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Association of serum complement (C3, C4) and immunoglobulin (IgG, IgM) levels with hormone replacement therapy in healthy post-menopausal women

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BACKGROUND: Recently published data suggest that hormone replacement therapy (HRT) may increase cardiovascular risk during the early months of therapy. Activation of the immune system is known to be involved in several types of cardiovascular disease. In this cross-sectional study, serum C3, C4, IgG and IgM levels were evaluated in healthy post-menopausal women receiving two different short-term HRT regimens, and in untreated women. METHODS: Serum C3, C4, IgM and IgG levels were assessed in 18 women receiving transdermal 17β-estradiol (50 μg/day) + continuous oral medroxyprogesterone acetate (MPA; 2.5 mg/day), in 56 women taking oral conjugated equine estrogen (CEE; 0.625 mg/day) + continuous MPA, and in 80 control women not receiving HRT. RESULTS: The mean serum C3 level was significantly higher in women using oral CEE + MPA than in women receiving transdermal 17β-estradiol + MPA, and those not on HRT ($P = 0.02$ and $P < 0.001$ respectively). Furthermore, women taking oral CEE + MPA had significantly higher mean levels of C4 compared with untreated women ($P < 0.01$). IgG and IgM levels were similar among women either of the two HRT regimens and between women not on HRT. CONCLUSIONS: Oral HRT may be involved in the development of cardiovascular disease through inflammatory mechanisms, as suggested by increased serum levels of C3 and C4.

Key words: C3/C4/hormone replacement therapy/immunoglobulin G/immunoglobulin M

Introduction

Hormone replacement therapy (HRT) relieves menopausal symptoms such as hot flashes and vaginal atrophy, and also prevents osteoporosis in post-menopausal women (Yasui *et al*., 2000). Numerous epidemiological studies have suggested that HRT reduces the risk of cardiovascular disease in post-menopausal women (Grodstein *et al*., 1997; Barret-Connor and Grady, 1998). In contrast, a pooled analysis of short-term randomized clinical trials indicated adverse effects, with a relative risk of 1.39 for cardiovascular events (Hemminki and McPherson, 1997). The recently published Women's Health Initiative (WHI) study showed that the proportion of women experiencing coronary heart diseases was increased by 29% for women taking estrogen plus progestin relative to placebo (Rossouw *et al*., 2002). The mechanism responsible for the increased cardiovascular risk with HRT use is unclear, but it has been hypothesized that this could be mediated in part through an effect on the inflammatory system, which is intensely involved in cardiovascular disease (Ross, 1999).

The complement system and immunoglobulins are the main components of humoral immunity. The activation of complement is known to be involved in a number of forms of cardiovascular disease. The results of some clinical studies have suggested that complement activation exacerbates myocardial defect following ischaemic injury (Gardinali *et al*., 1995), is involved in the generation of spontaneous atherosclerotic lesions (Seifert and Kazatchkine, 1988), and may indeed be an initiating factor in lesion formation (Torzewski *et al*., 1996). C3, the third complement component, is a cytokine and is produced by activated macrophages (Zimmer *et al*., 1982), which are the cells mainly concerned with the development of atherosclerotic plaques (Libby, 1995). Previous studies have indicated that serum C3 is a powerful indicator of the risk of myocardial infarction (Muscari *et al*., 1995; 1998; 2000). Furthermore, there is some evidence suggesting that patients with established atherosclerosis have elevated levels of IgA, IgE, IgG and IgM (Criqui *et al*., 1987; Muscari *et al*., 1988). Moreover, elevated levels of IgA, IgE

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Table I. General characteristics of patients in the control and study groups

Characteristic	Control group (n = 80)	Transdermal 17β-E_2 + MPA (n = 18)	Oral CEE + MPA (n = 56)
Age (years)	49.9 ± 4.9 (41–62)	51.9 ± 5.1 (46–62)	48.6 ± 4.7 (41–61)[a]
Duration of amenorrhoea (years)	4.1 ± 4.5 (1–20)	4.8 ± 5.3 (1–18)	3.2 ± 3.6 (1–20)
Duration of HRT use (months)	None	4.8 ± 2.5 (3–9)	5.8 ± 2.7 (3–9)
BMI (kg/m^2)	27.6 ± 3.5 (22.4–32.6)	27.6 ± 3.8 (22.6–31.5)	26.8 ± 2.8 (22.8–33.4)
FSH (mIU/ml)	64.5 ± 25.6 (31–109)	52.5 ± 20.6 (22–87)	34.7 ± 18.0 (13–90)[b]
Estradiol (pg/ml)	19.7 ± 10.8 (0–30)	50.2 ± 24.2 (21–108)[c]	64.3 ± 33.7 (12–122)[d]

