Effects of an oral and intravenous fat load on adipose tissue and forearm lipid metabolism

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Evans, Kevin, Mo L. Clark, and Keith N. Frayn. Effects of an oral and intravenous fat load on adipose tissue and forearm lipid metabolism. *Am. J. Physiol.* 276 (*Endocrinol. Metab.* 39): E241–E248, 1999.—We have studied the fate of lipoprotein lipase (LPL)-derived fatty acids by measuring arteriovenous differences across subcutaneous adipose tissue and skeletal muscle in vivo. Six subjects were fasted overnight and were then given 40 g of triacylglycerol either orally or as an intravenous infusion over 4 h. Intracellular lipolysis (hormone-sensitive lipase action; HSL) was suppressed after both oral and intravenous fat loads ($P < 0.001$). Insulin, a major regulator of HSL activity, showed little change after either oral or intravenous fat load, suggesting that suppression of HSL action occurred independently of insulin. The rate of action of LPL (measured as triacylglycerol extraction) increased with both oral and intravenous fat loads in adipose tissue ($P = 0.002$) and skeletal muscle ($P = 0.001$). There was increased escape of LPL-derived fatty acids into the circulation from adipose tissue, shown by lack of reesterification of fatty acids. There was no release into the circulation of LPL-derived fatty acids from skeletal muscle. These results suggest that insulin is not essential for HSL suppression or increased triacylglycerol clearance but is important in reesterification of fatty acids in adipose tissue but not uptake by skeletal muscle, thus affecting fatty acid partitioning between adipose tissue and the circulation, postprandial nonesterified fatty acid concentrations, and hepatic very low density lipoprotein secretion.

Intralipid; hormone-sensitive lipase; lipoprotein lipase; fatty acid reesterification

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ALTHOUGH AN ELEVATED PLASMA cholesterol concentration is a strong risk marker for coronary heart disease (CHD), many patients suffering myocardial infarction do not have particularly elevated cholesterol concentrations (38). This has prompted a search for other determinants of atherogenesis. One common feature of a number of conditions carrying increased risk of CHD is an increase in the plasma concentration of apolipoprotein B (hyper-apoB). Hyper-apoB is a stronger risk marker for CHD than the total cholesterol concentration (38).

Hyper-apoB is primarily due to an overproduction of apoB-containing particles by the liver (41). However, it may be reinforced by clearance defects as seen in insulin resistance (9, 31) and reported in some cases of familial combined hyperlipidemia (1). Hepatic very low density lipoprotein (VLDL) and, therefore, apoB secretion are strongly influenced by the delivery to the liver of fatty acids, the main substrate for VLDL-triacylglycerol (TAG) synthesis (17). Therefore, the major underlying defect in hyper-apoB may lie in the regulation of fatty acid supply in the circulation (37).

During the postprandial period, fatty acids arise from the action of lipoprotein lipase (LPL) in peripheral tissues, especially adipose tissue (13). The fatty acids released either enter the tissue for reesterification and storage, or they may be released into the systemic circulation. The release of LPL-derived fatty acids into the circulation has long been recognized from animal and in vitro experiments (4, 35), but only recently has its importance been appreciated in humans (13, 37). Because humans in Western societies spend much of each 24-h period in a postprandial state, regulation of this process might be of crucial importance in regulation of the integrated nonesterified fatty acid (NEFA) supply to the liver (and other tissues). In fact, it has been suggested that disruption of the normal coordinating role of insulin in the postprandial period underlies many of the lipid abnormalities associated with insulin resistance (12).

Little is known about regulation of the supply of LPL-derived NEFA into the circulation. Entrapment of fatty acids in adipose tissue (their alternative fate) is stimulated by insulin (15), which both suppresses the intracellular hormone-sensitive lipase (HSL) and increases esterification, thus apparently creating a concentration gradient down which fatty acids may flow (15). Both these effects of insulin may be impaired in insulin resistance (9). Regulation of the fate of fatty acids released by LPL action in the postprandial period is likely to be a major factor determining integrated NEFA supply and, thus, hepatic apoB secretion. It is of interest that conditions associated with increased CHD risk, including insulin resistance (9), type 2 diabetes mellitus (33), and familial combined hyperlipidemia (8), are all associated with elevated concentrations of NEFA in the postprandial period.

Fatty acids are an important source of energy for skeletal muscle. These fatty acids are obtained either from plasma NEFA or from circulating lipoproteins after hydrolysis by LPL. Skeletal muscle may be an important site for clearance of circulating TAG. Fatty acids released by LPL-mediated hydrolysis are taken up into the muscle. Unlike adipose tissue, there is no evidence that skeletal muscle releases NEFA into the circulation during hydrolysis of TAG by LPL (10), i.e., it does not act as a branch point as in adipose tissue.

