Available online at www.sciencedirect.com
ScienceDirect
Clinical Biochemistry 40 (2007) 623–628
CLINICAL BIOCHEMISTRY

Ameliorating effect of coenzyme Q_{10}, riboflavin and niacin in tamoxifen-treated postmenopausal breast cancer patients with special reference to lipids and lipoproteins

Srinivasan Yuvaraj [a], Vummidi Giridhar Premkumar [a], Kothandaraman Vijayasarathy [b], Sitthu Govindaswamy Dinakaran Gangadaran [b], Panchanatham Sachdanandam [a,*]

[a] *Department of Medical Biochemistry, University of Madras, Taramani Campus, Chennai – 600 113, Tamil Nadu, India*
[b] *Department of Medical Oncology, Government Royapettah Hospital, Kilpauk Medical College, Chennai – 600 014, Tamil Nadu, India*

Received 29 September 2006; received in revised form 12 January 2007; accepted 13 February 2007
Available online 19 March 2007

Abstract

Objectives: Tamoxifen (TAM), a non-steroidal anti-estrogen that is widely used in adjuvant therapy for all stages of breast carcinomas and in chemoprevention of high-risk group. The hepatic estrogenic effect of TAM induces hypertriglyceridemia by reduced activity of lipolytic enzymes (LPL) on triglycerides. Coenzyme Q_{10} (Co Q_{10}), riboflavin and niacin are proved to be potent antioxidant and protective agents against many diseases including cancer and cardiovascular diseases (CVD). In this context, the objective of the study is to find the effect of the combined modality of Co Q_{10} (100 mg), riboflavin (10 mg) and niacin (50 mg) with TAM (10 mg twice a day) on serum lipids and lipoprotein levels in postmenopausal women with breast cancer.

Design and methods: The vitamin supplementation with tamoxifen was given for a period of 90 days. Blood samples were collected at the base line, 45th and 90th day during the course of treatment. Plasma total cholesterol (TC), free cholesterol (FC), ester cholesterol (EC), phospholipids (PL), triglycerides (TGL), free fatty acids (FFA), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very low density cholesterol (VLDL-C) were estimated in 78 untreated, only TAM-treated and combinatorialy treated group along with 46 age- and sex-matched controls.

Results: Serum TGL and VLDL-C ($p < 0.001$) were found to be significantly elevated and LDL-C ($p < 0.01$), significantly reduced among TAM-treated patients as compared to the untreated breast cancer subjects. All the lipids and lipoprotein levels were found to be significantly altered in the untreated breast cancer patients when compared to their normal counterparts. All the lipid and lipoprotein abnormalities were reverted back to near normal levels on 90 days of treatment on combinatorial therapy.

Conclusion: The study figures the altered lipid and lipoprotein levels in the untreated and TAM-treated breast cancer patients. On combination therapy with Co Q_{10}, riboflavin and niacin, it counteracts the tamoxifen-induced hyperlipidemia to normal levels.
© 2007 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

Keywords: Breast cancer; Lipid; Lipoproteins; Tamoxifen; Riboflavin; Niacin; Coenzyme Q_{10}

Introduction

Breast cancer is the most common malignancy comprising 18% of all cancers in women [1]. The introduction of tamoxifen (TAM), a non-steroidal anti-estrogen in the early 1970s represented a landmark in the treatment of breast cancer [2]. Since 1990, death rates from breast cancer have decreased by over 25%, and this is at least partly due to adjuvant treatment with TAM [3]. Apart from its use in adjuvant treatment protocol, TAM has also shown to reduce the risk of contralateral breast cancers in carriers of *BRCA 1* or *BRCA 2* genes and in advance metastatic breast cancers [4,5]. The anti-tumor activity of TAM is largely believed to be due to its occupation of intracellular estrogen receptor sites in the target tissues and blocking the action of biologically active estrogen and estradiol [6].

Tamoxifen's effect on plasma lipids and lipoproteins, one of our earlier studies along with others has indicated that TAM

* Coresponding author.
E-mail address: psachdanandam2000@yahoo.co.in (P. Sachdanandam).