Values are mean ± SD (range).
[a]$P < 0.05$, difference between women receiving CEE + MPA and women receiving 17β-E_2 + MPA.
[b]$P < 0.05$, versus 17β-E_2 + MPA group and control group.
[c]$P < 0.01$, versus control group.
[d]$P < 0.001$, versus control group.
BMI = body-mass index; CEE = conjugated equine estrogen; HRT = hormone replacement therapy; MPA = medroxyprogesterone acetate; 17β-E_2 = 17β-estradiol.

and IgG are predictive of myocardial infarction (Kovanen *et al*., 1998).

In the present study, serum concentrations of complement (C3, C4), and immunoglobulin (IgM, IgG) were investigated in women treated with two different short-term HRT regimens for menopausal hormone deficiency symptoms, and in untreated women. To the best of the present authors' knowledge, this is the first study to determine the impact of HRT on serum complement (C3, C4) and immunoglobulin (IgM, IgG) levels.

Materials and methods

A total of 154 healthy post-menopausal patients aged between 41 and 62 years was enrolled in this cross-sectional study between October 2000 and March 2002. All the participating post-menopausal women were normotensive (blood pressure <140/90 mmHg), non-hysterectomized, and had been menopausal for at least 12 months before entering the study. Informed consent was obtained from all women before participating in the study.

Women with a history of cardiovascular, rheumatismal, autoimmune and malignant diseases, with acute or chronic infection, and those with a clinically relevant abnormality in laboratory tests of haematological, renal and hepatic functions were excluded from participation. Patients with a body mass index (BMI) >35 kg/m^2, caffeine consumption in excess of the equivalent of three cups of coffee per day, or who had been operated on within the past 6 months were also excluded. Additional exclusions included those women who had smoked cigarettes within the past year, had a medical history suggesting a possible contraindication to hormone use, a history of corticosteroid or any immunosuppressor drug use during the previous month. Those women with a plasma triglyceride level >200 mg/dl and total cholesterol level >240 mg/dl were also excluded.

In order to investigate the impact of short-term HRT use on the immune system, patients receiving oral or transdermal HRT for a duration of 3–9 months were involved in the study. For the analyses, 74 women receiving HRT were divided into two groups: group 1 (n = 18) received transdermal 17β estradiol (17β-E_2; 50 µg/day) plus continuous medroxyprogesterone acetate (MPA; 2.5 mg/day), while group 2 (n = 56) received oral conjugated equine estrogen (CEE; 0.625 mg/day) plus continuous MPA (2.5 mg/day). The control group comprised 80 healthy post-menopausal women who had not taken any HRT regimen within the previous 12 months, with a serum FSH level >30 mIU/ml, a serum estradiol level <30 pg/ml, and had been amenorrhoeic for at least 12 months. Women who had used HRT or any other hormonal therapy within the previous 12 months were excluded from the control group.

Plasma complement (C3, C4), immunoglobulin (IgM, IgG) and hormone status were evaluated in all participants, and results were compared between control and study groups, as well as among women receiving different HRT regimens.

Blood samples were taken after a fasting period of 10 h in the study and control groups. All laboratory parameters were measured as soon as blood samples were taken. Serum C3, C4, IgG, IgM levels were measured using turbidimetry kits (Biosystem, USA) with an automatic analyser (SPACE, Schiapparelli Biosystem, Inc., USA). This analytical method was applied according to the manufacturer's instructions. Normal ranges (provided by the manufacturers) for C3, C4, IgG and IgM were: 1.0–1.85 g/l, 0.2–0.45 g/l, 8.4–16.6 g/l and 0.5–2.2 g/l respectively. Daily calibrations were carried out for all measurements made during the study.

Statistical analysis

As many variables had a non-Gaussian distribution with positive skewness, statistical analysis was performed with non-parametric tests: Kruskal-Wallis and Mann–Whitney U-tests. The data were expressed as mean ± SD (range). Correlations between variables were calculated with Spearman's correlation coefficient. Statistical significance was set at $P < 0.05$. Data were collected in a Windows-based database (Microsoft Excel 2000) and analysed with the SPSS (Statistical Package for the Social Science, version 11.0) for Windows 98 (Microsoft Corp.).