Intralipid is a soya oil-based emulsion with particles composed of a TAG core surrounded by a phospholipid layer. Intralipid particles resemble chylomicrons in their metabolism (7) and acquire the apolipoproteins necessary for clearance rapidly once in the circulation (22). Previous studies have looked at the metabolic responses to a mixed meal containing fat. In a previous study, we looked at the metabolism of 10% Intralipid in peripheral tissues and concluded that it did not behave like native chylomicron particles (34). However, that study did not involve a direct comparison with chylomicron metabolism, and it used 10% Intralipid, which has a different phospholipid-to-TAG ratio. The main aim of this study was to investigate the effects of oral ingestion of a pure fat load or infusion of the TAG emulsion Intralipid on LPL and HSL action and the fate of LPL-derived fatty acids in vivo in the absence of an insulin response. In addition, Intralipid infusion might confer the benefit of avoiding variability in digestion and absorption. A subsidiary hypothesis was that LPL-derived fatty acids in skeletal muscle might enter the systemic circulation at a time of high LPL action (as would occur after a high-fat meal).

SUBJECTS AND METHODS

Subjects. Six healthy volunteers (4 male) aged 26–44 years with body mass index 21.1–26.4 kg/m^2 were each studied on two occasions after an overnight fast. All subjects were normolipidemic and normoglycemic. Their median fasting total cholesterol concentration was 4.2 mmol/l (2.8–5.9 mmol/l), with a median HDL-cholesterol concentration of 1.3 mmol/l (1.0–1.7 mmol/l) and a median TAG concentration of 0.7 mmol/l (0.2–1.1 mmol/l). Subjects refrained from strenuous exercise and alcohol for 24 h before each study and were given instructions to consume a low-fat meal on the evenings before the studies and then to fast from 8:00 PM, also avoiding caffeinated drinks. None of the subjects were smokers. The study was approved by the Central Oxford Research Ethics Committee, and all subjects gave informed consent.

Experimental methods. A 10-cm, 22-gauge Secalon Hydro-cath catheter (Ohmeda, Swindon, UK) was introduced over a guide wire into a superficial vein on the anterior abdominal wall and was threaded toward the groin so that its tip lay just superior to the inguinal ligament. As described previously (10), this provided access to the venous drainage from the subcutaneous abdominal adipose tissue, uncontaminated by muscle drainage and with a relatively minor contribution from skin. This adipose tissue depot has been shown to be representative of whole body adipose tissue (14). The cannula was kept patent by an infusion of 0.9% saline at 30 ml/h.

A cannula was placed retrogradely in an antecubital vein draining the forearm muscle. Another retrograde cannula was placed contralaterally to the deep venous cannula in a vein draining the hand, which was warmed in a hot-air box maintained at 60°C to obtain arterialized blood. A further cannula was placed in an antecubital vein on the arm with the warmed hand for infusion of Intralipid. The cannulas were kept patent by a slow infusion of 0.9% saline.

Adipose tissue blood flow (ATBF) was measured immediately after each blood sample with the ^{133}Xe washout method (26) after 2 MBq ^{133}Xe were injected into the region drained by the subcutaneous abdominal catheter. The first ATBF measurement was not taken until ≥30 min later to allow recovery from the hyperemic phase caused by the injection. Forearm blood flow was measured by strain-gauge plethysmography (18) on the arm with the deep antecubital cannula, just after each set of blood samples.

Two sets of basal blood samples, 20 min apart, were taken simultaneously from the arterialized vein, the abdominal vein, and the forearm vein. Before the deep venous samples were taken, a blood pressure cuff was inflated to a pressure of 200 mmHg around the wrist for 2 min to occlude superficial flow from the hand. After basal blood samples, one of two protocols was followed. On one occasion, the subjects received a 40-g fat load with only a small amount of carbohydrate in the form of tomato soup (Table 1). On the other occasion, they received an intravenous infusion of Intralipid at a constant rate over 4 h to provide the same fat load. Blood samples were taken 40, 80, 120, 180, 240, 300, and 360 min after the soup or start of the infusion. Additional arterialized samples were taken at 20 and 60 min for glucose and insulin measurement only.

Analytic methods. Blood samples were collected into heparinized syringes. A portion of each blood sample was rapidly deproteinized with 7% (wt/vol) perchloric acid. Plasma was separated rapidly from the remaining blood by centrifugation at 4°C. Plasma TAG and NEFA concentrations and blood glucose, glycerol, and 3-hydroxybutyrate concentrations were measured with enzymatic methods on an IL Monarch centrifugal analyzer (Instrumentation Laboratory, Warrington, UK). Plasma insulin was measured with a double-antibody radioimmunoassay (Kabi Pharmacia, Milton Keynes, UK).

Calculations and statistical methods. Arteriovenous differences (a−v) and venoarterial differences (v−a) were calculated to represent uptake and release of substances across tissue beds. Because there were no changes in adipose tissue or forearm blood flow during the studies, arteriovenous or venoarterial differences have been used throughout, rather than true fluxes.

