Food Sci. Biotechnol. Vol. 18, No. 4, pp. 884 ~ 888 (2009)

Food Science and Biotechnology

Effect of Citrus Pectin Oligosaccharide Prepared by Irradiation on High Cholesterol Diet B6.KOR-ApoE Mice

Ho Jin Kang[1], Joong Ho Kwon[1], Dong Uk Ahn[2], Ju Woon Lee[3], Wan Kyu Lee[4], and Cheorun Jo*

Department of Animal Science and Biotechnology, Chungnam National University, Daejeon 305-764, Korea
[1]*Department of Food Science and Technology, Kyungpook National University, Daegu 702-701, Korea*
[2]*Department of Animal Science, Iowa State University, Ames, IA 50010, USA*
[3]*Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup, Jeonbuk 580-185, Korea*
[4]*Laboratory of Veterinary Bacteriology, College of Veterinary Medicine, Chungbuk National University, Cheongju, Chungbuk 361-763, Korea*

Abstract Effect of citrus pectin oligosaccharides produced by irradiation was studied on the ability to improve lipid metabolism and hypercholesterolemia in mice fed high cholesterol diets. A total of 35 mice were divided into 5 groups and fed the following diets for 6 weeks: normal diet (C), 0.5% cholesterol (CH), 0.5% cholesterol+5% non-irradiated pectin (P), 0.5% cholesterol+5% irradiated pectin at 20 kGy (PIR), and 0.5% cholesterol+5% irradiated at 20 kGy and dialyzed (PIR-F). CH group had significantly higher serum triglycerides, total cholesterol, and low density lipoprotein (LDL)-cholesterol contents than pectin oligosaccharide-treated groups ($p<0.05$). Triglycerides and total cholesterol contents was the lowest in C and PIR-F and followed by PIR and P group, and CH group had significantly higher LDL-cholesterol. Serum high density lipoprotein (HDL)-cholesterol content in C group was not different from that in CH and P groups, but lower than that of PIR and PIR-F groups. These results suggest that pectin oligosaccharides produced by irradiation can reduce the levels of serum triglyceride, total cholesterol, and LDL-cholesterol in the blood of mice fed high-cholesterol diets and therefore, irradiation can be used as a tool to produce functional oligosaccharides from citrus pectin.

Keywords: citrus pectin, oligosaccharide, gamma irradiation, hypercholesterolemia, histopathological observation

Introduction

Citrus pectin is known to have hypocholesterolemic effects in humans (1). Oligosaccharides derived from pectin have shown to have applications as repressors of liver lipid accumulation in rats (2), antifungal phytoalexin elicitors in plants (3), and inducers of flowering and antibacterial agents (4). However, because of long dissolving time and high viscosity when the concentration of the pectin increased, the methods for lowering the molecular weight levels of pectin to oligomer has been proposed. Low viscosity and improved solubility of modified citrus pectin enable the preparation of highly concentrated solutions necessary for medical purposes (5). The chemical or enzymatic methods have been applied for the hydrolysis of pectin (6). Chemical treatment is a very common and fast method but it has some defects such as high cost, low yield, and acidic wastes by the use of HCl (7). The enzymatic processes are generally preferable over chemical reaction because the hydrolysis can be controlled more easily, but oligomers are not obtained in a good yield (8). Also, the method of enzymatic hydrolysis has a long processing time and very sensitive to pH change, and addition of chemicals or enzymes makes it difficult for industrial mass production.

Irradiation is a useful technology as one of sanitation treatments in food processing and preservation. Food irradiation has been proved to be a powerful process for a food and sterilization (9,10). And also, irradiation technology has positive effects in preventing decay of food ingredients by not only eliminating microorganisms but also improving the safety and shelf life without losing the nutritional or sensorial quality (11). Recently, irradiation effects on carbohydrates such as chitosan, sodium alginate, carrageenan, and cellulose have been investigated to enhance their use for recycling these bioresources and reducing the environmental pollution level. Nagasawa *et al*. (12) and Hien *et al*. (13) reported that alginate degraded by an irradiation could accelerate the growth of plants caused by the increase of the biological activity. Aliste *et al*. (14) studied the rheological and functional performance of water solutions of irradiated agar, alginates, and carrageenan. Prepared chitosan oligomer using irradiation has been also reported (15). It is well known that polysaccharides are degraded by irradiation due to the free radical induced scission of the glycosidic bonds. The irradiation of pectin in aqueous solutions caused the degradation of macromolecules rather than the radiation induced deesterification of polysaccharide chains reveals the decrease in viscosity of pectin solution (5).