Results

The general characteristics of the study and control groups are summarized in Table I. The mean age of patients was similar in the transdermal and control groups, and in the oral group and untreated women. However, CEE + MPA users were younger than transdermal 17β-E_2 + MPA users ($P < 0.05$). Women with or without HRT were similar with respect to duration of amenorrhoea and BMI. Mean FSH levels were lower in the oral CEE + MPA group compared with the transdermal 17β-E_2 + MPA group and those without treatment ($P < 0.05$). Mean estradiol levels were similar in the oral and transdermal groups.

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Table II. Immunological variables of patients in control and study groups

Immunological variable	Control group (n = 80)	Transdermal 17β-E_2 + MPA (n = 18)	Oral CEE + MPA (n = 56)
C3 (g/l)	1.34 ± 0.45 (0.47–2.35)	1.44 ± 0.42 (0.73–2.09)	1.71 ± 0.44 (0.81–2.76)[a,b]
C4 (g/l)	0.31 ± 0.15 (0.11–1.15)	0.34 ± 0.11 (0.15–0.50)	0.42 ± 0.29 (0.15–2.00)[c]
IgG (g/l)	11.67 ± 2.69 (5.88–18.70)	11.59 ± 2.75 (6.58–16.70)	12.28 ± 2.26 (7.04–17.70)
IgM (g/l)	1.07 ± 0.48 (0.27–2.49)	0.91 ± 0.34 (0.50–1.56)	1.12 ± 0.88 (0.25–4.78)

Values are mean ± SD (range).
[a]$P < 0.001$, versus control group.
[b]$P = 0.02$, versus 17β-E_2 + MPA group.
[c]$P < 0.01$, versus control group.
CEE = conjugated equine estrogen; MPA = medroxyprogesterone acetate; 17β-E_2 = 17β-estradiol.

When both study groups were compared with the control group, women receiving transdermal 17β-E_2 + MPA and without HRT had similar serum levels of C3 and C4, whereas women taking oral CEE + MPA had significantly higher mean values of C3 and C4 compared with controls (for C3, $P < 0.001$; for C4, $P < 0.01$; Table II). When the impact of HRT on complement levels with respect to the route of administration was compared, serum C3 levels were significantly higher in the oral group than the transdermal group ($P = 0.02$). Serum levels of C4 were higher in the CEE + MPA group than in the 17β-E_2 + MPA group, but not significantly so ($P = 0.229$) (Table II). No significant change was observed in any of the serum immunoglobulins (IgG or IgM) with HRT use, irrespective of the route of administration. The serum levels of immunoglobulins were also similar among HRT users and non-users (Table II).

Serum C3 and C4 concentrations were positively and strongly correlated ($r > 0.6$, $P < 0.0001$ in all groups). Finally, no significant correlation of C3 and C4 with estradiol, FSH, IgG and IgM levels was detected in any of the groups.

Discussion

The cross-sectional data obtained from the present studies indicated that plasma concentrations of C3 were significantly higher in healthy post-menopausal women using CEE + MPA than in similar women using transdermal 17β-E_2 + MPA and untreated post-menopausal women. In addition, serum levels of C4 were higher in women receiving CEE + MPA than in similar women not on HRT. No difference was detected in IgG and IgM levels between women taking any HRT regimen and women without HRT.

There is increasing evidence that post-menopausal HRT increases cardiovascular risk (Hemminki and McPherson, 1997; Rossouw *et al*., 2002). In the Heart and Estrogen/progestin Replacement Study (HERS), HRT appeared to increase the risk of new cardiovascular events in the first year, despite favourable effects on lipids (Hulley *et al*., 1998). Although the association between an increased cardiovascular risk and the administration of HRT has not yet been clarified, it was suggested that this might be mediated in part through an effect on the inflammatory system (Ross, 1999).