0009-9120/$ - see front matter © 2007 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.clinbiochem.2007.02.003

increases VLDL-C levels resulting in hypertriglyceridemia [7,8]. High TGL levels are proved to be independent and statistically significant risk factor for the development of cardiovascular complications [9]. Hence, this study is concentrated on reducing this contraindication of sole TAM treatment with Co Q_{10} (100 mg), niacin (50 mg) and riboflavin (10 mg), since combination of these vitamins could exert a profound influence on the fat-splitting enzymes that may titer the hypertriglyceridemic effect of TAM.

Co Q_{10} is a lipid-soluble benzoquionone found in all tissues and membranes. Co Q_{10} is particularly high in the inner mitochondrial membrane, where it functions as an electron carrier in oxidative phosphorylation. Co Q_{10} is an endogenously synthesized lipid-soluble anti-oxidant and protects membrane phospholipids and serum LDL-C from lipid peroxidation [10]. In the recent years, there is a growing body of evidence on protective role of Co Q_{10} in cardiovascular diseases (CVD) and cancer [11–13].

Riboflavin (vitamin B_2) a water soluble vitamin in its active coenzyme forms such as flavin mononucleotide (FMN^+) and flavin adenine dinucleotide (FAD^+), participates in redox processes involving one- and two-electron transition and in non-redox reactions such as photo-repair of thymidine dimers in photo-damaged DNA and the dehydration of non-activated organic substrates [14]. Niacin (nicotinic acid, Vitamin B_3) a water-soluble vitamin, serves as a precursor of nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Both NAD and NADP can be reduced to NADH and NADPH, respectively and these coenzymes participate in oxidation–reduction reactions catalyzed by dehyrogenase and oxidoreductase enzymes. The NAD/NADP-linked enzyme systems are involved in virtually every aspect of metabolic processes. Currently, niacin is one of the premier lipid lowering agents available to treat various CVD, with its first clinical utility, which dates back to 1954 [15]. In this context, efforts were undertaken to understand the benevolent role of Co Q_{10}, riboflavin and niacin on TAM-instigated lipid and lipoprotein abnormalities in postmenopausal breast cancer women.

Table 1
Characteristics of the patients and their disease (N=78 patients)

Characteristics		N	%
Age–year			
Mean	49 years		
Range	43–70 years		
Tumor size (T)			
$T1$		5	6.4
$T2$		31	39.7
$T3$		24	30.7
$T4$		18	23.0
Nodal status (N)			
$N0$		24	30.7
$N1$		41	52.5
$N2$		12	15.3
$N3$		1	1.2
$N4$		0	0
Metastasis (M)			
$M0$		78	100
$M1$		0	0
Clinical stage			
Stage I		5	6.4
Stage II		55	70.5
Stage III		18	23.0
Tumor histology type			
Ductal invasive		76	97.4
Lobular invasive		2	2.5
HER-2/neu			
Positive		19	24.3
Negative		59	75.6
Surgery			
Conservative		2	2.5
Mastectomy			
Simple		10	12.8
Radical		2	2.5
Modified radical		49	62.8
Patey's		9	11.5
Modified patey's		2	2.5
No surgery		4	5.1
Family history of cancer			
Yes		9	11.5
No		69	88.5
Diet			
Mixed		73	93.5
Vegetarian		5	6.5

N: number of patients; %: percentage.

Materials and method

Selection of patients

Seventy eight consecutively treated postmenopausal with resectable breast cancer were recruited from the Department of Medical Oncology, Government Royapettah Hospital, Chennai, India, through their physicians according to the process approved by the institutional human ethical review board. Informed consent was obtained from all the subjects with due explanation before entering into the study. The patient's characteristics are given in Table 1. Age: younger than 70 years with potentially curable and histopathologically confirmed breast cancer patients were recruited. Patients with diabetes mellitus, renal and hepatic diseases were excluded from the study. Patients who were on vitamin supplementation or hypolipidemic drugs were also excluded from the study. Age and socio-economically matched healthy controls were recruited from the hospital visitors, who were non-blood related to individuals with disease and who have no family history of cancer.

Blood sampling and study design

Approximately 5.0 mL E.D.T.A. blood was collected, and plasma was separated immediately by centrifugation at 3000×g for 10 min at 4 °C. Each sample was divided into suitable aliquots and processed immediately.