Recently Kang *et al*. (16) used irradiation to prepare a citrus pectin-oligosaccharide and investigated its antioxidant and cancer proliferation inhibition effect. Kang *et al.* (17) also reported that the average molecular weight of non-irradiated pectin solution showed the range of 500 kDa, while those of 10 and 20 kGy irradiated ones showed 30-40 kDa. Then, the average molecular weight was shown

*Corresponding author: Tel: +82-42-821-5774; Fax: +82-42-825-9754
E-mail address: cheorun@cnu.ac.kr
Received November 11, 2008; Revised January 19, 2009;
Accepted March 2, 2009

less than 37 kDa at 30 kGy or above. However, information regarding to the irradiation application on pectin and its derivatives is still limited.

The objective of this study was to apply irradiation technology for the production of functional oligosaccharide from citrus pectin. For this purpose, a pectin-oligosaccharide prepared by irradiation was investigated in terms of its pharmacochemical activities using hypercholesterolemic mice.

Materials and Methods

Preparation of pectin oligosaccharide Pectin (from citrus fruits) was purchased from the Sigma-Aldrich (St. Louis, MO, USA). Pectin was dissolved in distilled water (2%, w/v). The solution was separated into 3 groups; non-irradiated (P), irradiated at 20 kGy (PIR), and dialyzed by a membrane filter (Spectrum Laboratories Inc., Racho Dominguez, CA, USA) to achieve a molecular weight of less than 10,000 after an irradiation at 20 kGy (PIR-F).

Irradiation The solutions were subjected to an irradiation treatment at 20 kGy in a Co-60 irradiator (point source. AECL, IR-79; MDS Nordion International Inc., ON, Canada) in Korea Atomic Energy Research Institute, Daejeon, Korea. The temperature of the irradiation chamber was 14±1°C and the rate of an irradiation was 10 kGy/hr. Dosimetry was performed using 5-mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany). Gamma irradiation is capable of hydrolyzing chemical bonds, thereby cleaving large molecules of starch into smaller fragments of dextrin (18). Distilled water in a pectin solution was removed using a vacuum evaporator (Rotary Vacuum Evaporator N-11 Eyela; Tokyo Rikakikai Co., Ltd, Tokyo, Japan). Samples were then lyophilized in a laboratory lyophilizer (SEDSF12; Samwon Co., Ltd., Busan, Korea). The dried sample was collected and stored in screw capped glass bottles at an ambient temperature (18°C) and used as an ingredient for the experimental analysis. The PIR-F treatment was prepared by dialyzing the irradiated pectin solution (2%) through a membrane filter to achieve a molecular weight of less than 10,000. In detail, the membrane with a pectin solution was transferred to a plastic box (30×40×15 cm) with 10 folds of distilled water and incubated in a shaking incubator for 48 hr at 27°C. Dialyzed pectin (Mw<10,000) solution (outer solution) in distilled water was collected 3 times. The solution was evaporated using a vacuum evaporator and lyophilized using a laboratory lyophilizer. Lyophilzed pectin oligosaccharide powder was used for an analysis.

Table 1. Design of experimental mice group

Group[1)]	Diet condition
C	Basal diet
CH	High cholesterol diet (cholesterol 0.5%)
P	High cholesterol diet (cholesterol 0.5%)+pectin (0 kGy)
PIR	High cholesterol diet (cholesterol 0.5%)+pectin (20 kGy)
PIR-F	High cholesterol diet (cholesterol 0.5%)+pectin (20 kGy)

[1)]P, non-irradiated pectin (0 kGy); PIR, irradiated pectin (20 kGy); PIR-F, dialyzed by a membrane filter to achieve a molecular weight of less than 10,000 after irradiation at 20 kGy.