C3 is a cytokine and an acute-phase reactant protein produced by activated macrophages (Zimmer *et al*., 1982), liver and adipose tissue (Alper *et al*., 1969; Choy *et al*., 1992). C3 and its fragments are recognized regulators of the humoral immune response and B-cell proliferation (Lenhardt and Melchers, 1988). C3 complement is also heavily involved in the control of lipid and glucose metabolism through its fragment C3a-des-Arg (Baldo *et al*., 1993). In addition, serum C3 levels are a potent indicator of the risk of myocardial infarction in men (Muscari *et al*., 1995) and, together with C4 levels, have been found to be elevated in patients with severe angiographically assessed atherosclerosis who had already been affected by multiple ischaemic events (Muscari *et al*., 1988). A significant prevalence of the 'fast' allele of C3 has been demonstrated in atherosclerotic patients with respect to controls (Kristensen and Bruun Petersen, 1978). Furthermore, increasing amounts of data indicate that serum C3 and C4 levels strongly correlate with several cardiovascular risk factors such as age, triglycerides, blood glucose and systolic blood pressure (Muscari *et al*., 1988; 1995; 1998; 2000). Finally, complement activation is probably involved in the generation of spontaneous atherosclerotic lesions (Rus *et al*., 1986; Niculescu *et al*., 1987; Seifert and Kazatchkine, 1988; Seifert *et al*., 1989). Activated complement has cell-damaging and chemotactic properties (Marder *et al*., 1985), and therefore might support the development of intimal lesion or monocyte recruitment at the site of atheroma formation (Muscari *et al*., 1995).

From this perspective, the following two questions arise. First, might increased cardiovascular risk—as was demonstrated to emerge during the early period of HRT use in the HERS trial (Hulley *et al*., 1998)—be associated with the complement system? Second, in what way might the use of HRT affect the complement system? Limited data were available from the literature concerning the association of serum complement levels with HRT. However, HRT in the form of unopposed oral estrogen, or combined estrogen and progestin, increases serum C-reactive protein (CRP) concentrations in post-menopausal women (Cushman *et al*., 1999a;b; Ridker *et al*., 1999; Van Baal *et al*., 1999; Walsh *et al*., 2000; Luyer *et al*., 2001; Garnero *et al*., 2002). As a result of oral HRT use, hepatic synthesis of CRP increases (Van Baal *et al*., 1999; Herrington *et al*., 2001). Thus, serum levels of C3—which is an acute-phase reactant synthesized by the liver as CRP (Alper *et al*., 1969)—might also be raised following increased synthesis in liver due to the first-pass effect of oral

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HRT. Accordingly, no significant difference could be found between women receiving transdermal HRT and untreated women with respect to serum levels of C3 and C4. In transdermal HRT, hormones enter the systemic circulation without a hepatic first pass and, for this reason, serum C3 and C4 levels might not be affected.

The association of total immunoglobulins with cardiovascular disease remains the subject of debate. It has been proposed that serum IgA, IgG and IgE may be associated with cardiovascular disease in dyslipidemic men (Kovanen *et al*., 1998), but others (Muscari *et al*., 1988; 1995; 1999) have suggested that the increase in IgM and IgG levels might be a consequence of the primary involvement of C3, as there was no independent association of IgM and IgG with atherosclerosis and myocardial infarction. In agreement with previous findings (Bukh *et al*., 1987), in the present study no significant difference was found in IgG and IgM levels among women receiving oral or transdermal HRT, or in women not taking HRT.

The present study had certain limitations, the first being that the groups included only healthy women with a low risk of cardiovascular disease, and this may have affected the results. Second, the study was non-randomized, non-placebo-controlled and cross-sectional in design. Third, all of the participants enrolled had an intact uterus, and in order to protect the endometrium a combined HRT was used. The sole effect of progestins on the complement system or immunoglobulins is not known, but it may preclude the drawing of definitive conclusions regarding the effects of estrogen alone.

In summary, oral HRT use may cause significant changes in the serum levels of C3 and C4, which in turn may be associated with an increased risk of cardiovascular events during the early stages of treatment, as in the HERS trial. However, these are preliminary findings, and additional information must be derived from large, controlled studies before any clear-cut conclusions may be drawn.

References

Alper, C.A., Johnson, A.M., Birtch, A.G. and Moore, F.D. (1969) Human C′3: evidence for the liver as the primary site of synthesis. *Science*, **163**, 286–288.

Baldo, A., Sniderman, A.D., St-Luce, S., Avramoglu, R.K., Maslowska, M., Hoang, B., Monge, J.C., Bell, A., Mulay, S. and Cianflone, K. (1993) The adipsin-acylation stimulating protein system and regulation of intracellular triglyceride synthesis. *J. Clin. Invest.*, **92**, 1543–1547.

Barret-Connor, E. and Grady, D. (1998) Hormone replacement therapy, heart disease, and other considerations. *Annu. Rev. Public Health*, **19**, 55–72.