Blood samples were drawn from the disease-free, healthy, age-matched postmenopausal women (Group I), untreated breast cancer patients (Group II), breast cancer patients who were on tamoxifen (20 mg/day) therapy for more than a year (Group III), Group III patients after blood sample collection were recruited into Group IV and were supplemented with Co Q_{10} (100 mg), riboflavin (10 mg) and niacin (50 mg) along with tamoxifen

Table 2
Effect of coenzyme Q_{10}, niacin and riboflavin on serum lipid levels in postmenopausal breast cancer patients treated with tamoxifen

Parameters (mg/dL)	Group I control, normal age-matched, postmenopausal women (46)	Group II pretreatment untreated breast cancer women (78)	Group III treatment with tamoxifen (78)	Group IV 45 days after treatment with Co Q_{10}, riboflavin and niacin along with tamoxifen (78)	Group V 90 days after treatment with Co Q_{10}, riboflavin and niacin along with tamoxifen (78)
Total cholesterol	199.47±19	277.81±26.17^{a,***}	252.21±25.96^{b,NS,c,**}	209.50±19.97^{d,***}	202.09±19.09^{e,***,f,NS}
Free cholesterol	66.61±5.42	138.91±11.64^{a,***}	112.74±9.83^{b,**,c,***}	75.97±3.45^{d,***}	70.13±6.63^{e,***,f,NS}
Ester cholesterol	123.10±12.22	98.64±9.29^{a,**}	110.83±10.61^{b,NS,c,NS}	125.47±11.78^{d,***}	130.18±11.08^{e,***,f,NS}
Phospholipids	247.97±21.70	294.89±23.45^{a,**}	270.36±26.79^{b,NS,c,NS}	258.77±22.34^{d,**}	250.03±22.97^{e,*,f,NS}
Triglycerides	159.36±12.09	186.99±16.45^{a,**}	225.93±24.41^{b,***,c,**}	189.09±19.64^{d,***}	166.73±16.97^{e,***,f,NS}
Free fatty acids	93.11±8.81	141.39±12.44^{a,***}	129.11±12.22^{b,NS,c,***}	106.31±9.23^{d,**}	101.20±11.30^{e,***,f,NS}

Values are expressed as mean±SD. Number of subjects are indicated in parentheses.
Comparisons were made between: a—Group I and Group II; b—Group II and Group III; c—Group I and III; d—Group III and IV; e—Group III and Group V; f—Group I and V.
Statistical significance are expressed as; *$p<0.05$; **$p<0.01$; ***$p<0.001$; NS, not significant.

(20 mg/day) and blood samples were drawn the end of 45th day. Group IV (Gp) IV patients continued the same protocol and blood samples were collected at the end of 90th day (Group V).

Lipid analysis

The blood samples from all the subjects were analyzed within 3 days of sample collection using Bayer (RA-50, U.S.A.) semi-autoanalyzer. The Plasma TC was measured enzymatically at 520 nm by using a commercial kit (Agappe Diagnostics, India). Plasma TG was estimated at 520 nm by glycerol phosphate oxidase-*p*-aminophenazon (GPO-PAP) by using a commercial kit (Agappe). Plasma-free cholesterol was measured by the method of Leffler and McDougald using digitonin reagent and read at 540 nm [16]. Plasma FFA was estimated by the method of Horn and Menahan with color reagent used by Itaya [17]. Plasma PL was estimated by the method of Rouser et al. after digesting the lipid extract with perchloric acid [18]. The difference between total cholesterol and free cholesterol is the ester cholesterol content of plasma.

Lipoprotein analysis

Plasma HDL-C was determined by precipitating VLDL-C and LDL-C with heparin and manganese at pH 5.04 (Agappe). The HDL-C remaining in the supernatant was determined enzymatically as described for the measurement of the total cholesterol. Concentration of the plasma LDL-C was calculated by the standard Friedewald et al. equation [19].

Statistical analysis

The experimental data were analyzed for significant differences by independent Student's *t*-test and paired *t*-test using social sciences computer package (SPSS, version 11.0, Chicago, U.S.A.). Values were expressed as mean±standard deviation (SD). p value <0.05 was considered statistically significant.

