Clearance of lipoprotein remnant particles in adipose tissue and muscle in humans

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Abstract A major proportion of triglycerides in plasma triglyceride-rich lipoproteins (TRL) are removed in peripheral tissues by lipoprotein lipase, and hypothetically a minor proportion can also be removed by whole-lipoprotein particle uptake. This second removal pathway has not previously been directly demonstrated in humans. Simultaneous blood samples were drawn from arterialized blood, a vein draining the subcutaneous abdominal adipose tissue, and a deep antecubital vein of the forearm to provide arterio-venous gradients from blood-draining adipose tissue and muscle in seven male subjects. The men were given a fat-rich mixed meal containing vitamin A and the triglyceride and retinyl palmitate (RP) concentrations were quantified in the plasma. Density gradient ultracentrifugation was used to isolate TRL fractions, in which triglycerides, RP, apoB-48, and apoB-100 were quantified. There was clearance of triglycerides in muscle and adipose tissue and, in addition, removal of RP. By analysis of the TRL subfractions, the RP removal was likely to be confined to the largest chylomicron remnant particles. For the $S_f > 400$ fraction, the area under curve (AUC) relative to arterial for triglycerides were 79% (66–91%) and 81% (72–89%) in adipose tissue and muscle venous outflow, respectively (each $P < 0.02$ versus arterial). The corresponding values for RP were 87% (73–101%) and 85% (69–100%), respectively, (each $P < 0.05$ versus arterial). In the S_f 60–400 fraction there was further uptake of triglycerides, but not of RP. We hypothesize that the periphery could be of importance for removal of the largest chylomicron remnants, as their size might partially exclude them penetrating the fenestrated hepatic sinusoidal endothelium to reach the hepatic chylomicron remnant receptors.—**Karpe, F., S. M. Humphreys, J. S. Samra, L. K. M. Summers, and K. N. Frayn.** Clearance of lipoprotein remnant particles in adipose tissue and muscle in humans. *J. Lipid Res.* 1997. **38:** 2335–2343.

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Triglycerides are secreted in the form of triglyceride-rich lipoproteins (TRL) from liver and intestine to plasma for distribution to peripheral tissues. Lipoprotein lipase (LPL) hydrolyzes the triglycerides to free fatty acids, either for immediate use in muscles or for storage in adipose tissue. In humans, the secretion pattern of TRL is tissue-specific in that the liver only secretes very low density lipoproteins (VLDL) containing apolipoprotein (apo) B-100. After ingestion of fat, the intestine secretes chylomicrons, which have apoB-48 as their structural protein. The two apoB-containing lipoprotein species share the same lipolytic pathway, but the chylomicrons seem to be the favored substrate for LPL (1–3), resulting in accumulation of apoB-100 in the TRL fraction in the postprandial state (4–6). After lipolysis of triglycerides from the lipoprotein particle, a remnant is formed.

The hepatic and peripheral removal of chylomicron remnants is likely to depend on receptor-mediated processes. The variety and distribution of potential lipoprotein receptors on endothelial cells in the periphery is, however, restricted compared to hepatocytes. The LDL receptor is expressed on endothelial cells, but down-regulated by contact-inhibition, and therefore not likely to be highly expressed on normal endothelium (7, 8). Recently, the VLDL receptor, which is a non-hepatic receptor recognizing apoE as a ligand, has been shown to be expressed on endothelial cells (9, 10). Mice with a targeted disruption of the gene coding for the VLDL receptor do not develop hypertriglyceridemia, even after provocation with a carbohydrate-rich diet, but their total body weight and adipose tissue mass are slightly decreased compared to normal mice (11).

Abbreviations: AUC, area under curve; apo, apolipoprotein; HPLC, high performance liquid chromatography; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; LPL, lipoprotein lipase; LRP, LDL receptor-related protein; RP, retinyl palmitate; S_f, Svedberg flotation rate; TRL, triglyceride-rich lipoprotein; VLDL, very low density lipoprotein.

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These findings could imply a nonessential role for the VLDL receptor in the peripheral removal of TRL. The LDL receptor-related protein/α-2 macroglobulin receptor (LRP), which is a putative hepatic chylomicron remnant receptor, is not expressed on normal endothelial cells, but high expression is seen on endothelium of atherosclerotic arteries (12).