Animals and diets To investigate the effect of pectin oligosaccharide on the level of serum lipids, B6.KOR-ApoE mice (6-week old male) were obtained from Jung-Ang Laboratories Animal Inc. (Seoul, Korea) and left to acclimatize for 1 week before the experimental period. Animal care was in accordance to guidelines established by the Korean Food & Drug Administration (KFDA) using protocols approved by the Institutional Animal Care and Use Committee (IACUC). The mice were divided into 5 experimental treatment groups each contained 7 mice/ treatment group (Table 1).

All diets were based on American Institute of Nutrition Recommendations described in Table 2. Mice weighing 20-25 g were housed in stainless steel, wired cages individually (1 mouse/cage) in kept in an isolated room at a controlled temperature (20-22°C) and ambient humidity

Table 2. Nutritional composition (g/kg) of experimental diets

Ingredients	Experimental diet				
	C	CH	P	PIR	PIR-F
Casein	200	200	200	200	200
L-Cysteine	3	3	3	3	3
Corn starch	399.5	389.5	339.5	339.5	339.5
Sucrose	200	200	200	200	200
Cellulose	50	50	50	50	50
Soybean oil	100	100	100	100	100
t-Butylhydroquinone	0.014	0.014	0.014	0.014	0.014
Mineral mix[1)]	35	35	35	35	35
Vitamine mix[2)]	10	10	10	10	10
Choline bitartate	2.5	2.5	2.5	2.5	2.5
Cholesterol	0	5	5	5	5
Na-cholate	0	5	5	5	5
Sample	0	0	50	50	50

[1)]AIN93 mineral mixture.
[2)]AIN93 vitamine mixture.

(60-65%). Lights were maintained on an artificial 12-hr light-dark cycle. Mice had free access to water and feed throughout the study. All mice were weighed weekly and feed intake was recorded twice a week during 6 weeks. The feed efficiency ratio (FER) of each experimental group was calculated by dividing total body weight increase by total feed intake for the period.

Blood collection and analysis Each mouse was fasted for 12 hr before sacrifice and blood samples were collected from eyeball. The serum was separated from collected blood by centrifugation at 2,090×*g* for 15 min at 4°C. The concentration of serum triglyceride, total cholesterol, high density lipoprotein cholesterol (HDL-cholesterol), and low density lipoprotein cholesterol (LDL-cholesterol) were assayed automatically using ADVIA 1650 lipid analyzer (Bayer, Newbury, UK). Atherosclerogenic index (AI) was calculated as

AI=(total cholesterol−HDL-cholesterol)/HDL-cholesterol

Histopathological test The liver tissues were dissected after sacrificing the mice and fixed in 10% formalin at room temperature. The tissue was embedded into paraffin, sectioned by 3-4 mm thickness, and mounted on the glass microscope slides using standard histopathological techniques. The sections were stained with hematoxylin-eosin and examined using a light microscopy (Olympus, Tokyo, Japan).

Statistical analysis One way analysis of variance using SAS (SAS Institute, Cary, NC USA) software (SAS Institute, Inc., 1989) (19) and the Duncan's multiple range tests were used to compare the differences among mean values. Mean values with pooled standard errors of the mean (SEM) were reported with the significance defined at $p \leq 0.05$.

Results and Discussion

Feed intake, weight gain, and feed efficiency The results of feed intake and weight gain in each experimental group are shown in Table 3. Feed intake was not significantly different among the mice groups except for the only pectin group (P), which showed lower than other treatment groups. Feed intake and weight gain were not significantly different between control (C) and PIR-F treatment. Weight gain was the highest at group C (0.05 g/day), whereas P and PIR treatments were the lowest (0.01 g/day). The feed efficiency ratio (FER) of group C (0.014) was higher than CH, P, PIR-F, and PIR treatments.