Results

The values of plasma lipid levels are indicated in Table 2. The levels of all the lipid parameters were significantly altered in Group II ($p<0.01$), when compared to control Group I subjects. The levels of total cholesterol (TC), free fatty acids (FAA) and phospholipids (PL) were reduced in TAM-treated Group III patients as compared to Group II subjects, however, it was not statistically significant, whereas the triglycerides (TG) levels were significantly increased in the Group III subjects ($p<0.001$). After 90-day co-administration of tamoxifen with

Table 3
Effect of coenzyme Q_{10}, niacin and riboflavin on serum lipoprotein levels in postmenopausal breast cancer patients treated with tamoxifen

Parameters (mg/dL)	Group I control, normal age-matched, postmenopausal women (46)	Group II pretreatment untreated breast cancer women (78)	Group III treatment with tamoxifen (78)	Group IV 45 days after treatment with Co Q_{10}, riboflavin and niacin along with tamoxifen (78)	Group V 90 days after treatment with Co Q_{10}, riboflavin and niacin along with tamoxifen (78)
HDL-cholesterol	52.16±3.37	44.49±4.14^{a,**}	47.67±4.24^{b,NS,c,NS}	57.82±4.28^{d,***}	66.1±5.58^{e,***,f,***}
LDL-cholesterol	108.83±18.51	177.01±16.37^{a,***}	146.66±21.95^{b,**,c,NS}	111.48±10.38^{d,***}	106.59±10.55^{e,***,f,NS}
VLDL-cholesterol	38.59±3.94	55.78±4.38^{a,***}	70.12±6.57^{b,***,c,***}	40.59±3.73^{d,***}	36.08±3.20^{e,***,f,NS}

Values are expressed as mean±SD. Number of subjects are indicated in parentheses.
Comparisons were made between: a—Group I and Group II; b—Group II and Group III; c—Group I and III; d—Group III and IV; e—Group III and Group V; f—Group I and V.
Statistical significance are expressed as; *$p<0.05$; **$p<0.01$; ***$p<0.001$; NS, not significant.

Co Q_{10}, riboflavin and niacin, the TG levels were reverted back to near normal levels in Group V patients.

The levels of plasma lipoproteins are indicated in Table 3. The plasma HDL-C levels were significantly reduced in untreated Group II subjects ($p < 0.01$) as compared to normal Group I subjects. The plasma LDL-C and VLDL-C values were significantly elevated in Group II ($p < 0.001$) as compared to Group I patients. The VLDL-C levels were found to be significantly raised in the TAM-treated Group III ($p < 0.001$), when compared to untreated Group II patients. After combinatorial therapy, the VLDL-C levels were reverted back to near normal levels with no significant difference between Group IV and Group V subjects.

Discussion

In the last 30 years, TAM has been shown to be effective not just as an adjuvant therapy after surgery, chemo or radiation therapy in early and advanced breast cancer cases, but also in chemoprevention in high-risk pre- and postmenopausal women. Some results of aromatase (enzyme responsible for synthesis of estrogen) inhibitors have been shown to be encouraging in postmenopausal women; however, they have contraindicated results in premenopausal women with functioning ovaries [20]. These findings are preliminary with a median follow-up of only 31 months and no survival data are available. Until further data emerge, adjuvant TAM therapy would remain the standard for most of the breast cancer patients. The NSABP P-I study [21], the International Breast Intervention Study I (IBISI) [22], the Italian Breast Cancer Prevention Study [23] and the Royal Marsden Hospital Breast Cancer Prevention Trial [24] were some of the major trials conducted with TAM. The meta-analysis of the data from the above four studies by Cuzick et al., together with the Oxford overview of contralateral breast cancer reduction by adjuvant TAM, revealed a net 38% reduction in invasive and pre-invasive cancers [25].

Abnormal lipid synthesis or defective degradation of lipids is implicated in pathological conditions like diabetes, atherosclerosis and cancer [26–28]. The observed increase in plasma lipid in breast cancer patients may be due to an altered lipid metabolism associated with the disease [7]. The exact mechanism by which lipid and lipoprotein contribute to carcinogenesis is unclear. However, reports suggest that lipid peroxidation products, malondialdehyde may cross-link proteins and DNA on the same and opposite strands [29]. An earlier study reported that lipid might primarily affect gonads and subsequently higher estradiol secretion could influence the development of malignancies in the mammary glands and lymphoid system [30].

