Comparison of the effects of dietary saturated, mono-unsaturated and polyunsaturated fatty acids on very-low-density lipoprotein secretion when delivered to hepatocytes in chylomicron remnant-like particles

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Abstract
The effect of chylomicron remnant-like particles (CRLPs) enriched in saturated, mono-unsaturated or n-6 polyunsaturated fatty acids (derived from palm, olive or corn oil, respectively) on the secretion of VLDL (very-low-density lipoprotein) by rat hepatocytes in culture was investigated. CRLPs were incubated with cultured hepatocytes for 5 h. The medium was then removed and the secretion of cholesterol and triacylglycerol (TAG) into the whole medium during the following 16 h was determined. After exposure of the cells to olive oil as compared with corn and palm oil CRLPs, secretion of TAG into the medium was decreased. The TAG content of the cells was also lower in experiments with olive oil as compared with corn oil CRLPs. The levels of apoB48 (apolipoprotein B48) found in the medium remained unchanged after the exposure of the cells to the different types of remnants. These findings indicate that the type of fat in the diet directly affects VLDL lipid secretion on delivery to the liver in chylomicron remnants.

Introduction
It is well known that the various fatty acids in the diet exert differential effects on serum lipid and lipoprotein concentrations. SFAs (saturated fatty acids) are thought to increase cardiovascular disease risk because they elevate serum total and LDL (low-density lipoprotein)-cholesterol concentrations relative to MUFAs (mono-unsaturated fatty acids) and PUFAs (polyunsaturated fatty acids) [1]. The role of hypertriglyceridaemia, however, has been more controversial, but it is now generally accepted as a risk factor for coronary heart disease [2].

Lipid from the diet is carried from the intestine to the liver in CMRs (chylomicron remnants) and previous work in our laboratory has shown that the fatty acid composition of these lipoproteins reflects that of the fat consumed [3]. Thus, in order to study the direct effects of dietary fats on hepatic VLDL (very-low-density lipoprotein) secretion, a major contributor to blood TAG (triacylglycerol), it is necessary to deliver the fatty acids to hepatocytes in CMRs. In previous work, we have shown that exposure of liver cells to CMRs enriched in n-3 as compared with n-6 PUFAs decreases the secretion of TAG, cholesterol, cholesteryl ester and apoB48 (apolipoprotein B48) in VLDL [4], but further investigation is needed to fully establish the relative effects of SFAs, MUFAs and n-6 PUFAs.

Experimental
CRLPs (CMR-like particles) containing TAG enriched in SFAs, MUFAs or n-6 PUFAs obtained from palm, olive or corn oil were prepared by sonication of a lipid mixture followed by ultracentrifugation, and bound to rat apoE (apolipoprotein E) by incubation with rat plasma as described previously [5].

Hepatocytes were isolated from the livers of male Wistar rats by perfusion with collagenase and cultured in RPMI 1640 medium as before [4,6]. The cultures were incubated in the presence of corn, olive or palm oil CRLPs (0.3 μmol of TAG/ml) for 5 h. The medium was then replaced with fresh medium and the incubation continued for 16 h. TAG was determined after extraction of cell or medium samples with chloroform/methanol (2:1, v/v). ApoB48 was quantified by Western blotting coupled to ECL® (enhanced chemiluminescence) analysis [7].

Results
The content of TAG, TC (total cholesterol) and the TAG/TC ratio was similar in all three types of CRLPs, and our previous work has shown that CRLPs prepared with TG from corn, olive or palm oil are enriched in n-6 PUFAs, MUFAs or SFAs. The three types of particles also contained rat apoE as assessed by SDS/PAGE.
Figure 1 | Cellular content and secretion of TAG into the medium after incubation of hepatocytes with corn, olive or palm oil CRLPs

Hepatocytes were incubated with or without (control) corn, olive or palm oil CRLPs (0.3 μmol of TAG/ml) for 5 h, the medium was then removed and replaced with fresh medium without CRLPs, and the incubation was continued for a further 16 h. After this time the medium was collected and the cells were harvested and the content of TAG was determined. Data are reported as means ± S.E.M. for ten separate hepatocyte preparations. Significance limits: *P < 0.01 compared with palm oil CRLPs; †P < 0.05 compared with corn oil CRLPs, §P < 0.05 compared with control.

After incubation of hepatocytes with the three types of CRLPs, the amount of TAG secreted into the medium was significantly increased in comparison to cells incubated without CRLPs in the case of corn and palm, but not olive CRLPs (Figure 1). TAG secretion was also significantly lower in the presence of olive oil CRLPs as compared with corn or palm oil CRLPs. In addition, cellular levels of TAG were lower in hepatocytes exposed to olive oil as compared with corn or palm oil CRLPs. The levels of apoB48 secreted into the medium were not significantly changed by any of the CRLP types tested.

Discussion
The CRLPs derived from olive, corn and palm oil used in the present work resemble physiological remnants in size, density and lipid composition [8] and also contain rat apoE. We have demonstrated previously that the fatty acid composition of CRLPs containing TAG from palm, olive and corn oil is similar to both that of the parent oils and that of rat physiological remnants derived from them [3], so that the CRLPs were enriched in SFAs, MUFAs and n−6 PUFAs respectively. Any differential effects of the three types of particles on lipid and apoB48 secretion by cultured rat hepatocytes observed in our experiments can therefore be attributed to the difference in their fatty acid composition.

Our previous work has shown that exposure of rat hepatocytes to CMRs enriched in n−6 PUFAs from corn oil obtained from rats [9] increases the secretion of TAG into the medium compared with control cells incubated without lipoproteins (Figure 1), while the secretion of apoB48, which is essential for the assembly and secretion of hepatic VLDL, is unaffected. The findings reported here are in agreement with this and demonstrate further that palm oil CRLPs (enriched in SFAs) have a similar effect. In contrast, hepatocytes treated with olive oil CRLPs (enriched in MUFAs) secreted significantly less lipid than either untreated cells or those treated with CRLPs high in SFAs or n−6 PUFAs (Figure 1). Secretion of apoB48, however, was not changed, suggesting that delivery of MUFAs as compared with SFAs or n−6 PUFAs to the hepatocytes in CMRs leads to the secretion of lipid-depleted particles.

In addition to decreased secretion of TAG into the medium, intracellular levels of TAG were also lower in hepatocytes incubated with MUFA-enriched CRLPs. Since we have shown previously that CMRs derived from palm, olive or corn oil are taken up at similar rates by hepatocytes [10,11], it seems unlikely that this effect can be explained by a decreased rate of uptake of the particles enriched in MUFAs. However, our earlier studies have shown that a greater proportion of the TAG taken up by the tissue from CMRs derived from olive as compared with corn or palm oil is used for oxidation [10], thus it is possible that less fatty acid from the olive oil CRLPs is available for TAG production. It is also possible that the different types of remnants may affect the synthesis of TAG in the cells, altering its availability for VLDL secretion.

In summary, the results reported here demonstrate that dietary MUFAs as compared with n−6 PUFAs and SFAs cause decreased secretion of VLDL TAG directly when they are delivered to the liver in CMRs, and this may contribute to the hypolipidaemic effects of these fatty acids.

References
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