Bukh, A., Jensen, H.K., Andersen, H.J., Eriksen, P.B. and Moller, P.H. (1987) Search for circulating immune complexes and activation of the complement system in relation to estrogen treatment. *Acta Obstet. Gynecol. Scand.*, **66**, 703–708.

Choy, L.N., Rosen, B.S. and Spiegelman, B.M. (1992) Adipsin and an endogenous pathway of complement from adipose cells. *J. Biol. Chem.*, **267**, 12736–12741.

Criqui, M.H., Lee, E.R., Hamburger, R.N., Klauber, M.R. and Coughlin, S.S. (1987) IgE and cardiovascular disease: results from a population-based study. *Am. J. Med.*, **82**, 964–968.

Cushman, M., Legault, C., Barrett-Connor, E., Stefanick, M.L., Kessler, C., Judd, H.L., Sakkinen, P.A. and Tracy, R.P. (1999a) Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) study. *Circulation*, **100**, 717–722.

Cushman, M., Meilahn, E.N., Psaty, B.M., Kuller, L.H., Dobs, A.S. and Tracy, R.P. (1999b) Hormone replacement therapy, inflammation and hemostasis in elderly women. *Arterioscler. Thromb. Vasc. Biol.*, **19**, 893–899.

Gardinali, M., Conciato, L., Cafaro, C. and Agostino, A. (1995) Complement system in coronary heart disease: a review. *Immunopharmacology*, **30**, 105–117.

Garnero, P., Jamin, C., Benhamou, C.L., Pelissier, C. and Roux, C. (2002) Effects of tibolone and combined 17β-estradiol and norethisterone acetate on serum C-reactive protein in healthy post-menopausal women: a randomised trial. *Hum. Reprod.*, **17**, 2748–2753.

Grodstein, F., Stampfer, M.J., Colditz, G.A., Willet, W.C., Manson, J.E., Joffe, M., Rosner, B., Fuchs, C., Hankinson, S.E., Hunter, D.J. *et al*. (1997) Postmenopausal hormone therapy and mortality. *N. Engl. J. Med.*, **336**, 1769–1775.

Hemminki, E. and McPherson, K. (1997) Impact of postmenopausal hormone therapy on cardiovascular events and cancer: pooled data from clinical trials. *Br. Med. J.*, **315**, 149–153.

Herrington, D.M., Brosnihan, K.B., Pusser, B.E., Seely, E.W., Ridker, P.M., Rifai, N. and MacLean, D.B. (2001) Differential effects of E and droloxifene on C-reactive protein and other markers of inflammation in healthy postmenopausal women. *J. Clin. Endocrinol. Metab.*, **86**, 4216–4222.

Hulley, S., Grady, D., Bush, T., Furberg, C., Herrington, D., Riggs, B. and Vittinghoff, E. (1998) Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) research group. *JAMA*, **280**, 605–613.

Kovanen, P.T., Manttari, M., Palosuo, T., Manninen, V. and Aho, K. (1998) Prediction of myocardial infarction in dislipidemic men by elevated levels of immunoglobulin classes A, E, and G, but not M. *Arch. Intern. Med.*, **158**, 1434–1439.

Kristensen, B.O. and Bruun Petersen, G. (1978) Association between coronary heart disease and the C3F-gene in essential hypertension. *Circulation*, **58**, 622.

Lenhardt, W. and Melchers, F. (1988) The role of C3 and its fragments in the control of S phase entry of activated mouse B lymphocytes via the complement receptor type 2. *Exp. Clin. Immunogenet.*, **5**, 115–122.

Libby, P. (1995) Molecular bases of the acute coronary syndromes. *Circulation*, **91**, 2844–2850.

Luyer, M.D.P., Khosla, S., Owen, W.G. and Miller, V.M. (2001) Prospective randomised study of effects of unopposed estrogen replacement therapy on markers of coagulation and inflammation in postmenopausal women. *J. Clin. Endocrinol. Metab.*, **86**, 3629–3634.

Marder, S.R., Chenoweth, D.N., Goldstein, I.M. and Perez, H.D. (1985) Chemotactic responses of human peripheral blood monocytes to the complement-derived peptides C5a and C5a desarg. *J. Immunol.*, **134**, 3325–3331.

Muscari, A. and Puddu, P. (1999) Immunoglobulins, C3, and the risk of myocardial infarction. *Arch. Intern. Med.*, **159**, 2364.

Muscari, A., Bozzoli, C., Gerratana, C., Zaca, F., Rovinetti, C., Zauli, D., La Placa, M. and Puddu, P. (1988) Association of serum IgA and C4 with severe atherosclerosis. *Atherosclerosis*, **74**, 179–186.