TAM has been shown to inhibit cholesterol biosynthesis [31], to inhibit acyl-CoA:cholesterol acyl-transferase, to interact with P-glycoprotein, a protein involved in intracellular cholesterol trafficking and to protect LDL-C from peroxidation, a mechanism that could account for its cardioprotective action [32]. In the current study too, a similar kind of hypolididemic action of TAM with respect to TC and LDL-C was observed. Despite TAM's favorable impact on overall lipid profiles, these benefit do not translate into a cardioprotective effect, as there has been a severe hypertriglyceridemia with high VLDL-C in the TAM-treated group (Gp III), a known independent risk factor for CVD [33].

In the current study, it has been observed that the combination therapy reduces the TG and VLDL-C levels. Our results with the above combinatorial therapy with vitamin on serum lipids and lipoproteins are in line with the recent observation of Ayfer Yalcin et al. [11]. Co Q_{10} is the only endogenously synthesized lipid-soluble antioxidant and protects membrane phospholipids and serum LDL-C from lipid peroxidation. Two different mechanisms of Co Q_{10} antioxidant function have been postulated: (1) it may act independently as a chain-breaking antioxidant, providing hydrogen atoms to reduce peroxyl and/or alkoxyl radical [34], or (2) it may form a redox interaction with other lipid-soluble antioxidant such as vitamin E by regenerating it from its phenoxyl radical form [35], which increases the LPL activity. The raised LPL activity causes further increase in VLDL-C and IDL (intermediate density lipoprotein) catabolism, thereby decreasing TG and VLDL-C cholesterol levels. Recent experiments have shown Co Q_{10} anti-atherogenetic mechanism by improvement in the endothelial function of conduit arteries [36] and upregulation of ubiquitin mRNA expression in heart muscles [37], and FAD, which serves as a coenzyme for glutathione reductase and other enzymes. Glutathione reductase mediates the regeneration of reduced glutathione, which plays an important role in scavenging free radicals and reactive oxygen species [38]. Riboflavin may exert its hypolididemic activity indirectly by preventing lipid peroxidation with reduced glutathione [39]. Riboflavin is also essential for synthesis of other B vitamins and coenzymes especially niacin and pyridoxine from tryptophan. Riboflavin along with other B vitamins and folic acid checks hyperhomocysteinemia, which is another risk factor for CVD [40,41].

There has been a significant increase in the serum HDL-C levels in the vitamin co-administered group (Gp IV and V) when compared to the sole tamoxifen-treated group (Gp III) or the untreated group (Gp II), which may be due to the modulating hypolipidemic action of niacin, as it increases the HDL-C levels and at the same time decreases LDL-C and TG levels. The rise in the HDL-C (known as good cholesterol) initiates cholesterol efflux and facilitates the removal of excess cholesterol from the arteries and delivers it to the liver for removal through reverse cholesterol transport pathway [42]. Niacin lowers triglycerides and apolipoprotein-B containing lipoproteins (e.g., VLDL-C and LDL-C) mainly by decreasing fatty acid mobilization from adipose tissue triglycerides stores and by inhibiting hepatocyte diacylglycerol acyltransferase and triglycerides synthesis leading to increased intracellular apo B degradation and subsequent decreased secretion of VLDL-C and LDL-C particles [43]. The mechanism by which niacin raises HDL-C is by decreasing the fractional catabolic rate of HDL-apo AI without affecting the synthetic rates [15,44]. The extended presence of HDL-C on combinatorial therapy enhances fibrinolysis, inhibits platelet aggregation, inhibits LDL-C oxidation, inhibits proinflamatory cytokine overexpression and acts as an anti-oxidant in association with paraoxonase and platelet activation factor acetyl hydrolase [44]. Recent in vitro studies using Hep G2 cells have

shown that niacin selectively inhibits the uptake/removal of HDL-apo AI (but not HDL-cholesterol ester) by hepatocyte, thereby increasing the capacity of retained HDL-apo AI to augment cholesterol effux through reverse cholesterol pathway [45].

In conclusion, a significant increase in triglycerides and VLDL-C has been observed in the TAM-treated breast cancer patients, with complete lipid and lipoprotein derangements in untreated breast cancer patients. TAM on co-administration with Co Q_{10}, riboflavin and niacin, significantly decreased the TAM-induced lipid abnormalities.