Quantification of TRL in postprandial plasma is a challenge. By measuring triglycerides a discrimination between hepatic and intestinal TRL is not possible. In humans, specific quantification of apoB-48 and apoB-100 in TRL is likely to give an accurate estimate of the relative contribution of intestinal and hepatic lipoproteins and several methods have recently been devised for this purpose (13–16). An additional way to quantify intestinal lipoproteins is to use the specific metabolic route for absorption of vitamin A and retinyl esters as retinyl palmitate (RP) (17). After secretion of chylomicrons from the intestine, RP is carried in the lipoprotein core and is thought not to leave plasma until final whole-lipoprotein particle removal ensues. Supplementation of a lipid-rich test meal with vitamin A has therefore often been used as a means of quantifying lipoproteins of intestinal origin in the postprandial state. Unfortunately, this method has a number of limitations, one of them being transfer of RP to apoB-100 lipoprotein particles at late time-points after ingestion of fat (18). Furthermore, it reflects apoB-48 levels in TRL fractions poorly (19) and the absorption of RP is labile (20).

Some of the apoB-100-containing lipoproteins seem to be particularly efficient in liberating the triglyceride core components, as they can form triglyceride-poor, cholesterol-rich LDL particles as remnants. This does not seem to be the case for most of the large VLDL particles, which instead are removed from plasma before they reach a particle size comparable to LDL (21). Chylomicrons essentially do not generate LDL-sized lipoproteins in humans, i.e., apoB-48 is generally not found in LDL. Furthermore, Karpe and coworkers (22) have recently postulated that a major proportion of chylomicron remnants are removed from plasma after transient lipolysis as very large particles (greater than Svedberg flotation rate (S_f) 400 or greater than 75 nm in diameter) in humans. Studies in animals have shown that the liver seems to be the principal organ for uptake of chylomicron remnants, but the relative importance of the hepatic uptake shows profound heterogeneity among studies, with estimations ranging from 20% to 80% (23). Accordingly, other tissues than liver also take part in the removal of chylomicron remnants. Hypothetically, the periphery could be of specific importance for removal of the largest remnants, as they are too large to easily penetrate through the fenestrated hepatic sinusoidal endothelium to reach the hepatic chylomicron remnant receptors (24).

We hypothesize that the triglycerides of large TRL particles can be removed in peripheral tissues not only via lipolysis by LPL, but also by whole-lipoprotein particle removal. To test this hypothesis, we designed a study in which quantification of triglycerides was made along with accurate estimations of TRL particles in the postprandial state in both arterialized and venous plasma draining either muscle or adipose tissue in humans. A net extraction across the tissue of triglycerides together with RP or apoB would be a sign of lipoprotein particle uptake.

METHODS

Protocol and subjects

Seven healthy male subjects aged 21–47 years were studied. Their body mass indices ranged from 19.3 to 27.9 kg/m^2 (median 23.2 kg/m^2). They were instructed to ingest an essentially fat-free dinner before 7 PM the preceeding evening and arrived at 7.30 AM in the morning. In order to study the arterio-venous difference in concentration of lipoprotein particle constituents, intravenous lines were established at the following points. A 10-cm/22-gauge catheter (Secalon Hydrocath, Viggo-Spectramed, Swindon, UK) was inserted anterogradely into a vein draining the subcutaneous abdominal adipose tissue, as described previously (25). A cannula was placed retrogradely in a vein draining a warmed hand to provide arterialized blood (26). A third cannula was inserted retrogradely in a deep antecubital vein of the forearm to provide venous blood draining muscle (27, 28). All lines were kept patent by slow infusions of saline.

Subjects were served a mixed meal at time 0, which was normally 1–2 after arrival to provide the basis for endogenous chylomicron and chylomicron remnant formation. The meal consisted of 85 g carbohydrate, 60 g fat, and 13 g protein, which was made up of a milk shake containing 60 g corn oil, 100 ml skimmed milk, 100 ml sugar-free strawberry squash, and 50 ml ice cream containing 0.5% fat (0.25 g). Subjects also ate breakfast cereal consisting of 40 g cornflakes with 200 ml skimmed milk, plus a cup of decaffeinated tea or coffee with milk from the above allowance. Vitamin A (Arovit, Roche, Basle, Switzerland) in the form of RP in tablets (in total 250.000 IU) was given with the meal. The subjects were instructed to chew the tablets carefully and to distribute them evenly over the whole meal. One subject did not receive the tablets and data for RP measurement is therefore n = 6.