Extraction of TAG was assumed to represent the action of LPL. This probably represents a small overestimation of true LPL action, as there is evidence for removal of intact lipoprotein particles in both adipose and forearm tissues (24). Fractional TAG extraction was calculated as the arteriovenous difference as a percentage of the arterial concentration. The rates of action of LPL and HSL were calculated on the basis of assumptions discussed in detail previously (9). The calculations were as follows

$$\text{LPL action} = (\text{a–v})_{\text{TAG}}$$

$$\text{HSL action} = (\text{v–a})_{\text{glycerol}} - \text{LPL action}$$

The rates of action of LPL and HSL calculated in this way are expressed as micromoles of glycerol per liter, rather than absolute activity units.

Table 1. *Nutrient composition of test meal*

	Carbohydrate	Fat	Protein
Dried diced onion, 4 g	2.8	0.1	0.4
Tomato puree, 27 g	3.5	0.1	1.2
Drained tinned tomatoes, 133 g	3.3	0.1	1.2
Intralipid 20%, 200 ml	0	40	0
Total	9.6	40.3	2.8

All nutrients measured in grams and determined from manufacturer's data and food tables (20).

The net inward flow of fatty acids from capillary to tissue (transcapillary exchange) was calculated as

$$3 \times (a\text{--}v)_{TAG} - (v\text{--}a)_{NEFA}$$

This represents the net movement of fatty acids across the capillary wall, expected to be outward (negative) in the fasting state and inward (positive), representing fat storage, in the postprandial state. This net exchange includes fatty acids derived from plasma TAG as well as plasma NEFA.

Overall reesterification of fatty acids within the tissue was calculated on the assumption that hydrolysis of each mole of TAG releases 1 mol of glycerol and 3 mol of fatty acid, as

$$\text{Overall tissue reesterification} = 3 \times (v\text{--}a)_{glycerol} - (v\text{--}a)_{NEFA}$$

where $3 \times (v-a)_{glycerol}$ represents total NEFA production, i.e., the sum of LPL and HSL action, and $(v-a)_{NEFA}$ represents NEFA release from adipose tissue. Reesterification of fatty acids calculated in this way includes fatty acids derived from both HSL and LPL action. For reesterification calculations, plasma NEFA concentrations (P) were converted to those in whole blood (B) with the hematocrit (H)

$$B = P \times (1 - H)$$

Repeated-measures analysis of variance (ANOVA) with SPSS (SPSS UK, Chertsey, UK) was used to test for the significance of changes in plasma concentrations and arteriovenous differences with time, studies (oral vs. intravenous fat loads), and time by study interactions. NEFA reesterification was tested by paired t-tests on areas under the curve.

RESULTS

There were no significant changes in either adipose tissue or forearm blood flow after either the oral fat load or intravenous fat infusion (Fig. 1). The results are therefore presented as arteriovenous concentration differences rather than fluxes.

Glucose concentrations were slightly higher after the oral fat load, and this persisted throughout the study (Fig. 2A). Insulin concentrations showed a very small rise to peak at 8.9 mU/l at 60 min after the oral fat load and a gradual decrease in concentration during and after the infusion (Fig. 2B).

3-Hydroxybutyrate concentrations rose after both the oral fat load and intravenous fat infusion, with concentrations increasing earlier with the infusion (Fig. 3).

TAG concentrations in arterialized plasma rose with both oral fat load and intravenous fat infusion, with a greater rise with the infusion (Fig. 4A). TAG arteriovenous differences in both forearm and adipose tissue increased with both oral fat load and intravenous infusion (Fig. 4, B and C), with no significant differences between oral and intravenous fat loads. The increase in TAG extraction appears to be delayed in forearm compared with adipose tissue, but this was not significant. The percentage extraction of TAG in adipose tissue was significantly less with the infusion than with the oral fat load (Fig. 5A). In the forearm, there was a significant increase in TAG fractional extraction
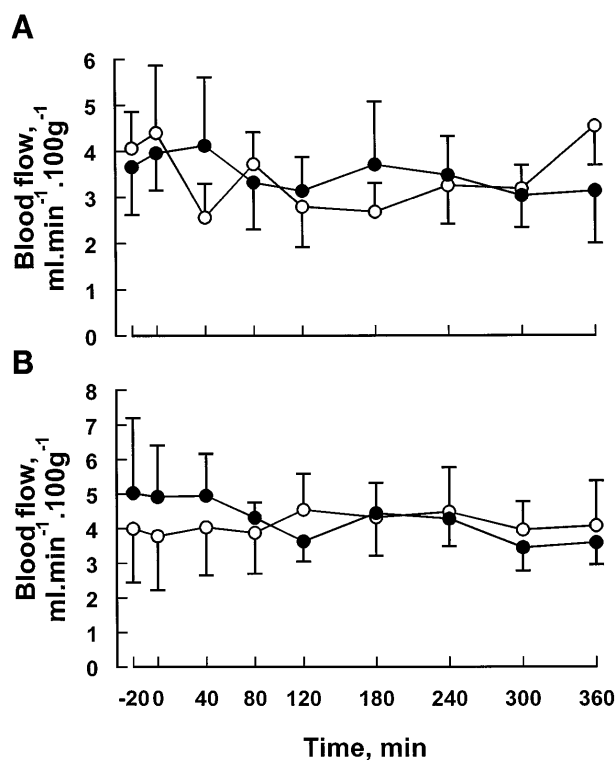

Fig. 1. Changes in adipose tissue (*A*) and forearm (*B*) blood flow with oral (●) and intravenous (○) fat load.

with both the infusion and oral fat load, with a greater increase after the oral fat load (Fig. 5B).