Acknowledgments

The authors whole-heartedly thank Kaneka Corp., Japan, for its philanthropic gift of coenzyme Q_{10} samples and Madras Pharmaceuticals for the niacin and riboflavin samples. The technical expertise provided by Dr. K. Rengaswamy and Dr. AL. Arun Kumar of Appaswamy Hospital and Isotope Diagnostic Center, Chennai, is greatly acknowledged.

References

[1] McPherson K, Steel CM, Dixon JM. ABC of the breast diseases, breast cancer epidemiology, risk factors and genetics. BMJ 2000;321:624–8.
[2] Clemons M, Danson S, Howell A. Tamoxifen ('Nolvadex'); a review. Cancer Treat Rev 2002;28:165–80.
[3] Rutqvist LE, Johansson H, Signomklao T, Johansson U, Fornande T, Wilking N. Adjuvant tamoxifen therapy for early stage breast cancer and second primary malignancies. J Natl Cancer Inst 1995;87:645–51.
[4] Narod SA, Brunet J-S, Ghadirian P, et al. Tamoxifen and risk of contralateral breast cancer in *BRCA1* and *BRCA2* mutation carriers: a case-control study. Lancet 2001;356:1876–81.
[5] Fossati R, Confalonieri C, Torri V, et al. Cytotoxic and hormonal treatment for metastatic breast cancer: a systematic review of published randomized trials involving 31,510 women. J Clin Oncol 1998;16:3439–60.
[6] Howell A, Osborne CK, Morris C, Wakeling AE. ICI 182,780 (Faslodex™). Development of novel, "pure" antiestrogen. Cancer 2000;89:817–25.
[7] Thangaraju M, Kumar K, Gandhirajan R, Sachdanandam P. Effect of tamoxifen on plasma lipids and lipoproteins in post-menopausal women with breast cancer. Cancer 1994;73:653–63.
[8] Liu CL, Yang TL. Sequential changes in serum triglycerides levels during adjuvant tamoxifen therapy in breast cancer patients and the effect of dose reduction. Breast Cancer Res Treat 2003;79:11–6.
[9] Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. Am J Cardiol 1998;81:7B–12B.
[10] Nohl H, Gille L, Kozlou AV. Critical aspects of the antioxidant function of coenzyme Q in biomembranes. BioFactors 1999;9:155–61.
[11] Yalcin A, Kilinc E, Sagcan A, Kultursay H. Coenzyme Q_{10} concentration in coronary artery disease. Clin Biochem 2004;37:706–9.
[12] Bhagavan HN, Chopra RK. Potential role of ubiquinone (coenzyme Q_{10}) in pediatric cardiomyopathy. Clin Nutr 2005;24:331–8.
[13] Perumal SS, Shanthi P, Sachdanandam P. Augmented efficacy of tamoxifen in rat breast tumorigenesis when gavaged along with riboflavin, niacin and Co Q_{10} : effects on lipidperoxidation and antioxidants in mitochondria. Chem-Biol Interact 2005;152:49–58.
[14] Imada Y, Iida H, Ono S, Murahashi S. Flavin catalyzed oxidation of sulfides and amines with molecular oxygen. J Am Chem Soc 2003;125: 2868–69.
[15] Ganji SH, Kamanna VS, Kashyap ML. Niacin and cholesterol: role in cardiovascular disease (Review). J Nutr Biochem 2003;14:298–305.
[16] Leffer HH, McDougald CH. Colorimetric method for the estimation of cholesterol. Am J Clin Pathol 1963;39:311–3.
[17] Horn WT, Menahan LA. A sensitive method for the determination of free fatty acids in plasma. J Lipid Res 1981;22:377–81.
[18] Rouser G, Fleisher S, Yamanoro A. Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spits. Lipids 1970;5:494–6.
[19] Friedald WT, Levy RI, Fredriekson DS. Estimation of concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1976;18:499–502.
[20] ATAC Trialists Group. Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomized trial. Lancet 2002;359:2131–9.
[21] Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-I. J Natl Cancer Inst 1998;90:1371–88.
[22] IBIS Investigators Writing Committee. First results from the International Breast Cancer Intervention Study (IBIS-I): a randomized prevention trial. Lancet 2002;360:817–24.