Blood samples were drawn simultaneously from the three sites into heparinized syringes (Monovettes, Sarstedt, Leicester, UK) at −30, 0, 40, 80, 120, 160, 200, and 240 min, and immediately put on ice. Time 0 was just before the meal. Plasma was recovered after a low speed centrifugation within 30 min at 4°C.

Lipoprotein fractionation

TRL were subfractionated by cumulative density gradient ultracentrifugation (29). Plasma was adjusted to density 1.10 kg/l by mixing 3.0 ml of plasma with 1.5 ml of NaBr 1.42 kg/l. A density gradient consisting of 4 ml of 1.10 kg/l plasma and 3 ml each of 1.065, 1.020, and 1.006 kg/l NaCl solutions was then formed in Beckman Ultraclear tubes (volume 13.4 ml) which had been coated with polyvinyl alcohol (BDH Chemicals Ltd, Poole, England) (30). Ultracentrifugation was performed in a Beckman SW40 Ti swinging bucket rotor at 40,000 rpm and 15°C. Consecutive runs calculated to float $S_f > 400$ (32 min), S_f 60–400 (3 h 28 min) and S_f 20–60 (16 h) particles were made. After each centrifugation, the top 0.5 ml of the gradient containing the respective lipoprotein subclass was aspirated, and density 1.006 kg/l salt solution was used to refill the tube before the next run. The S_f 12–20 fraction (intermediate density lipoprotein, IDL) was recovered after the last ultracentrifugal run by slicing the tube 29 mm from the top after the S_f 20–60 lipoproteins had been aspirated. All salt solutions used to prepare the density gradients were adjusted to pH 7.4 and contained 0.02% sodium azide and 0.01% EDTA.

Analytical methods

Triglyceride concentrations were measured using an enzymatic method (31). The concentration of apoB-100 and apoB-48 in fractions of TRL was determined with an analytical SDS-PAGE method (14). Lipoprotein fractions were immediately delipidated and dissolved in SDS-phosphate buffer and frozen for later analyses, which were performed within a month. The protein content of the IDL fraction was determined on fresh samples according to Lowry et al. (32). RP was analyzed in whole plasma as well as TRL fractions by HPLC (19). These TRL fractions were frozen with glycerol (final concentration 10%) to minimize lipoprotein particle aggregation. Samples for RP analyses were shipped in frozen condition from Oxford to Stockholm and analyzed within a month.

As small differences in concentration of particles between arterialized and venous plasma are being studied, it is important to confirm that these do not reflect changes in hemoconcentration during passage through the tissues, for instance because of lymphatic drainage from the tissues. A set of unpublished data from previously published studies were reanalyzed to test this. Other details of the studies have been reported previously (25, 33, 34). The experimental set-up was very similar to the present study. The data consisted of hematocrit measurements in arterialized, adipose and forearm venous blood from three baseline samples and for 6 h after a test meal in 15 subjects. There were 11 measurements per subject, each of simultaneous hematocrit determinations from the three sites, and thus a total of 495 individual measurements. The hematocrit levels from the three respective sites at time zero were 41.8 ± 3.9%, 41.8 ± 4.1%, and 41.9 ± 4.1%. Due to repeated blood sampling during the experiments mounting to the same blood volumes as in the present experiment, the hematocrits decreased significantly to 40.5 ± 4.3%, 40.6 ± 4.0%, and 40.6 ± 4.2% at 240 min after meal intake, respectively. Accordingly, there were no consistent differences in hematocrit among the sites either before or after the meal. This powerful dataset thus argues against any detectable changes in hemoconcentration across the tissues studied.

Calculation and statistics

All derived data parameters were calculated for individual samples. Data were analyzed as areas under curve (AUC), as recommended by Matthews (35). AUCs were calculated from time 0, thus omitting the −30 timepoint. For each subject, the AUC for the venous concentration of a substance was expressed as a percentage of the AUC for the arterial concentration, to give a relative area under the curve. Means are shown in graphs and table. The non-parametric Wilcoxon's signed ranks test was used to compare concentrations or AUCs in arterial and venous blood.