Muscari, A., Bozzoli, C., Puddu, G.M., Sangiorgi, Z., Dormi, A., Rovinetta, C., Descovich, G.C. and Puddu, P. (1995) Association of serum C3 levels with the risk of myocardial infarction. *Am. J. Med.*, **98**, 357–364.

Muscari, A., Massarelli, G., Bastagli, L., Poggiopollini, G., Tomassetti, V., Volta, U., Puddu, G.M. and Puddu, P. (1998) Relationship between serum C3 levels and traditional risk factors for myocardial infarction. *Acta Cardiol.*, **53**, 345–354.

Muscari, A., Massarelli, G., Bastagli, L., Poggiopollini, G., Tomassetti, V., Drago, G., Martignani, C., Pacilli, P., Boni, P. and Puddu, P. (2000) Relationship of serum C3 to fasting insulin, risk factors and previous ischaemic events in middle-aged men. *Eur. Heart J.*, **21**, 1081–1090.

Niculescu, F., Hugo, F., Rus, H.G., Vilaicu, R. and Bhakdi, S. (1987) Quantitative evaluation of the terminal C5b-9 complement complex by ELISA in human atherosclerotic arteries. *Clin. Exp. Immunol.*, **69**, 477–483.

Ridker, P.M., Hennekens, C.H., Rifai, N., Buring, J.E. and Manson, J.E. (1999) Hormone replacement therapy and increased plasma concentration of C-reactive protein. *Circulation*, **100**, 713–716.

Ross, R. (1999) Atherosclerosis an inflammatory disease. *N. Engl. J. Med.*, **340**, 115–126.

Rossouw, J.E., Anderson, G.L., Prentice, R.L., La Croix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A., Howard, B.V., Johnson, K.C. *et al*. (2002) Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health

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Initiative randomized controlled trial. Writing Group for the Women's Health Initiative Investigators. *JAMA*, **288**, 321–333.

Rus, H.G., Niculescu, F., Constantinescu, E., Cristea, A. and Vlaicu, R. (1986) Immunoelectron-microscopic localization of the terminal C5b-9 complement complex in human atherosclerotic fibrous plaque. *Atherosclerosis*, **61**, 35–42.

Seifert, P.S. and Kazatchkine, M.D. (1988) The complement system in atherosclerosis. *Atherosclerosis*, **73**, 91–104.

Seifert, P.S., Hugo, F., Hansson, G.K. and Bhakdi, S. (1989) Prelesional complement activation in experimental atherosclerosis. Terminal C5b-9 complement deposition coincides with cholesterol accumulation in the aortic intima of hypercholesterolemic rabbits. *Lab. Invest.*, **60**, 747–754.

Torzewski, J., Oldroyd, R., Lachmann, P., Fitzsimmons, C., Proudfoot, D. and Bowyer, D. (1996) Complement induced release of monocyte chemotactic protein from human smooth muscle cells a possible initiating event in atherosclerotic lesion formation. *Arterioscler. Thromb. Vasc. Biol.*, **16**, 673–677.

Van Baal, W.M., Kenemans, P., Van der Mooren, M.J., Kessel, H., Emeis, J.J. and Stehouwer, C.D.A. (1999) Increased C-reactive protein levels during short-term hormone replacement therapy in healthy postmenopausal women. *Thromb. Haemost.*, **81**, 925–928.

Walsh, B.W., Paul, S., Wild, R.A., Dean, R.A., Tracy, R.P., Cox, D.A. and Anderson, P.W. (2000) The effects of hormone replacement therapy and raloxyfene on C-reactive protein and homocystein in healthy postmenopausal women: a randomised, controlled trial. *J. Clin. Endocrinol. Metab.*, **85**, 214–218.

Yasui, T., Irahara, M. and Aono, T. (2000) Effects of oral and transdermal hormone replacement therapy in relation to serum estrogen levels. In Studd, J. (ed.), *The Management of the Menopause. The Millennium Review 2000.* Parthenon Publishing Group Press, London, UK, pp. 27–33.

Zimmer, B., Hartung, H.P., Scharfenberger, G., Bitter-Suermann, P. and Hadding, U. (1982) Quantitative studies of the secretion of complement C3 by resident, elicited and activated macrophages. Comparison with C2, C4 and lysosomal enzyme release. *Eur. J. Immunol.*, **12**, 426–430.

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