactic acid bacteria possess various types of protease. The characteristics and types of enzymes differ between the different species of bacteria. Therefore, a variety of peptides can be produced in cheese, depending on the types of starters used, or whether or not non-starter bacteria exist [1]. The main proteases that contribute to hydrolysis are coagulants (chymosin, pepsin, fungal acid protease, plant acid protease) used for cheese making and plasmin which originates from raw milk. In addition, cathepsins originating from lysosome, peptidase originating in starters, di- and tri-aminopeptidase with carboxypeptidase and proline specific exopeptidase contribute in a small way. Various physical properties and flavors are produced as different types of cheese contain different ratios of each enzyme, and the reactivity of enzymes relies upon a wide range of manufacturing conditions and environmental factors. There have been many reports regarding the peptides found in cheese [2-5]. Table 1 represents only the structures of peptides, of which bioactivity has been suggested (Table 1).

Effect of cheese consumption on abdominal adipose accumulation and serum adiponectin levels

We conducted an animal experiment to examine the effects of Gouda type cheese produced using *L. helveticus* on some biological markers of the metabolic syndrome [6]. Because *L. helveticus* has higher protease activity than that of other starter microorganisms, we focused some peptides derived from the cheese, and also investigated the effects of the peptides on the production of adiponectin from primary cultures of rat abdominal adipose cells. In order to investigate the effect of ingestion of a Gouda-type cheese on abdominal adipose accumulation and the production of adiponectin, we conducted an animal experiment, in which the animals were divided into an experimental cheese diet group, fed a diet containing cheese, and a control group, fed a diet consisting of raw materials and nutritional ingredients containing casein and butter oil. Four weeks after study initiation, a significant difference in serum cholesterol in rats with versus without cheese intake for 8 weeks was noted in both rat groups, and it was revealed that the rats with cheese intake had significantly lowered serum cholesterol. Analysis of lipoproteins was performed in the serum samples collected at 8 weeks after the start of the experiment. The contents of triglyceride and cholesterol were measured in each of the 4 classes of lipoproteins, namely, chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density

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Table 1. Bioactive peptides in cheese

Amino acid sequence of peptide	Bioactivity	Casein segment	Source cheese
RPKHPIKHQGLPQ	anti-hypertension	αs1 (1-13)	Gouda
HPIKHQGLPQ	anti-oxidation	αs1 (4-13)	Gouda
VNELSKDIGSE*	anti-oxidation	αs1 (37-47)	Chedder
DIGSESTEDQAMEDIKEMEAESISSSEEIVPNSVEEK*	anti-cariogenesis	αs1 (43-79)	Chedder
EDVPSER	anti-oxidation	αs1 (84-90)	Chedder
VPSERYL	anti-hypertension	αs1 (86-92)	Manchego
KKYNVPQL	anti-hypertension	αs1 (102-109)	Manchego
LEIVPK	anti-hypertension	αs1 (109-114)	Manchego
EIVPNSAEERL*	anti-oxidation	αs1 (110-120)	Cheddar
ITVDDKHY	anti-oxidation	αs2 (86-93)	Cheddar
IPY	anti-hypertension	αs2 (202-204)	Manchego
VRYL	anti-hypertension	αs2 (205-208)	Manchego
RELEELNVPGEIVESLSSSEESITRINK*	anti-cariogenesis	β (1-28)	Chedder
SSEESITR*	anti-oxidation	β (17-25)	Chedder
SSSEESITR*	anti-oxidation	β (16-25)	Chedder
FQSEE*	anti-oxidation	β (33-37)	Blue
YPFPGPI	Opioid activity	β (60-66)	Cheddar, Swiss, Blue, Brie
YPFPGPIPN	anti-hypertension	β (60-68)	Gouda
EMPFPKYP	anti-oxidation	β (108-115)	Chedder
MPFPKYPVQPF	anti-hypertension	β (109-119)	Gouda
FPKYPVEP	anti-oxidation	β (111-118)	Chedder
VLPVPQK	anti-oxidation	β (170-176)	Chedder
VRGPFP	anti-hypertension	β (199-204)	Manchego