NEFA concentrations in arterialized plasma increased during the infusion and decreased to baseline after the end of the infusion (Fig. 6A). After the oral fat load, arterialized plasma NEFA concentrations decreased and then gradually returned to baseline (Fig. 6A). There were no significant changes in either arterio-adipose venous (Fig. 6B) or arterio-forearm venous concentration differences with time; neither were there any significant differences between the oral and intravenous fat loads. NEFA transcapillary exchange in adipose tissue (representing net fatty acid uptake) became significantly less negative with both oral and intravenous fat loads but showed no differences between oral and intravenous fat loads (Fig. 7A).

There was no significant reesterification of fatty acids in adipose tissue with either oral or intravenous fat load (Fig. 7B). There was no significant difference between oral and intravenous fat loads (repeated-measures ANOVA, $P = 0.2$ for meal effect).

With both the oral and intravenous fat loads, there was a fall in HSL action in adipose tissue to close to zero after 3–4 h, followed by an increase back to baseline (Fig. 8). There was no significant difference in HSL action between the oral and intravenous fat loads.

DISCUSSION

The most striking feature of this study is the downregulation of HSL action during infusion of Intralipid. This downregulation is also seen after a mixed meal,
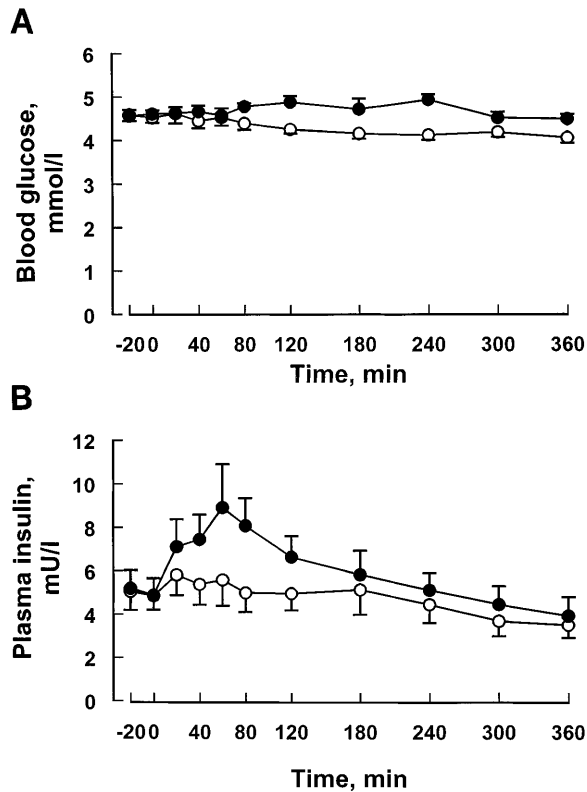
Fig. 2. Changes in glucose (*A*) and insulin (*B*) concentrations with oral (●) and intravenous (○) fat load. Repeated-measures ANOVA $P < 0.001$ for glucose time by study interaction; $P < 0.001$ for insulin time effect; and $P = 0.032$ for insulin time by study interaction.

Fig. 4. Changes in arterialized (*A*), adipose arteriovenous (a−v) difference (*B*) and forearm a−v difference (*C*) plasma triacylglycerol (TAG) concentrations with oral (●) and intravenous (○) fat load. Repeated-measures ANOVA, $P < 0.001$ (time effect) and $P < 0.001$ (time by study interaction) for arterialized plasma; $P = 0.002$ (time effect) for adipose a−v difference; $P = 0.001$ (time effect) for forearm a−v difference.

when there is increased insulin action and increased reesterification of fatty acids in adipose tissue. In our study, downregulation of HSL action occurred in the absence of increased insulin action and without fatty acid reesterification in adipose tissue.

