A partially purified preparation of oxidases from human liver, containing 8 m-units of THC-CoA oxidase, 16 m-units of palmitoyl-CoA oxidase, and 4.5 mg of protein in 2 ml of 10 mM-pyrophosphate buffer, pH 9.0, 10 μM-FAD and 1 mM-EDTA was applied to a hydroxylapatite column (0.9 cm x 12 cm). The preparation was left to adsorb for 30 min, then eluted with a linear 10–200 mM-phosphate gradient. This elution buffer contained 10% (v/v) ethylene-glycol, 10 μM-FAD, 1 mM-EDTA and was adjusted to pH 7.5. Fraction volumes were 0.95 ml and elution rate was 20 ml/h. Recovery of both oxidase activities was 85%. THC-CoA oxidase, Δ; palmitoyl-CoA oxidase, □; phosphate concentration, ---.

The three enzymes could be distinguished on the basis of differences in molecular mass, pI values and binding to affinity and adsorption matrices. In this study, we wished to investigate whether human liver also contains three acyl-CoA oxidases.

Homogenates or subcellular fractions enriched in peroxisomes from human liver were sonicated in hypotonic buffer and the released peroxisomal oxidases were partially purified by means of ammonium sulphate fractionation and heat treatment in the presence of FAD. The partially purified oxidases were applied to a chromatofocusing column (PBE 94 gel) and eluted with a pH gradient. Both palmitoyl-CoA oxidase and THC-CoA oxidase eluted at pH 7.4. When the same preparation of partially purified oxidases was applied to a hydroxylapatite column and eluted with a linear phosphate gradient, a complete separation of THC-CoA oxidase and palmitoyl-CoA oxidase was obtained, the former enzyme eluting at the lower and the latter at the higher phosphate concentrations (Fig. 1).

Fig. 1. Separation of human liver fatty acyl-CoA oxidase and THC-CoA oxidase by hydroxylapatite column chromatography

Differential effects of N-ethylmaleimide, zinc acetate, lithium chloride and other salts, and of storage in ethylene glycol, on the oxidase activities in whole homogenates or in partially purified preparations provided additional proof for the existence of separate acyl-CoA oxidases for fatty acyl-CoAs and THC-CoA.

The molecular masses of fatty acyl-CoA oxidase and THC-CoA oxidase, as estimated by gel filtration, were 160 kDa and 140 kDa, respectively.

Our findings demonstrate that human liver peroxisomes contain separate acyl-CoA oxidases for fatty acyl-CoA and THC-CoA, which seems to explain why in a number of patients a deficient peroxisomal β-oxidation of fatty acids is not accompanied by an abnormal bile acid metabolism and vice versa [7]. Our experiments, however, do not provide any indication for the presence of two separate fatty acyl-CoA oxidases in human liver.


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Plasma lipoproteins of normal Golden Retrievers

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Lipid keratopathy, the deposition of lipid in one or both corneas, is an unusual condition of natural occurrence in both humans and dogs [1–3].

While lipid keratopathy is usually associated with some form of primary ocular disorder, notably anterior segment inflammation, the Golden Retriever can develop the condition in the apparent absence of ocular inflammatory changes [2]. This observation has led us to examine the plasma lipoprotein profile of normal and affected Golden Retrievers in some detail, in case there are any variations of plasma lipoproteins which might help to explain why lipid keratopathy may develop in a population of Golden Retrievers in the absence of inflammatory eye disease.

As all the Golden Retrievers with lipid keratopathy have been pet animals living under normal domestic conditions, we have examined pet animals of similar age range and environment to establish the plasma lipoprotein profile of normal Golden Retrievers.

Virtually all previous reports of canine lipoproteins have been restricted to laboratory animals, kept under artificial conditions of diet and lifestyle. Often sex, age and breed of dog have not been recorded. There has been little or no data presented for pets living under normal domestic conditions.

Our group of normal dogs was chosen from animals in pet homes, or those referred to the School of Veterinary Science at Bristol for treatment of conditions totally unrelated to either ocular or other disorders known to affect blood lipids. Blood was obtained from the cephalic vein after an overnight fast and taken into EDTA. Plasma was separated immediately and sodium azide added as preservative, then stored at 4°C until analysis.

Abbreviations used: HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein.

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Lipoproteins were isolated from plasma, usually within 24 h using a combination of precipitation and ultracentrifugation [4]. High-density lipoproteins (HDL) were isolated in the supernatant after precipitation of very-low- and low-density lipoproteins (VLDL and LDL, respectively) using sodium phosphotungstate–magnesium chloride. VLDL were obtained by ultracentrifugation of plasma (density 1.006 g/ml) at 132 000 g for 3.5 h in the Beckman Airfuge at room temperature. HDL₁ and HDL₂ subfractions were separated from HDL after adjustment of density to 1.25 g/ml using potassium bromide and ultracentrifugation at 132 000 g for 3.5 h. HDL in the supernatant was removed by tube slicing and HDL₄ in the infranatant was retained for analysis. Cholesterol and triacylglycerols were measured enzymically using Boehringer Test Kits (nos. 23669 and 644200, respectively) in plasma, VLDL and LDL, and cholesterol only in HDL₄. Levels in LDL and HDL₂ were obtained by difference. Isolated lipoprotein fractions were stored at −20°C before analysis in batches.