lipoprotein (HDL). The amount of cholesterol contained in the VLDL was significantly ($p<0.01$) lower in the cheese diet group compared with that in the control group. VLDL is a triglyceride rich lipoprotein synthesized in the liver, and it is known to be increased by high-fat loading. VLDL is also a precursor of LDL, known as bad cholesterol. Therefore, this result suggested that cheese intake inhibits the synthesis of VLDL in the body. Furthermore, the lipoproteins were divided into 20 fractions based on their molecular diameter, and subclass analysis was performed. The amount of cholesterol in the VLDL was lower in the cheese diet group for all molecular radii of the lipoproteins. The serum level of LDL was lower in the cheese diet group in the molecular radius range of large, medium and very small of the apolipoprotein. The serum HDL was lower in the cheese diet group for the molecular diameters of small and very small. The epidemiological investigation described in the past articles found that the risk of developing cardiovascular disease had increased 15-fold when large VLDL particles and small HDL particles were both increased [7,8]. In our study, the amounts of both large VLDL particles and small HDL particles were controlled in the group with cheese intake, which suggested that the cheese intake might reduce the risk of developing cardiovascular disease. Moreover, we measured the amount of adiponectin in blood at the 4th week and the 8th week. The group with cheese intake maintained the amount of adiponectin, while the level had significantly decreased in the group without cheese intake. The visceral fat tissues, that is, the mesenteric, perinephric, peritesticular, and posterior abdominal wall adipose tissues were removed separately and weighed, and the weight of the mesenteric adipose tissue, which is the major white adipose tissue, was significantly lower in the cheese diet group. The results of previous studies have revealed the role of abdominal adipose tissue as the largest endocrine tissue in the living body, and it is understood that maintenance of normal homeostasis of the abdominal adipose tissue is important for the prevention of metabolic syndrome and inhibition of the development of atherosclerosis [9,10]. It has been suggested that excessive abdominal adipose accumulation is associated with disruption of regulation of the secretion of adipocytokines, which are secreted from the abdominal adipose tissue, resulting in the development of various clinical diseases [11]. In particular, it is known that abdominal adipose accumulation is associated with lower serum levels of adiponectin, which is specifically secreted from the adipose tissue and usually exists in the blood at high concentrations. Because adiponectin may have a physiological role in the prevention of diabetes mellitus, atherosclerosis, inflammation, hypertension, etc, it is considered very important to increase the concentration of adiponectin in the blood or attenuate any decrease in its concentration in order to avoid the metabolic syndrome [12]. The above data confirmed that, when given a 20% higher caloric intake than an ordinary diet, the cheese intake reduced visceral fat and also maintained a good balanced between serum cholesterol and adiponectin, suggesting that cheese intake might be an effective prophylaxis against the metabolic syndrome.

Effect of antioxidant peptides on adiponectin production of abdominal adipose cells

We searched for the presence of antioxidant activity in 20 types of cheeses because most of the well-known antioxidants are plant-based substances such as those in tea and vegetables [13]. Although milk protein before ripening had little antioxidant activity, it was revealed that the ripened cheeses such as Gouda, Parmesan and Camembert, had antioxidant activities. Antioxidant activity was particularly high in the moldy cheeses. Then, we searched the origin of antioxidant activity in cheese, and then identified some antioxidant peptides.

The antioxidant peptides contained in the ripened cheeses were shown in Table. These peptides were decomposed into smaller peptides by digestive enzymes, and it was revealed these decomposed substances also had antioxidant activity. In addition, it was confirmed that the activity of the cheese-based antioxidant peptides is relevant to tea catechin, a well-known antioxidant, and furthermore, it had twice the activity of the marketed antioxidant peptide known as carnosine. The amino acid sequence of the peptide with high antioxidant activity separated from the water-soluble fraction of the Gouda-type cheese

produced using *L. helveticus* as the starter corresponded to the sequence from the 4th to the 13th amino acids at the N-terminal end of αs1-casein, and was a decapeptide, His-Pro-Ile-Lys-His-Gln-Gly-Leu-Pro-Gln [6]. The decapeptide was broken down into two peptide fragments (His-Pro-Ile-Lys and His-Gln-Gly-Leu-Pro-Gln) by artificial digestive juices, and the antioxidant activities of these peptides were comparable to that of tea catechin in an equivalent molar ratio.

We investigated the effects of the antioxidant peptide on the production of adiponectin from primary cultures of the rat abdominal adipose cells [6]. It was evaluated using VAC01, a kit for primary culture of the rat abdominal adipose adipose cells. When the antioxidant decapeptide was added to the culture medium in the concentration range of 10, 50, and 100 mM, the production of adiponectin was significantly increased as compared with that in the culture not treated with the decapeptide. The concentration of adiponectin was the highest when 50mM of the peptide was added to the medium. Ford, *et al.* [14] reported that subjects with the metabolic syndrome have significantly lower serum concentrations of antioxidants, such as carotenoids, vitamin C and vitamin E. Furukawa, *et al.* [15] demonstrated that exposure of visceral fat to oxidant stress affects the regulation of adipocytokine production, thereby increasing the risk of development of metabolic syndrome and atherosclerosis. The antioxidant peptide contained in the cheese potentially contributed, at least in part, to the effect of the dietary cheese consumption on the production of serum adiponectin and reduction of abdominal adipose accumulation.

Conclusion

There have been many reports that cheese consumption is suggested to have a variety of health-promotive effects. Human intervention studies have demonstrated that cheese dose not increase plasma cholesterol concentration [16-18]. Comparison of the effects of consumption of butter and cheese prepared using an identical amount of milk fat revealed that consumption of cheese was associated with lower serum cholesterol levels than the consumption of butter with an identical amount of fat [17]. With regards to homeostasis, it was shown that women with the highest cheese consumption had beneficially lower concentration of plasminogen activator inhibitor 1 [19].

The French paradox is still unresolved and continues to be a matter of debate and controversy. Most notably this would involve milk bioactive peptides and biomolecules from cheese molds. Advances in molecular science have revealed a wide variety of secondary metabolites of *Penicillium roqueforti* as well as roquefortine, whose ability to inhibit cholesterol biosynthesis and bacterial growth might be a key mechanism in favoring their therapeutic potential for cardiovascular disease [20]. Additional human studies will support the "cheese" hypothesis further by elucidating the mechanism of biological functions.

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