Ethical considerations

The studies were approved by the Central Oxford Research Ethics Committee, and all subjects gave informed consent.

RESULTS

Plasma concentrations

The plasma triglyceride concentration was consistently higher in arterialized plasma compared to the venous outflow, both in the fasting as well as the postprandial state (**Fig. 1**). The mean fasting triglyceride level decreased from 0.90 ± 0.36 in arterial plasma to 0.86 ± 0.36 mmol/l ($P < 0.01$) in muscle venous outflow and to 0.86 ± 0.37 mmol/l ($P < 0.05$) in adipose tissue venous outflow. The plasma triglyceride concentration

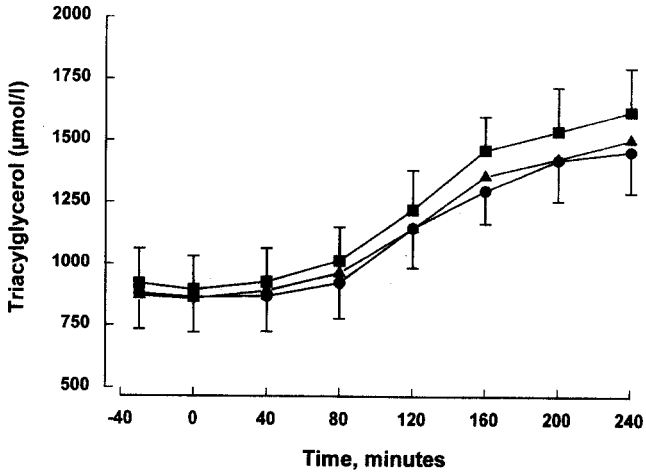

Fig. 1. Triglyceride concentrations in arterialized plasma (■), venous outflow from muscle (●), and adipose tissue (▲) after ingestion of the mixed meal at time 0.

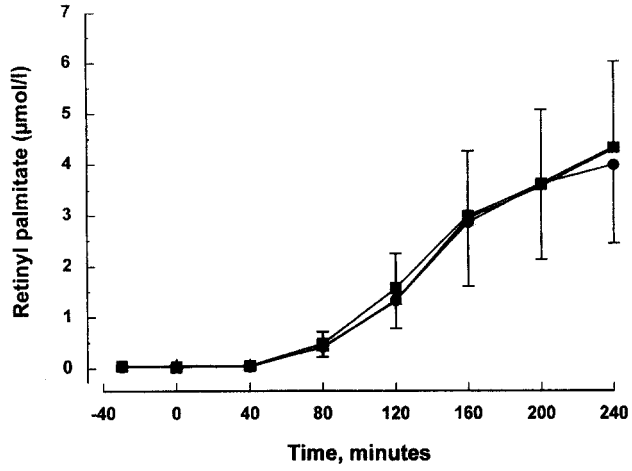

Fig. 2. Retinyl palmitate (RP) concentrations in arterialized plasma (■), venous outflow from muscle (●), and adipose tissue (▲) after ingestion of the vitamin A-containing mixed meal at time 0.

increased by approximately 50% in response to the meal. The difference between the arterial and venous plasma triglyceride concentrations increased in the postprandial state, arguing for an increased extraction of triglycerides by tissues in the postprandial state. The mean peak triglyceride level (+240 min) decreased from 1.62 ± 0.46 mmol/l in arterial plasma to 1.46 ± 0.44 mmol/l ($P < 0.01$) in muscle venous outflow and to 1.51 ± 0.49 mmol/l ($P < 0.01$) in adipose tissue venous outflow. Thus, similar patterns were observed comparing muscle and adipose tissue. Based on calculations of triglyceride AUC, on average 7–9% of the plasma triglycerides were extracted during passage through the tissues (**Table 1**), although the relative extraction was lower in the fasting state and the beginning of the postprandial state, compared to the late postprandial phase, which exhibited an extraction greater than 10% (Fig. 1).