Content of serum of triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol, and atherosclerogenic index The amount of serum triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol of mice was shown in Table 4. Triglyceride, total cholesterol, and LDL-cholesterol contents were significantly ($p<0.05$) lower in group C than that in high cholesterol and pectin oligosaccharide-treated groups. Triglyceride contents decreased in order of P<PIR<PIR-F, and LDL-cholesterol content was the highest in CH, high cholesterol diet only (169.5 mg/dL), whereas an addition of pectin or pectin oligosaccharide including P, PIR, and PIR-F group (100.0, 92.8, and 89.8 mg/dL, respectively) decreased the level of LDL-cholesterol although they did not show difference among them ($p>0.05$). Serum HDL-cholesterol contents in C, CH, and pectin diet groups were not different ($p>0.05$) however, the contents increased by feeding PIR and PIR-F group diet (74.2 and 77.8 mg/dL). Many reports have been introduced the natural agents of significant activities on reducing and/or regulating serum cholesterol and triglycerides levels over the years (20). Among them, soluble dietary fibers are known to have high activity for depressing the cholesterol content and it is known that some degree of polymerization is necessary for this activity. Mokady (21) has reported that highly viscous pectin had a stronger effect to repress the level of the cholesterol content of blood. Yamaguchi *et al.* (2) suggested that a molecular weight at least equal to or larger than that of low molecular weight pectin would be necessary for repressing the serum cholesterol level. These effects may be come from the inhibition of lipid absorption or cholesterol synthesis by the production of volatile fatty acids (2). One of the major mechanisms reported to explain the dietary fiber-mediated lowering of serum cholesterol is the greater excretion of bile acids and steroids, which leads to the regulation of bile acid biosynthesis. Gallaher *et al* (22) reported that dietary fibers decreased adsorption of bile acids and bile salts, and increased fecal bile acid excretion. These activities decreased the serum cholesterol

Table 3. Feed intake, weight gain, and feed efficiency ratio of B6.KOR-Apoe mice fed pectin oligosaccharide prepared from irradiation

Group	Feed intake (g/day)	Weight gain (g/day)	Feed efficiency ratio (FER)
C	3.51$^{a1)}$	0.05^{a}	0.014^{a}
CH	3.39^{a}	0.01^{ab}	0.002^{b}
P	3.02^{b}	-0.01^{b}	-0.003^{ab}
PIR	3.21^{ab}	-0.02^{b}	-0.006^{c}
PIR-F	3.48^{a}	0.01^{ab}	-0.003^{ab}
SEM$^{2)}$	0.007	0.004	0.006

$^{1)a-c}$Different letters within the same column differ significantly ($p<0.05$).
$^{2)}$Standard errors of the mean ($n=35$).

Table 4. Content (mg/dL) of triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol in the serum of B6.KOR-Apoe mice fed pectin oligosaccharide prepared from irradiation

Group	Triglyceride	Total cholesterol	HDL-cholesterol	LDL-cholesterol
C	145.5$^{c1)}$	139.8^{d}	69.1^{b}	70.7^{c}
CH	255.2^{a}	239.0^{a}	69.5^{b}	169.5^{a}
P	154.1^{b}	171.8^{b}	71.8^{ab}	100.0^{b}
PIR	149.9^{bc}	166.2^{c}	74.2^{a}	92.0^{b}
PIR-F	143.7^{c}	167.6^{c}	77.8^{a}	89.8^{b}
SEM$^{2)}$	0.87	1.29	0.67	2.31

$^{1)a-d}$Different letters within the same column differ significantly ($p<0.05$).
$^{2)}$Standard errors of the mean ($n=35$).

Table 5. Atherosclerogenic index (AI) in serum of B6.KOR-Apoe mice fed pectin oligosaccharide prepared from irradiation

Experimental diet group					
C	CH	P	PIR	PIR-F	SEM[1)]
1.02[c2)]	2.44[a]	1.39[b]	1.23[bc]	1.15[c]	0.241

[1)]Standard errors of the mean (n=35).
[2)]Different letters within the same row differ significantly (p<0.05).

Table 6. Histopathological observations of the liver of B6.KOR-Apoe mice fed pectin oligosaccharide prepared from irradiation

Group	Histopathological findings of the liver	
	Swelling of hepatic cell	Fat degeneration
C	1.00[b1)]	1.43[ab]
CH	2.86[a]	2.86[a]
P	1.86[ab]	2.00[ab]
PIR	0.71[b]	0.71[b]
PIR-F	0.71[b]	0.71[b]
SEM[2)]	0.519	0.485

[1)a-b]Different letters within the same column differ significantly (p<0.05).
[2)]Standard errors of the mean (n=7).

level as a result of increased cholesterol turnover and authors indicated the wide range applications of dietary fiber in foods as a hypocholesterolemic agent (22,23). A physiological mechanism of cholesterol-lowering effect could be explained by the interaction of bile acids with dietary fiber as a bile acid sorbent (23,24) and through the viscosity of dietary fiber within the gastrointestinal tract (22). Yoo *et al.* (25) and Hwang and Park (26) reported that bile acid sorption capacity of pectin and guar galactomannan increased with an increase in concentration. The increase of the bile acid sorption capacity of pectin can be explained based on the results of high solution viscosities and strong interactions between biopolymers and bile acids (25).

