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FERROXIDASE II ACTIVITY AND SERUM CHOLESTEROL

S.K. MACFARLANE, M.C. DOHERTY and T.L. DORMANDY

Department of Chemical Pathology, Whittington Hospital, London N19 5NF, UK

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Ferroxidase II (Fox II) was developed in serum by acid incubation for 24 h. The resulting activity showed a strong positive correlation with the serum cholesterol concentration in normal subjects and patients with hyperlipidaemia. The potentiating effect of cholesterol on developed Fox II has been confirmed by the in-vitro addition of cholesterol to serum. There was no significant correlation between developed Fox II and caeruloplasmin (ferroxidase I) or between cholesterol and caeruloplasmin.

KEY WORDS: Caeruloplasmin, cholesterol, ferroxidase II, lipid peroxidation.

INTRODUCTION

Ferroxidase II (Fox II) has been characterised as a serum protein complex with iron-oxidising ability.[1] It can be distinguished from caeruloplasmin (ferroxidase I) by its resistance to azide. It has been shown that both copper and a lipid component are essential for enzymic activity.[2] Mainly because Fox II activity in serum requires several hours incubation to develop, the significance of this activity and its possible relation to caeruloplasmin have remained controversial. The present work is based on a clinical study aimed at investigating the relationship between Fox II activity, caeruloplasmin and serum lipids.

MATERIALS AND METHODS

Fox II activity was developed by acidifying fresh serum to pH 5.5 with 0.2 mol/l sodium acetate buffer and incubating at 37°C for 24 h. Fox II was measured by a modification of the method of Johnson *et al.*[3] Caeruloplasmin was measured by radial immuno-diffusion (Hoechst U.K. Ltd). Cholesterol and triglycerides were measured by standard enzymic methods. Where cholesterol was added *in vitro*, water soluble polyoxyethanyl-cholesteryl sebacate (Sigma Chemical Company, U.K.) was added directly to serum or a water blank. Cholesterol levels were determined prior to acidification.

RESULTS

Lipid Levels and Developed Fox II

Cholesterol, triglycerides and developed Fox II were measured on fresh serum from 23 normal subjects and 12 patients attending a hyperlipidaemia clinic. The serum cholesterol ranged from 2.6 to 10.1 mmol/l and triglycerides from 0.33 to 6.10 mmol/l. There was no significant correlation between triglyceride concentration and developed Fox II. In contrast, developed Fox II showed a strong positive correlation with serum cholesterol ($r = 0.835$, $p < 0.001$) as shown in Fig. 1.

Variation of Developed Fox II with Cholesterol in Individuals

To establish if developed Fox II varies with changes in cholesterol in individual subjects, serial samples were obtained at different times from a group of 9 patients admitted in diabetic ketoacidosis. The series of samples ranged from 3 samples taken within 8 hours of admission to 8 samples obtained over a period of 3 days. The cholesterol concentration ranged from 2.0 mmol/l to 9.7 mmol/l. The overall correla-