The plasma RP concentration started to increase at 80 min after meal intake (**Fig. 2**). Arterial concentrations seemed to be slightly above venous concentrations, although the relative difference was considerably smaller than for triglycerides. The AUC for RP was, however, significantly lower (approximately 95% of arterial plasma) in the two venous sites compared to arterial plasma, arguing for tissue removal of chylomicron remnant particles. (Table 1).

Lipoprotein fractions

The plasma triglyceride extraction was most prominent in the $S_f > 400$ fraction (**Fig. 3**). The triglyceride extraction continued further down in the lipolytic cascade, with significant extraction from the S_f 60–400 fraction in both tissues (Fig. 3, Table 1). There was no consistent difference between arterial and venous triglyceride concentrations in the S_f 20–60 fraction (Fig. 3, Table 1). The tissue removal of RP was most promi-

TABLE 1. Areas under curves for triglycerides, RP, and apoB in venous outflow from adipose tissue and skeletal muscle relative to arterial plasma

	Adipose Tissue		Skeletal Muscle	
Fraction	Triglycerides	RP	Triglycerides	RP
Plasma	93 (90–97)[a]	96 (94–98)[a]	91 (86–96)[a]	94 (89–99)[a]
$S_f > 400$	79 (66–91)[a]	87 (73–101)[a]	81 (72–89)[a]	85 (69–100)[a]
S_f 60–400	97 (94–100)[a]	104 (95–112)	92 (87–98)[a]	103 (91–115)
S_f 20–60	102 (96–108)	101 (75–127)	97 (94–101)	101 (78–123)
	ApoB-100	ApoB-48	ApoB-100	ApoB-48
$S_f > 20$	93 (85–100)[a]	96 (88–104)	92 (90–95)[a]	91 (84–99)[a]

Values are AUCs for venous plasma and lipoprotein fractions expressed as a percentage of the equivalent arterial AUC. They are shown as mean (95% confidence interval).

[a] $P < 0.05$, statistical difference between arterial and venous AUCs are calculated using Wilcoxon's signed ranks test.