The result of atherosclerogenic index (AI) is also support the finding (Table 5). AI of normal diet and PIR-F group was lower than those of other treatment groups (p<0.05). AI of the mice fed high cholesterol diet group CH in B6.KOR-Apoe mice was the highest. The LDL of hypercholesterolemic rabbits was more susceptible to oxidative modification than that of normal rabbits (27). This oxidative modification of LDL is involved in the initiation and promotion of atherosclerosis (28). Thus, an increased cholesterol level can be a significant predictor of the development of coronary artery disease, and serum LDL-cholesterol levels should be used as the basis for initiating and monitoring treatment of patients with elevated blood cholesterol (29). According to these studies, a pectin oligosaccharide prepared from irradiation of pectin solution and dialysis step is beneficial for the lipid metabolism in high cholesterol diet mice.

Histopathological observations Histopathological observation of the liver was examined by observing remarkable impressions of fat degeneration by cellular swelling of liver cell and fat droplet accumulation in cell. The histopathological findings for the liver taken from those mice ranged from 4 (severe degree) to 0 (without normal limit). The liver of the mice fed normal diet had slight degree of swelling of hepatic cell and fat degeneration, whereas that of the mice fed high cholesterol diet was found to be severe hepatic cell swelling and fat degeneration (Table 6). The histopathological investigation showed a large difference among individual mouse in P group but it was still lower chance of metabolic disorder than high cholesterol only group. In the pectin oligosaccharide treatment groups, on the other hand, the liver from mice fed PIR and PIR-F were not found any fat degeneration and cellular swelling of liver cell with minor exceptions (Table 6). These results suggested that pectin oligosaccharide prepared by irradiation may be able to reduce the swelling of hepatic cell and fat degeneration on the mouse liver. The results of present study also suggest that supplement of pectin oligosaccharide reduced levels of serum triglyceride, total cholesterol, and LDL-cholesterol of blood in B6.KOR-Apoe mice fed with high cholesterol diets.

In conclusion, the present study has demonstrated that an irradiation technology can be applied to produce functional pectin oligosaccharide without any chemical treatment and the pectin oligosaccharide prepared by irradiation and followed by dialysis to separate the lower molecular weight products can be a potential health beneficial effect.

Acknowledgments

This work was supported by the Korea Research Foundation grant funded by the Korean Government (MOEHRD) (KRF-2006-214-F00020).

References

1. Kay RM, Truswell AS. Effect of citrus pectin on blood lipids and faecal steroid excretion in man. Am. J. Clin. Nutr. 30: 171-175 (1977)
2. Yamaguchi F, Shimizu N, Hatanaka C. Preparation and physiological effect of low-molecular-weight pectin. Biosci. Biotech. Bioch. 58: 679-682 (1994)
3. Bishop PD, Pearce G, Bryant JE, Ryan CA. Isolation and characterization of the proteinase inhibitor-inducing factor from tomato leaves. J. Biol. Chem. 259: 13172-13177 (1984)
4. Iwasaki K, Inoue M, Matsubara Y. Continuous hydrolysis of pectate by innobilized endo-polygalacturonase in a continuously stirred tank reactor. Biosci. Biotech. Bioch. 62: 262-272 (1998)
5. Zegota H. The effect of gamma irradiation on citrus pectin in N_2O and N_2O/O_2 saturated aqueous solutions. Food Hydrocolloid 13: 51-58 (1999)
6. Cho M, Kim BY, Rhim JH. Degradation of alginate solution and powder by gamma-irradiation. Food Eng. Prog. 7: 141-145 (2003)
7. Jeon YJ, Kim SK. Production of chitooligosaccharides using an ultrafiltration membrane reactor and their antibacterial activity. Carbohyd. Polym. 41: 133-141 (2000)
8. Marie CR, Monique AVA, Jean FT. Gelation properties of extruded lemon cell walls and their water-souble pectins. Carbohyd. Res. 26: 271-282 (1994)
9. Waje C, Kim MY, Nam KC, Jo C, Kim DH, Lee JW. Effect of irradiation on the color, microbiological quality, and sensory attributes of frozen ground beef, pork, and chicken after 6 months at −6°C. Food Sci. Biotechnol. 17: 212-215 (2008)
10. Yun HJ, Lim SY, Yang SH, Lee WY, Kwon JH, Lim BL, Kim DH. Effect of gamma irradiation on the growth and patulin production of *Penicillium griseofulvum* in an apple model system. Food Sci. Biotechnol. 17: 723-727 (2008)