Blood flow. The lack of response of adipose tissue and forearm blood flow to Intralipid is unusual (Fig. 1). Adipose tissue and forearm blood flow increases substantially after a meal (3, 39), although an increase in forearm blood flow has not been seen in all studies (10). Insulin is thought to act as a vasodilator of skeletal

muscle (2), although it is not clear whether insulin acts as a direct vasodilator in adipose tissue (39). The lack of blood flow response could be due to the lack of an insulin response after the intravenous lipid load and only a very small insulin response after the oral fat load (Fig. 2). A previous study showed a greater fall in systemic and calf vascular resistance after a high-fat meal together with insulin infusion when compared

Fig. 3. Changes in arterialized 3-hydroxybutyrate concentration with oral (●) and intravenous (○) fat load. Repeated-measures ANOVA, $P < 0.001$ for time effect and time by study interaction.
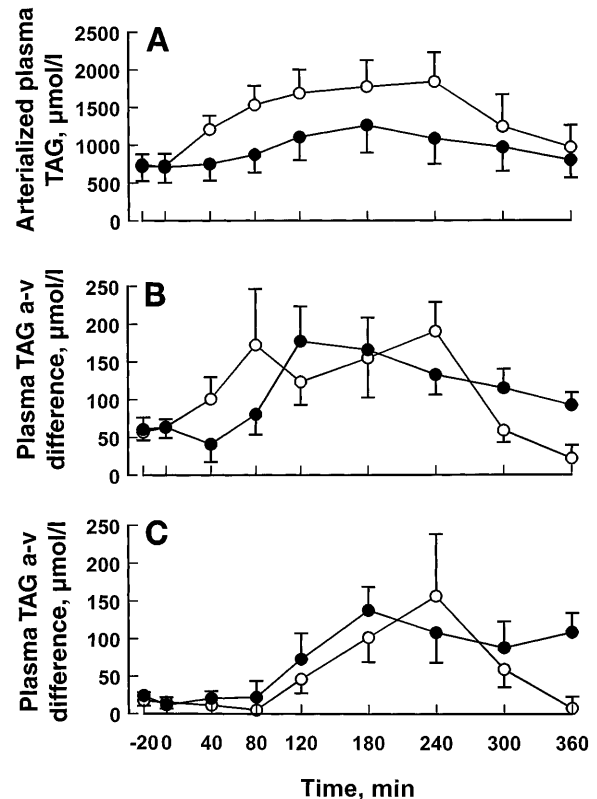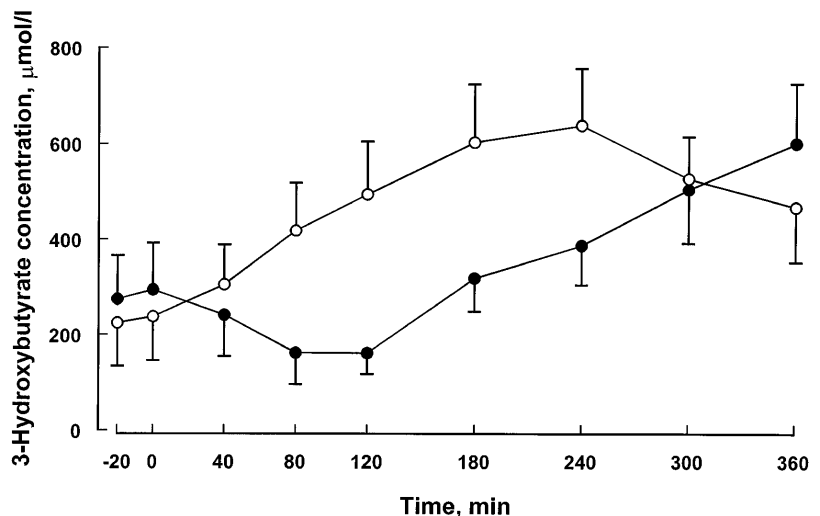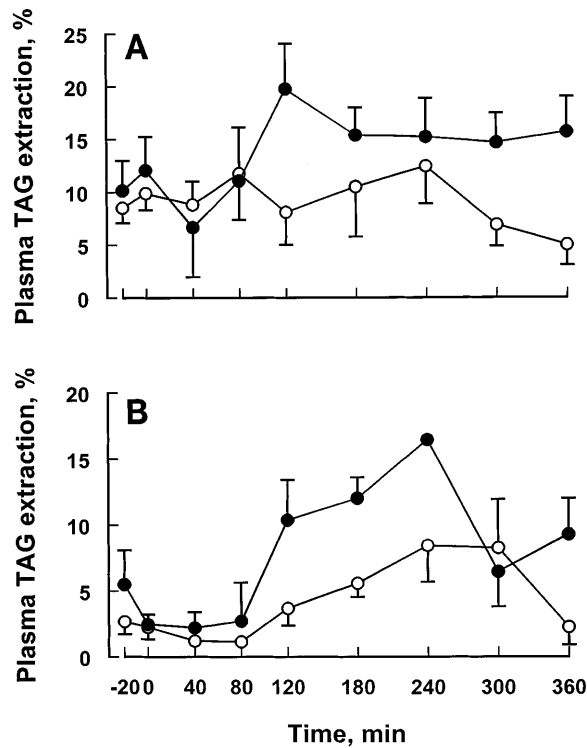
Fig. 5. Changes in percentage extraction of TAG in adipose tissue (*A*) and forearm (*B*) with oral (●) and intravenous (○) fat load. Repeated-measures ANOVA: adipose tissue, $P = 0.004$ for time by study interaction; forearm, $P = 0.001$ for time effect, and $P = 0.041$ for study effect.

with a high-fat meal alone (25). The lack of response of blood flow to an oral lipid load suggests that the usual increase in blood flow after a meal is not mediated by gut hormone stimulation due to the presence of food but may be a direct action of the rise in insulin concentrations after a mixed meal.