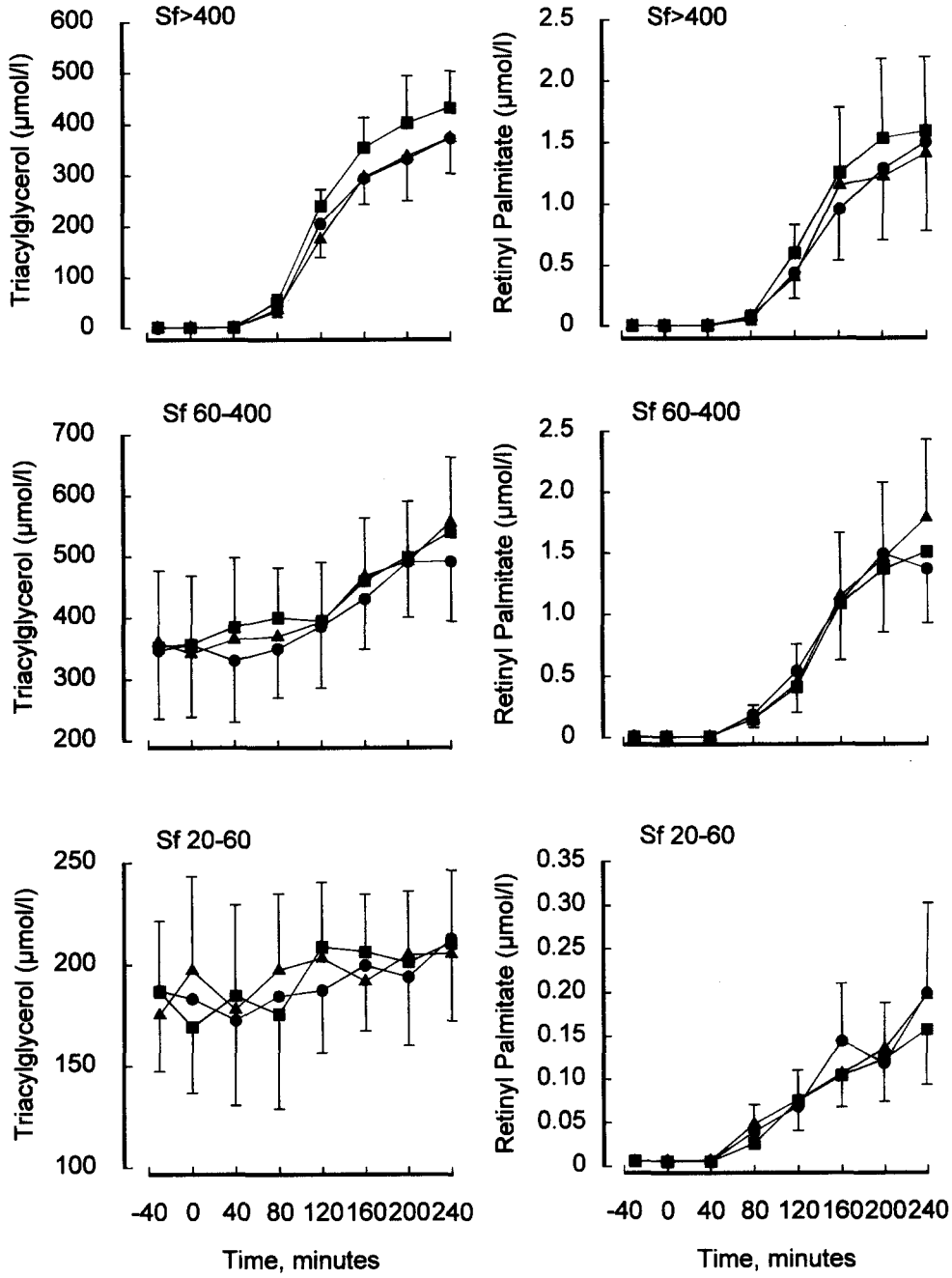

Fig. 3. Retinyl palmitate (RP) and triglyceride concentrations in the $S_f > 400$, S_f 60–400, and S_f 20–60 fractions isolated from arterialized plasma (■), venous outflow from muscle (●), and adipose tissue (▲) after ingestion of the vitamin A-containing mixed meal at time 0.

nent in the $S_f > 400$ fraction (Fig. 3, Table 1). As the lipolytic process leads to transformation of larger particles to smaller species, appearance of RP in the S_f 60–400 fraction might therefore be anticipated. However, this was not seen, as the RP levels were almost identical in arterial and venous plasma in the S_f 60–400 fraction (Fig. 3, Table 1). The concentrations of RP in the S_f 20–60 fraction were very low and no significant differences between arterial and venous plasmas were seen (Fig. 3, Table 1). In all, the RP data argue for tissue removal of chylomicron remnants only from the $S_f > 400$ fraction. There was no major difference between adipose tissue and muscle (Table 1).

ApoB-48 was present in fasting plasma in the S_f 60–400 and S_f 20–60 fractions and increased in response to meal intake. The levels of apoB-48 were very low in