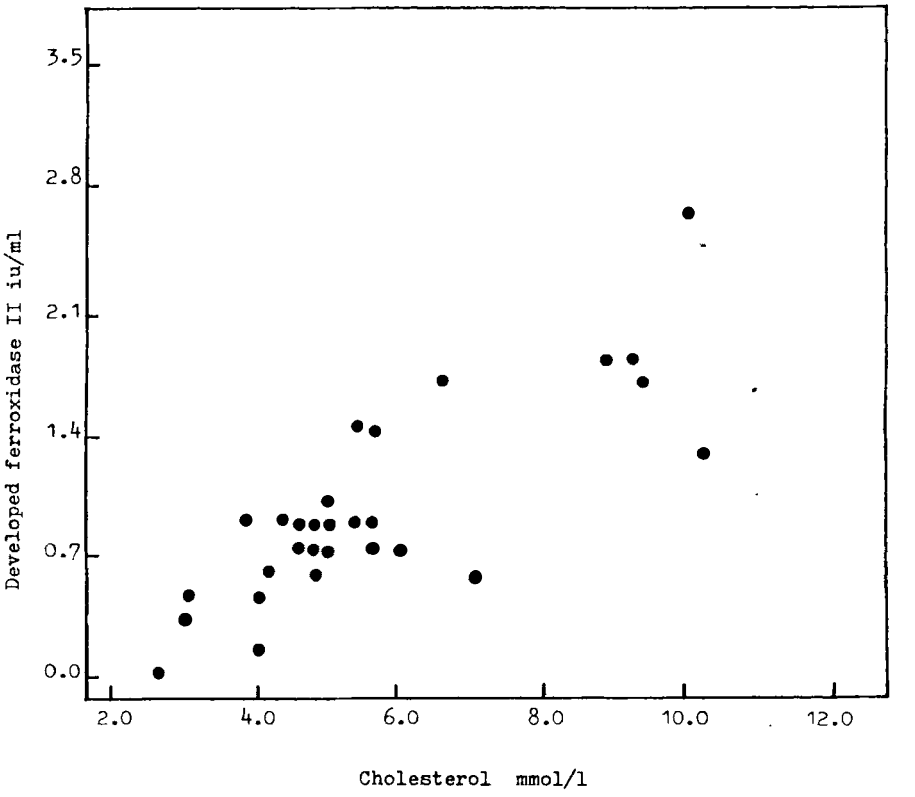

FIGURE 1 The relationship between developed ferroxidase II and serum cholesterol in normal subjects and patients with hyperlipidaemia. $n = 35$; Regression equation $y = 2.72 + 3.04x$; Correlation coefficient $r = 0.835$.

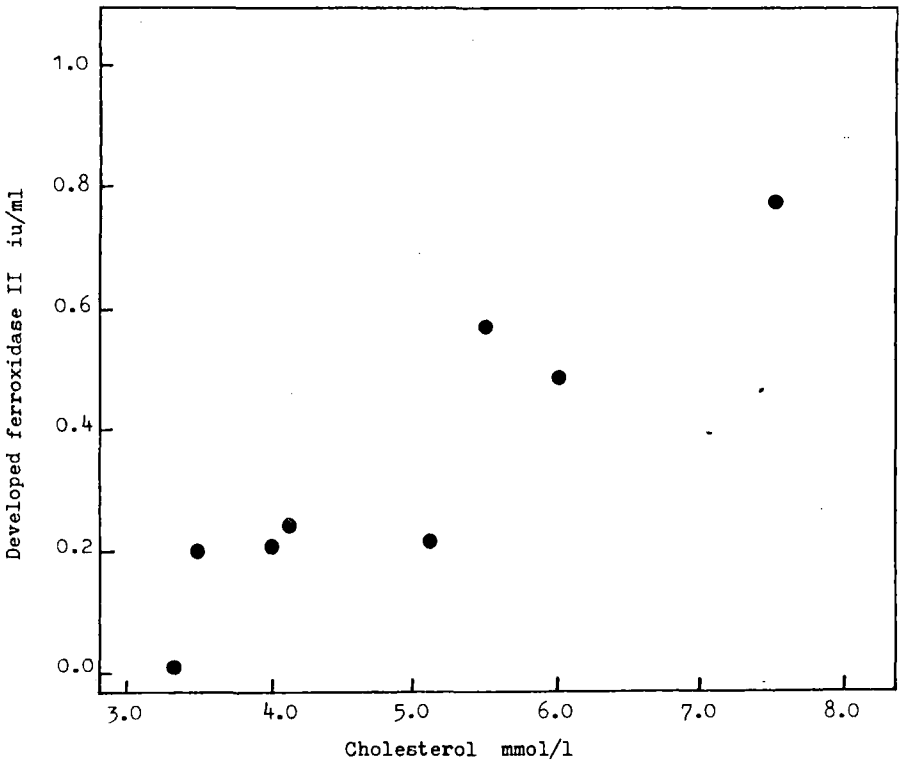

FIGURE 2 The relationship between developed ferroxidase II and serum cholesterol in a single diabetic subject. Samples were obtained at different time intervals over a period of 3 days after admission. $n = 8$; Regression equation $y = 3.13 + 5.26x$; Correlation coefficient $r = 0.911$.

tion between developed Fox II and cholesterol in the 42 samples obtained from this group was again significant ($r = 0.749$, $p < 0.001$).

Figure 2 illustrates cholesterol concentration plotted against developed Fox II in a series of 8 samples from a single diabetic subject obtained at different time intervals over a period of 3 days. The relationship between cholesterol and developed Fox II was highly significant ($r = 0.911$, $p < 0.001$).

Cholesterol Addition in-vitro

To confirm the relationship between cholesterol concentration and developed Fox II, varying amounts of water soluble cholesterol were added to fresh pooled serum. The resulting cholesterol concentration was determined and the samples were incubated at 37°C for 24 h after acidification to pH 5.5. Figure 3 shows cholesterol plotted against developed For II in this series. It can be seen that as the cholesterol level increases, developed Fox II is also enhanced. The correlation between the measured cholesterol concentration and developed Fox II is highly significant ($r = 0.990$, $p < 0.001$). No Fox II could be developed in the absence of serum when cholesterol