The dog is particularly resistant to the development of atherosclerosis. It has been shown [5] that dogs need to be made hypothyroid, and be maintained on a high-cholesterol diet for periods in excess of 6 months to achieve plasma cholesterol levels of approximately 20 mmol/l, and only under these conditions do arterial lesions occur. This is in sharp contrast to humans whose plasma cholesterol levels are now thought to be undesirable high if greater than 5.2 mmol/l [6].

Although canine lipoproteins correspond in density to human lipoproteins [7], the major carrier of cholesterol in canine plasma is HDL, in contrast to humans, where LDL, directly related to risk of ischaemic heart disease, performs this function. The almost equal distribution of HDL subfractions in the Golden Retriever contrasts with humans in whom HDL₄, cholesterol, believed to be inversely related to risk of atherosclerotic heart disease, represents approximately one-third of the total HDL fraction. Triacylglycerol levels in the dogs are invariably low, with a percentage distribution very similar to those of humans. Our results indicate that in the normal Golden Retriever there are essentially no sex differences in lipoprotein lipids (Table 1).

These results will provide the basis for a full comparative study of lipid keratopathy in this breed and also when comparing with other less susceptible breeds.

Table 1. Plasma and lipoprotein cholesterol and triacylglycerol in normal Golden Retrievers

<table>
<thead>
<tr>
<th>Value</th>
<th>Males (n = 12)</th>
<th>Females (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.35 ± 0.40</td>
<td>5.73 ± 0.20</td>
</tr>
<tr>
<td>VLDL</td>
<td>3.27 ± 0.04</td>
<td>4.18 ± 0.20</td>
</tr>
<tr>
<td>LDL</td>
<td>2.33 ± 0.13</td>
<td>2.95 ± 0.23</td>
</tr>
<tr>
<td>HDL₁</td>
<td>1.32 ± 0.16</td>
<td>1.23 ± 0.11</td>
</tr>
<tr>
<td>HDL₂</td>
<td>1.32 ± 0.20</td>
<td>1.37 ± 0.08</td>
</tr>
<tr>
<td>HDL₃</td>
<td>1.32 ± 0.10</td>
<td>1.37 ± 0.08</td>
</tr>
</tbody>
</table>

A differential effect of phorbol ester on the internalization of iron and transferrin by HL 60 cells

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The accepted paradigm for iron accumulation by eukaryotic cells is by receptor-mediated endocytosis of diferric transferrin (Fe³⁺-TRF). Briefly, circulating Fe³⁺-TRF binds to a specific cell surface receptor, the complex is internalized into specialized endocytic vesicles and the iron is released as a consequence of the acidic pH in the endosome. The iron-free apotransferrin is returned to the cell surface in association with the recycling transferrin receptor, but rapidly dissociates from the receptor on re-exposure to physiological pH [1]. An alternative model, however, has proposed that iron is released from Fe³⁺-TRF at the cell surface by a transmembrane oxidoreductase [2]. In this model, the transferrin receptor serves to bring Fe³⁺-TRF into close juxtaposition with the oxidoreductase and so the amount of iron delivered to the cell is dependent on the number of cell surface receptors. This model would also account for the ability of transformed cells to grow and divide in the presence of soluble iron salts instead of Fe³⁺-TRF [3]. Both models can account for the relationship between transferrin receptor expression and the growth rate of cells since, in each case, the rate of iron accumulation, and thus of growth, would be dependent on the number of cell surface receptors [4]. We have elected to study the effect of phorbol 12-myristate 13-acetate (PMA) on the binding and internalization of both ¹²⁵I-Fe³⁺-TRF and ⁴⁰KFe⁺⁺-TRF by HL60 cells. We have concluded that internalization by these cells is not dependent on the internalization of transferrin.

The preparation of non-radiolabelled diferric transferrin (Fe³⁺-TRF), ¹²⁵I-Fe³⁺-TRF and ⁴⁰KFe⁺⁺-TRF has been described previously [5]. HL60 cells were cultured in serum-free RPMI 1640 medium supplemented with 10 mm-glutamine, 5 μg of insulin/ml and 5 μg of Fe³⁺-TRF/ml. The cells were pelleted by centrifugation at 850 g for 1 min, washed with Earle's basal salt solution and resuspended at a density of approx. 5 x 10⁶ cells/ml before experimentation. Five microlitres of dimethyl sulfoxide (DMSO) alone or containing either PMA or 4α-phorbol 12,13-didecanoate to give a final concentration of 20 nm was added to aliquots of HL60 cells 30 min before the presentation of appropriate ligands. Analysis of the binding and uptake of both ¹²⁵I-Fe³⁺-TRF and ⁴⁰KFe⁺⁺-TRF using an acidic, iron-chelating buffer to distinguish between surface and internalized ligands has all been described previously [5, 6].

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