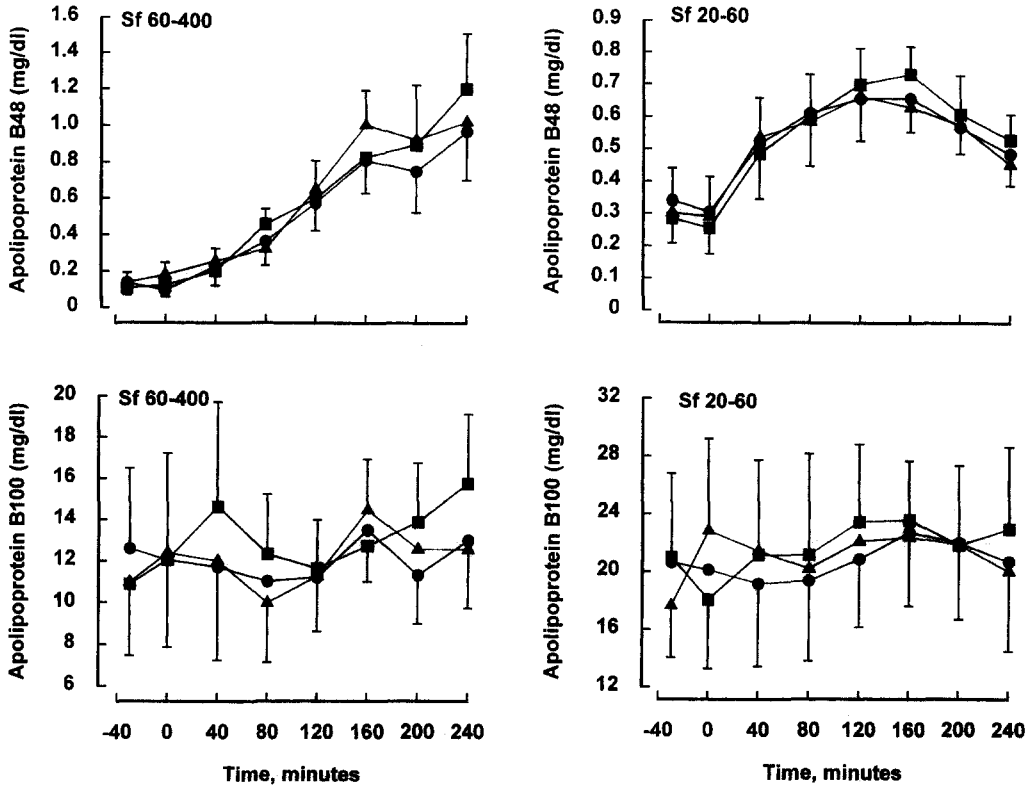

Fig. 4. ApoB-48 and apoB-100 concentrations in the S_f 60–400 and S_f 20–60 fractions isolated from arterialized plasma (■), venous outflow from muscle (●), and adipose tissue (▲) after ingestion of the mixed meal at time 0.

the $S_f > 400$ fraction and three of seven subjects had levels below the detection limit (<0.2 mg/l) at all postprandial time-points (data not shown). There was no major difference in the apoB-48 concentration between arterial and venous plasmas in the S_f 60–400 fraction (**Fig. 4**). The arterial concentrations of apoB-48 seemed to be higher in the postprandial phase (from +80 min and onwards) in the S_f 20–60 fractions (Fig. 4).

There were no major differences in the apoB-100 concentrations between arterial and venous plasmas in the S_f 60–400 fraction, but the postprandial concentrations (from +80 min and onwards) of apoB-100 in the S_f 20–60 fraction seemed to be slightly higher in arterial plasma compared to both venous plasmas (Fig. 4).

The AUC of apoB in $S_f > 400$, S_f 60–400, and S_f 20–60 were added for apoB-48 and apoB-100 separately yielding a summary fraction, i.e., $S_f > 20$. The AUC for apoB-48 was significantly smaller in muscle venous outflow compared to arterial plasma and the apoB-100 concentration was significantly smaller for both muscle and adipose tissue outflow (Table 1). Obviously, apoB-100 particles in the S_f 60–400 and S_f 20–60 fractions can be converted to even smaller particles, thus appearing in the S_f 12–20 fraction (IDL) and thereby explaining lower concentrations in venous plasma compared to the arterial ones. There was, however, no difference in arterial and venous plasma concentrations for total IDL protein. The AUC measure for the three sites were arterial; 242 ± 117 mg/l × h, adipose tissue venous outflow: 241 ± 113 mg/l × h and muscle venous outflow: 245 ± 119 mg/l × h, respectively.

DISCUSSION

The present work shows the expected clearance of triglycerides in muscle and adipose tissue and, in addition, tissue removal of several markers for TRL particles, i.e., RP, apoB-100, and apoB-48. In posprandial samples the lipoprotein particle removal was confined to the largest chylomicron remnant particles. There was no major difference between muscle and adipose tissues in this respect.

The methodology used in this investigation has proven to be very useful in the investigation of muscle and adipose tissue substrate fluxes (36–38). By determination of arterio-venous concentration differences of

triglycerides, non-esterified fatty acids, and glycerol, the rates of action of both hormone-sensitive lipase and LPL have been calculated (39). However, in the light of the present findings, it must be concluded that the previous estimation of the rate of action of LPL probably represented a small, but significant, overestimation.