[23] Veronesi U, Maisonneuve P, Sacchini V, et al. Tamoxifen for breast cancer among hysterectomised women. Lancet 2002;359:1122–4.
[24] Powles T, Eeles R, Ashley S, et al. Interim analysis of the incidence of breast cancer in the Royal Marsden Hospital tamoxifen randomized chemoprevention trial. Lancet 1998;352:98–101.
[25] Cuzick J, Powles T, Veronesi U, et al. Overview of the main outcomes in breast-cancer prevention trials. Lancet 2003;361:296–300.
[26] Stolar MW. Atherosclerosis in diabetes: a role of hyperinsulinemia. Metab Clin Exp 1988;37:1.
[27] Carloson LA, Bottiger LE. Ischemic heart disease in relation to fasting values of plasma TG and cholesterol. Cancer 1972;1:865–8.
[28] Kumar K, Arivazhagan R, Sachdanandam P. Studies on the changes in the plasma lipids and lipoprotein in patients with benign and malignant breast cancer. Biochem Int 1991;23:581–9.
[29] Summerfield FW, Tappel AL. Determination of fluorescence quenching of the environment of DNA cross-links made by malondialdehyde. Biochem Biophys Acta 1983;740:276–82.
[30] Stepsenwol J. Carcinogenic effect of cholesterol in mice. Proc Soc Exp Biol Med 1966;121:168–71.
[31] Holleran AL, Lindenthal B, Aldaghlas TA, Kelleher JK. Efect of tamoxifen on cholesterol synthesis in HepG2 cells and cultured rat hepatocytes. Metabolism 1998;47:1504–13.
[32] Callaghan R, Higgins CF. Interaction of tamoxifen with multidrug resistance P-glycoprotein. Br J Cancer 1995;71:294–9.
[33] Assmann G, Schulte H, Funke H, Von Eckardstein A. The emergence of triglycerides as a significant independent risk factor in coronary artery disease. Eur Heart J 1998;19(Suppl M):M8–M14.
[34] James AM, Robin Smith AJ, Murphy PM. Antioxidant and prooxidant properties of mitochondrial coenzyme Q. Arch Biochem Biophys 2004;423: 47–56.
[35] Kagan V, Serbinov E, Packer L. Antioxidant effects of ubiquiones in microsomes and mitochondria are mediated by tocopherol recycling. Biochem Biophys Res Commun 1990;169(3):851–7.
[36] Watts GF, Playford DA, Croft DK, et al. Coenzyme Q(10) improves endothelial dysfunction of the brachial artery in Type II diabetes mellitus. Diabetologia 2002;45:420–6.
[37] Maulik N, Yoshida T, Engelman RM, et al. Dietary coenzyme Q(10) supplement renders swine hearts resistant to ischemia-reperfusion injury. Am J Physiol: Heart Circ Physiol 2000;278:1084–90.
[38] Rivlin RS, Riboflavin. In: Browman BA, Russell RM, editors. Present knowledge in nutrition. Washington, DC: ILSI Press; 2001. p. 191–8.
[39] Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr 2004;134:489–92.
[40] Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 1991;324: 1149–55.
[41] Hustad S, et al. Riboflavin as determinant of plasma total homocysteine: effect modification by the methylenetetrahydrofolate reductase C677T polymorphism. Clin Chem 2000;46(8 Part 1):1065–71.
[42] Kashyap ML. Mechanistic studies of high density lipoprotein. Am J Cardiol 1998;82:42U–8U.

[43] Carlson LA, Oro L, Ostman J. Effect of single dose of nicotinic acid on plasma lipids in patients with hyperlipoproteinemia. Acta Med Scand 1968;183:457–65.
[44] Jin FY, Kamanna VS, Kashyap ML. Niacin decreases removal of high density lipoprotein A-I but not cholesterol ester by Hep G2 cells. Implications for reverse cholesterol transport. Arterioscler Thromb Vasc Biol 1997;17:2020–8.
[45] Jin FY, Kamanna VS, Kashyap ML. Niacin accelerates intracellular apo B degradation by inhibiting triacylglycerol synthesis in human hepatoblastoma (Hep G2) cells. Arterioscler Thromb Vasc Biol 1999;19:1051–9.