Glucose, insulin, and 3-hydroxybutyrate concentrations. The carbohydrate content of the meal can explain the slightly higher glucose concentrations and the small peak in insulin concentrations after the oral fat load (Fig. 2). This was necessary to make the intravenous lipid emulsion palatable for oral consumption. Intralipid contains a large amount of free glycerol. This may result in increased hepatic glucose production. After oral Intralipid, the glycerol is directed to the liver via the portal vein. There is therefore no increase in systemic arterial glycerol concentrations, in contrast to that seen with intravenous Intralipid infusion. It is not clear what effects, if any, this glycerol will have on the metabolism of Intralipid.

The increase in 3-hydroxybutyrate concentrations with both oral and intravenous lipid loads (Fig. 3) suggests a considerable increase in hepatic fatty acid oxidation. The earlier rise with intravenous infusion is probably a result of earlier delivery of TAG to the circulation. After a mixed meal, there is a fall in 3-hydroxybutyrate concentrations followed by a later rise (10).

TAG concentrations and clearance. The elevation of plasma TAG with both oral and intravenous load was

as expected (Fig. 4*A*). The fractional extraction of plasma TAG across adipose tissue after the oral fat load increased from a fasting level of 10% to a peak at ~20% (Fig. 5), in agreement with previous studies with mixed meals in healthy subjects (10). Whereas the fractional extraction of TAG was lower after intravenous infusion than after oral fat load, the absolute extraction as determined by TAG arteriovenous difference was the same (Fig. 4*B*).

The greater rise in plasma TAG concentrations with an intravenous fat load can be explained by reduced clearance (i.e., fractional extraction) as well as a more rapid delivery of TAG into the circulation. The delivery of the oral fat load to the circulation would be affected by digestion and absorption; therefore, the time course of delivery to the circulation may be different from that of the intravenous fat load. It is possible that with intravenous infusion the greater rate of delivery of TAG to the circulation saturated LPL. This is unlikely, as previous work has shown that TAG clearance is not saturated at the concentrations achieved in this study (28, 29), and steady-state concentrations of TAG, rather than the continuously rising TAG concentrations that would be expected if the clearance mechanisms were saturated, were obtained with the infusion. Reduced clearance of TAG with intravenous fat load implies impaired LPL action with intravenous fat load.

Intralipid particles are smaller than the larger chylomicrons (27), and larger particles may be cleared from the circulation more efficiently, both for chylomicrons (32) and emulsions (27). The reticuloendothelial system is an important site for TAG emulsion clearance from
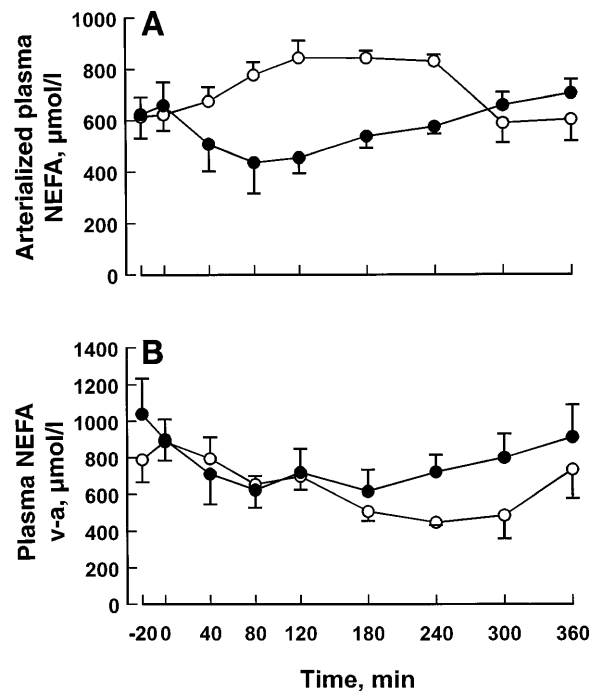