It is likely that the removal of lipoprotein remnant particles found in the present study also leads to a small non-lipolytic tissue removal of triglycerides. For several reasons the design of the present study does not allow quantification of this, presumably minor, removal pathway. First, the most significant (in a statistical sense) and reliable (whole plasma measurement; fractionation increases analytical error) TRL particle measure, which showed signs of tissue removal, was RP. It has previously been shown that RP is not uniformly distributed between apoB-48 particles, as large chylomicrons/chylomicron remnants ($S_f > 400$) contain 5- to 10-fold more RP molecules per particle compared to the smaller species (S_f 60–400) (22). Second, the retinyl palmitate disappearing from the $S_f > 400$ fraction may go in two directions, either removal of particles from the plasma compartment or a lipolytic conversion to a more dense lipoprotein fraction, i.e., S_f 60–400. Interestingly, RP and triglycerides behaved somewhat differently in this respect as RP was removed from the $S_f > 400$ fraction but there was no removal, nor an increase of RP, in the S_f 60–400 fraction. In contrast, triglycerides were removed from both $S_f > 400$ and S_f 60–400 fractions, which implies a much higher triglyceride removal than lipoprotein particle removal. Third, apoB-48 and apoB-100 were only quantified in lipoprotein fractions and one cannot exclude the possibility that the lipolytic action on TRL in muscle and adipose tissue results in transfer of TRL particles from one fraction to another one further downstream in the lipolytic cascade. To partially circumvent this problem in the interpretation of the present data, we summarized the apoB-100 and apoB-48 contents in all the TRL fractions. As the AUCs of these measures were consistently smaller in venous plasmas compared to arterial, the apoB-data give support to the more robust whole plasma quantification of RP. We deliberately chose not to measure apoB-100 in whole plasma, as it would be very unlikely that a difference in concentration of apoB-100 between arterial and venous plasmas would be detected. Normally, less than 10% of apoB-100 in plasma is in the TRL fraction in normolipidemic males, and a fractional removal of greater than 10% would be very unlikely, i.e., removal of less than 1% of total plasma apoB-100.

There are some inherent problems in the use of RP as surrogate marker for chylomicrons/chylomicron remnants. RP is, for example, known to appear in apoB-100 particles in the late postprandial phase (6–8 h after fat intake) (18). Furthermore, RP has been shown to be sensitive to LPL-mediated hydrolysis in vitro (40), but this hydrolysis was seen to begin at a point at which all triglycerides had first been consumed (23, 40). This situation is not likely to occur under the present experimental conditions. As we have only been tracing the plasma RP levels during the first four postprandial hours and no signs of complete triglyceride hydrolysis in fractions were recorded, we consider RP as a useful marker of intestinally derived TRL in the present study. Furthermore, the labile absorption of RP (20), should not be a problem in this setting as we are studying arterio-venous differences in individual subjects.

The removal of RP may have been facilitated by receptor-mediated processes. LRP, a putative chylomicron remnant receptor, is not, however, expressed on normal endothelium (12). In rodents, it has been argued that the LDL receptor is vital for the removal of chylomicron remnants (41), but proof of this does not exist in humans (42). In any case, the LDL receptor is likely to be poorly expressed on normal endothelium (7, 8). In contrast, the VLDL receptor is expressed on endothelium and strongly binds the pertinent ligand (apoE) of chylomicron remnants. Furthermore, the presence of this receptor may have some importance for utilization of nutrients as body weight and development of adipose tissue mass were decreased in mice lacking the VLDL receptor (11). The tissue removal of some of the presumably large chylomicron remnants seen in this study could therefore be mediated by the VLDL receptor.

An alternative explanation for the present findings is that the large ($S_f > 400$) particles were not removed from the vascular bed, but bound transiently to the endothelium. This process of margination has been shown directly in humans (22). In the present study we quantified the arterio-venous difference in concentration for the analytes up to 240 min after start of formation of chylomicrons/chylomicron remnants. Theoretically, the arterio-venous difference in AUC could have been smaller than estimated if the whole peak had been integrated (also the decline of RP) as there might be a time-lag implied in the process of margination of chylomicrons. Material initially bound could have been released later. Margination probably represents binding of particles to LPL and its heparan sulfate anchor. If LPL had become attached to the particles, then this might in itself have acted as a further ligand for the VLDL receptor (43, 44). LPL seems to be carried by TRL particles in plasma (45). We therefore postulate that the largest chylomicron remnants could have a peripheral removal process via the VLDL receptor (46), or via the LDL receptor if it were to be expressed (47, 48), which could be further facilitated by LPL.

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