11. Kwon JH. Effect of gamma irradiation and methyl bromide fumigation on the quality of fresh chestnuts during storage. Food Sci. Biotechnol. 14: 181-184 (2005)
12. Nagasawa N, Mitomo H, Yoshii F, Kume T. Global exposure models for automotive coating photo-oxidation. Polym. Degrad. Stabil. 69: 279-306 (2000)
13. Hien NQ, Nagasawa N, Tham LX, Yoshii F, Dang VH, Mitomo H, Makuuchi K, Kume T. Growth-promotion of plants with depolymerized alginates by irradiation. Radiat. Phys. Chem. 59: 97-101 (2000)
14. Aliste AJ, Vieira FF, Del Mastro NL. Radiation effects on agar, alginates, and carrageenan to be used as food additives. Radiat. Phys. Chem. 57: 305-308 (2000)
15. Choi WS, Ahn KJ, Lee DW, Byun MW, Park HJ. Preparation of chitosan oligomers by irradiation. Polym. Degrad. Stabil. 78: 533-538 (2002)
16. Kang HJ, Jo C, Kwon JH, Jeong IY, Byun MW. Physicochemical characteristics and biological activity of irradiated pectin solution. Korean J. Food Sci. Technol. 37: 741-745 (2005)
17. Kang HJ, Jo C, Kwon JH, Son JH, An BJ, Byun MW. Antioxidant and cancer cell proliferation inhibition effect of citrus pectin oligosaccharide prepared by irradiation. J. Med. Food 9: 313-320 (2006)
18. Kang IJ, Byun MW, Yook HS, Bae CH, Lee HS, Kwon JH, Chung CK. Production of modified starches by gamma irradiation. Radiat. Phys. Chem. 54: 425-430 (1999)
19. SAS Institute, Inc. SAS User's Guide. SAS Institute, Inc., Cary, NC, USA (1989)
20. Jahromi F, Ray AB, Chansouria JPN. Antihyperlipidemic effect of flavonoids from *Pterocarpus marsupium*. J. Nat. Prod. 56: 989-994 (1993)
21. Mokady S. Effect of dietary pectin and algin on blood cholesterol level in growing rats fed a cholesterol-free diet. Nutr. Metab. 15: 290-294 (1973)
22. Gallaher DD, Hassel CA, Lee KK, Gallaher CM. Viscosity and fermentability as attributes of dietary fiber responsible for the hypocholesterolemic effet in hamsters. J. Nutr. 123: 244-252 (1993)
23. Muzzarelli RAA. Chitosan-based dietary foods. Carbohyd. Polym. 29: 309-316 (1996)
24. Story JA, Kritchevsky D. Comparison of the binding of various bile acids and bile salts *in vitro* by several types of fiber. J. Nutr. 106: 1292-1294 (1976)
25. Yoo BS, Lee SM, Choi MJ, Hwang JK. Adsorption capacity of pectin and guar galactomannan mixtures as bile acid sorbents. Food Sci. Biotechnol. 10: 562-565 (2001)
26. Hwang JK, Park CH. Chitosan and guar galatomannan mixtures as bile acid sorbents: An *in vitro* study. Food Sci. Biotechnol. 8: 213-216 (1999)
27. Nenseter MS, Gudmundsen O, Malterud KE, Berg T, Drevon CA. Effect of cholesterol feeding on the susceptibility of lipoproteins to oxidative modification. Biochim. Biophys. Acta 1213: 207-214 (1994)
28. Steinberg D. Antioxidant vitamins and coronary heart disease. New Engl. J. Med. 328: 1487-1489 (1993)
29. The expert panel. The role of triglycerides as a CHD risk factor. J. Am. Med. Assoc. 269: 3015-3023 (1993)