Fig. 6. Changes in arterialized (*A*) and adipose a−v difference (*B*) nonesterified fatty acid (NEFA) concentrations with oral (●) and intravenous (○) fat load. Repeated-measures ANOVA, $P < 0.001$ for time by study interaction for arterialized plasma; no significant differences for adipose a−v differences.
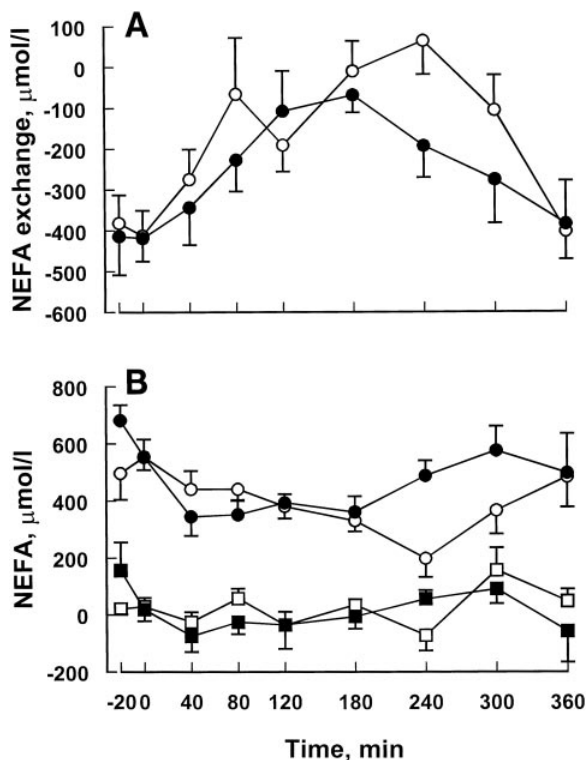
Fig. 7. Changes in transcapillary exchange of fatty acids in adipose tissue (*A*) with oral (●) and intravenous (○) fat load. Repeated-measures ANOVA $P < 0.001$ for time effect. *B*: reesterification of fatty acids in adipose tissue with oral (●, ■) and intravenous (○, □) fat load. ■ and □, reesterification; ● and ○, total fatty acid release. Area under the curve for reesterification, paired *t*-tests for difference from zero; $P = 0.7$ for oral fat load; $P = 0.13$ for intravenous fat load.

the plasma and may be more important for clearance of larger particles (27). There may be increased hepatic TAG clearance, and this is supported by the early and marked rise in plasma 3-hydroxybutyrate concentrations with intravenous lipid infusion (Fig. 3), a marker of increased hepatic fatty acid oxidation. Emulsion particles are more likely to be removed from the circulation with little prior lipolysis compared with chylomicrons (21).
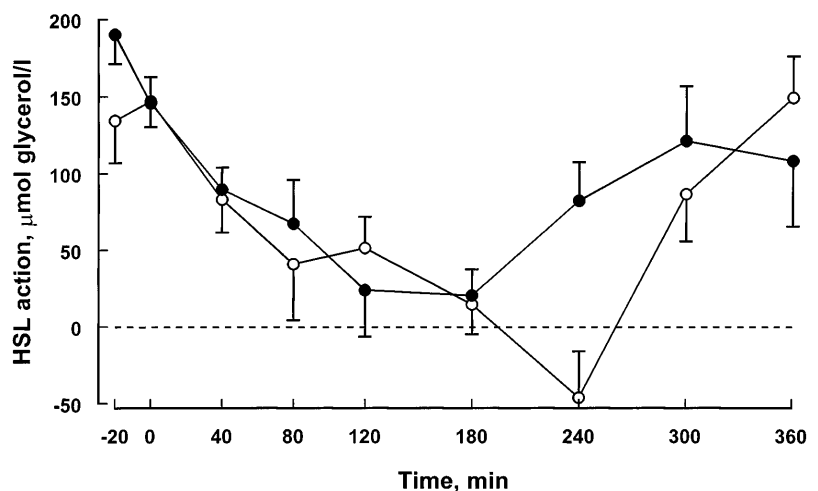
There is evidence that Intralipid particles in the circulation acquire the necessary apolipoproteins for removal (22), and it has been assumed that their clearance reflects that of chylomicrons (21). The present results suggest that removal of Intralipid particles by adipose tissue is less efficient than that of native chylomicrons. The small rise in insulin concentration after the oral fat load is unlikely to be sufficient to explain the difference between the oral and intravenous lipid loads.

Similar to the effects seen in adipose tissue, there was increased TAG extraction across skeletal muscle with both oral and intravenous fat loads, although again with less of an increase after intravenous fat load (Fig. 4*C*). These results are in agreement with previous results showing that, with an intravenous fat infusion, TAG is hydrolyzed and taken up into skeletal muscle (19), and there is an increase in muscle LPL activity with a high-fat diet (11). Muscle LPL activity either falls or shows no change after a mixed meal and falls during an euglycemic clamp (11).

NEFA concentrations. The rise in arterialized plasma NEFA concentrations during Intralipid infusion (Fig. 6*A*) is well recognized (28). This increase in NEFA concentration presumably arises from the hydrolysis of TAG by LPL in peripheral tissues, and this has been demonstrated by the change in composition of the NEFA pool during infusion of Intralipid or other exogenous TAG emulsions (36). During rapid hydrolysis of lipid emulsions, the high rate of TAG hydrolysis can lead to a large production of NEFA, which exceed the capacity of assimilation by tissues. The local accumulation of fatty acids inhibits LPL activity and displaces the enzyme from its endothelial binding sites (29), releasing it into the circulation (29). In such conditions, the rise of plasma NEFA concentration results from spillage of NEFA produced by endothelial lipolysis and possibly from additional TAG hydrolysis taking place directly into the circulation.