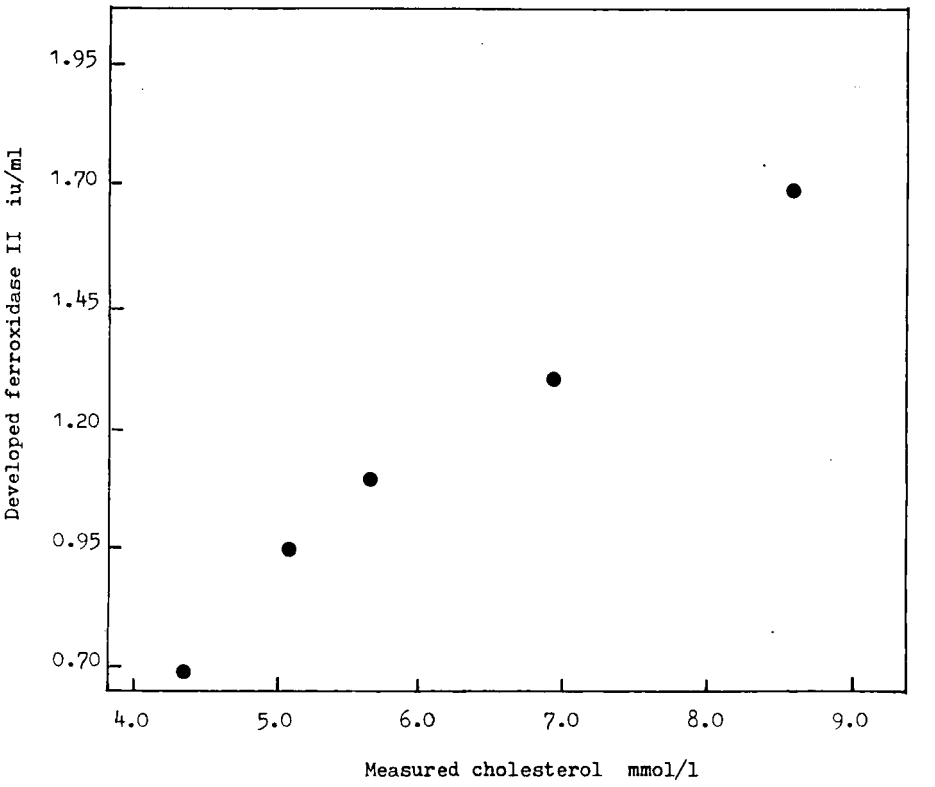

FIGURE 3 The relationship between developed ferroxidase II and measured cholesterol in pooled serum to which varying concentrations of water soluble cholesterol had been added. $n = 5$; Regression equation $y = 0.938 + 4.45x$; Correlation coefficient $r = 0.990$.

was added to deionised water and the samples acidified and incubated in a similar manner.

Relationship Between Fox II, Cholesterol and Caeruloplasmin

To establish if a relationship existed between caeruloplasmin and either developed Fox II or cholesterol, fresh serum was obtained from 33 subjects. Developed Fox II, cholesterol and caeruloplasmin concentrations were determined as described in Materials and Methods. Developed Fox II ranged from 0.02 to 2.66 iu/ml, cholesterol from 2.6 to 10.1 mmol/l and caeruloplasmin from 0.22 to 0.55 g/l. There was no significant correlation between developed Fox II and caeruloplasmin or between cholesterol and caeruloplasmin.

DISCUSSION

Ferroxidase II has been described as an "artefact" mainly on the ground that it requires acidification and incubation to be measurable.[4] A similar "charge" could be

made against a number of widely used diagnostic tests: e.g. the serum acid phosphatase can be measured only at a highly "unphysiological" pH. The argument can be resolved only by evidence which suggests that the measurement reflects a clinical state or that it correlates with a physiological variable. The striking correlation between developed Fox II and the serum cholesterol concentration, but not between developed Fox II and other serum lipid fractions, does not entirely settle the argument since the significance and mechanism of a raised serum cholesterol itself remain unclear. A raised serum cholesterol is nevertheless an accepted and valuable "marker" of lipid-related diseases: and the highly significant short- and long-term relationship between developed Fox II and the cholesterol concentration provide prima-facie evidence pointing to the biological significance of the former.

The correlation between developed Fox II and cholesterol is the more unexpected since the development of Fox II is thought to require lipid peroxidation (one explanation for the need for pre-incubation) and cholesterol is the least autoxidisable of the main serum lipid classes. There is nevertheless evidence that cholesterol affects the peroxidisability of lipid membranes in a strictly concentration-dependent manner.[5] The serum cholesterol concentration is clinically significant because it probably reflects the cholesterol concentration in membrane structures: and it seems not unreasonable to suggest that the same may be true of developed Fox II. It may be noted that neither developed Fox II, nor any of the serum lipid classes, nor indeed any lipid-related clinical abnormality correlates with the main extracellular ferroxidase, caeruloplasmin.

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