During intravenous Intralipid infusion, the rise in arterialized NEFA concentrations was not accompanied by an increase in NEFA release into the circulation

Fig. 8. Changes in action of hormone-sensitive lipase (HSL) in adipose tissue with oral (●) and intravenous (○) fat load. Repeated-measures ANOVA, $P < 0.001$ for time effect.

from adipose tissue (Fig. 6*B*), and the normally close relationship between arterial NEFA concentrations and rate of NEFA release from adipose tissue seen in previous studies (14) was not seen. This can be explained either by NEFA release from outside adipose tissue or by impairment of NEFA clearance during Intralipid infusion due to saturation of hepatic fatty acid esterification and oxidation pathways. After the oral fat load, arterialized NEFA concentrations were suppressed (Fig. 6*A*), although not to the same extent as after a mixed meal (10). It has previously been shown that even a small increase in insulin concentrations can produce significant decreases in plasma NEFA concentrations (5), and this would explain the suppression of NEFA concentrations after the oral fat load. The normal relationship between arterialized NEFA concentrations and NEFA release from adipose tissue was maintained.

Arteriovenous NEFA concentration differences across the forearm did not change with either oral or intravenous lipid load. This suggests that there is no release into the circulation of LPL-derived fatty acids from skeletal muscle. This efficient entrapment of fatty acids in skeletal muscle occurred despite a high-fat load and lack of an insulin response. This is in contrast to what is seen in adipose tissue, where there is no significant trapping of fatty acids in the tissue with either oral or intravenous fat load. The difference in LPL-derived fatty acid uptake may be explained by a fatty acid concentration gradient between the circulation and skeletal muscle (42) that is not present in adipose tissue. There are also differences in the control of LPL activity in these two tissues, with fasting reducing LPL activity in adipose tissue but having no effect or even increasing LPL activity in skeletal muscle (40).

LPL and HSL action and NEFA reesterification. Intracellular lipolysis, as shown by the calculated rate of HSL action, was suppressed almost completely with both oral and intravenous fat load (Fig. 8). Insulin is a major regulator of HSL activity, yet this showed only a slight increase after the oral lipid load and a gradual decrease during and after the intravenous load. It seems that suppression of HSL activity can occur without insulin. Catecholamines are important in controlling HSL action. There is evidence that α_2-adrenoceptor activation is strongly antilipolytic (16) and may account for the suppression of HSL activity without increased insulin action seen in this study. Alternatively, it may be that the fatty acids produced by LPL-mediated hydrolysis of chylomicrons or Intralipid particles caused suppression of HSL activity. There is some evidence, at least in cell-free systems, that fatty acids and their coenzyme A esters can inhibit HSL activity (23). In starvation, there is increased HSL activity together with elevated plasma NEFA concentrations. However, the situation in the current study differs in that we gave a large fat load, and suppression of HSL activity is therefore appropriate.

TAG extraction (a reflection of LPL action) in adipose tissue increased with both oral and intravenous lipid loads (Fig. 4*B*), similar to the pattern seen after a mixed meal. A marked increase in LPL activity has previously been seen with a pure fat infusion (30). This increase in LPL action (without an increase in insulin concentration) is in contrast to the lack of increase in LPL action in obese subjects (where there is a state of insulin resistance) (31), implying that the lack of an increase in LPL action in obese subjects cannot be simply a lack of effective insulin action. Overall NEFA release from adipose tissue into the circulation did not change with oral or intravenous Intralipid (Fig. 6*B*), despite HSL suppression, implying increased escape of LPL-derived fatty acids into the circulation. This is shown by the lack of reesterification of NEFA within adipose tissue (Fig. 7*B*). This is in contrast to what is seen after a mixed meal, when there is increased reesterification of LPL-derived fatty acids (15). With both oral and intravenous fat load, NEFA transcapillary flux across adipose tissue was strongly negative (outward flow) in the fasting state, becoming close to zero at 180- 240 min before becoming strongly negative again (Fig. 7*A*). After a mixed meal, NEFA transcapillary flux becomes positive after 60 min and stays strongly positive until at least 300 min (15). The different responses can be attributed partly to the lack of reesterification after fat loading and partly to the earlier return of HSL activity.

These results suggest that insulin is not essential for HSL suppression or LPL activation in the postprandial period but may be important in reesterification of NEFA in adipose tissue, thus affecting NEFA partitioning between adipose tissue and the circulation. Circulating plasma NEFA concentrations strongly influence hepatic VLDL and, therefore, apoB secretion. This provides a link between defective fatty acid reesterification in adipose tissue and hyper-apoB. The upregulation of reesterification by insulin has been shown previously in experiments involving both glucose infusion (43) and insulin infusion (6) and after the eating of a normal meal (9). Insulin may therefore be necessary to give precise control to the pathway of net fat storage. In contrast, fatty acid uptake into skeletal muscle occurs efficiently in the absence of increased insulin action, with no escape of LPL-derived fatty acids into the circulation, even in the presence of a high-fat load.

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