# Co-ordination of hepatic and adipose tissue lipid metabolism after oral glucose

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**Abstract The integration of lipid metabolism in the splanchnic bed and in subcutaneous adipose tissue before and after ingestion of a 75 g glucose load was studied by Fick's principle in seven healthy subjects. Six additional subjects were studied during a hyperinsulinemic euglycemic clamp. Release of non-esterified fatty acids (NEFA) from adipose tissue and splanchnic NEFA extraction followed a similar time-course after oral glucose, and there was a highly significant relationship between adipose tissue NEFA release and splanchnic NEFA uptake. There was no immediate inhibition of splanchnic very low density lipoprotein (VLDL)-triacylglycerol (TAG) output when plasma insulin levels increased after glucose. Adipose tissue extraction of VLDL-TAG tended to vary in time in a manner similar to splanchnic VLDL-TAG output and the two were significantly related. The area-under-curves (AUC) for splanchnic extraction of NEFA was significantly lower than that for output of VLDL, implying depletion of hepatic TAG stores during the experiment. In the hyperinsulinemic clamp experiments, there was on average suppression of splanchnic VLDL-TAG output although between-person variability was marked. This suppression could be explained by a very low supply of NEFA during the clamp. We conclude that there is an integrated pattern of metabolism in splanchnic and adipose tissues in the postabsorptive and post-glucose states. Flux of NEFA from adipose tissue drives splanchnic NEFA uptake. Splanchnic VLDL-TAG secretion appears to be regulated by a number of factors and in turn controls TAG extraction in adipose tissue. Insulin does not seem to play a key role in the acute regulation of hepatic VLDL metabolism under these particular conditions in vivo.**—Bülow, J., L. Simonsen, D. Wiggins, S. M. Humphreys, K. N. Frayn, D. Powell, and G. F. Gibbons. **Co-ordination of hepatic and adipose tissue lipid metabolism after oral glucose. *J. Lipid Res.* 1999.** 40: **2034–2043.**

**Supplementary key words** very low density lipoproteins • apolipoprotein B • particle size • splanchnic secretion • adipose tissue uptake • metabolic cycling

The regulation of lipid and lipoprotein metabolism in the liver is usually integrated with that of adipose tissue and the effectiveness of this relationship is essential for the maintenance of normolipidemia. Together, these tissues represent the poles of a large metabolic cycle in which metabolites of each tissue are used as substrates for the other. Thus, hepatic very low density lipoprotein (VLDL) is a precursor of triacylglycerols (TAG) stored in adipose tissue, and fatty acids released from adipose tissue TAG are important precursors of hepatic VLDL. The control of whole body lipid metabolism is, to a large extent, dependent on the efficient regulation of this cycle. In the postprandial state, insulin plays a major role in the co-ordination of whole-body lipid metabolism. In adipose tissue, insulin promotes TAG storage by stimulating plasma lipoprotein uptake (1) and preventing TAG release as fatty acids in the plasma (2). Studies, in vitro, with animal and human hepatocytes have suggested that insulin may also promote hepatic TAG storage by preventing TAG release as VLDL (3–5). On the basis of this latter work we proposed that the hepatic sensitivity to the inhibitory effect of insulin was a major determinant of lipemia, and, furthermore, in insulin-resistant subjects, that the decreased sensitivity to the inhibitory effect of insulin was an important contributory factor to the hypersecretion of VLDL (6, 7). Recent work in human subjects has provided support for this hypothesis. In those studies, although the secretion of VLDL apolipoprotein B (apoB) was suppressed during an euglycemic, hyperinsulinemic clamp in normal subjects, this was not the case in subjects with insulin resistance such as those who were obese (8) or diabetic (9).

Excessive postprandial lipemia is strongly associated with risk of coronary artery disease (10, 11). Elevated VLDL of hepatic origin is one component of postprandial lipidemia (12), and the studies described above suggested that, in insulin resistance, lipidemia may be potentiated by inefficient postprandial suppression of VLDL output

Abbreviations: VLDL, very low density lipoprotein; apoB, apolipoprotein B; TAG, triacylglycerol; NEFA, non-esterified fatty acids; 3-OHB, 3-hydroxybutyrate; AUC, area under curve.

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by insulin. The validity of this hypothesis depends, of course, on the assumption that the postprandial surge of insulin normally suppresses hepatic output of VLDL. Despite strong circumstantial evidence, this assumption has never been tested directly in vivo in humans.

We therefore sought to investigate the integration of lipid metabolism in the splanchnic bed and in adipose tissue with particular emphasis on events occurring in the period after glucose ingestion, and in additional studies during an euglycemic hyperinsulinemic clamp. Measurements of arterio-venous differences across the splanchnic bed have been previously used by other groups to determine VLDL-TAG output in the fasting state (13, 14). As far as we are aware, however, no such studies have been carried out after oral ingestion of carbohydrates, and they have not been combined with simultaneous studies of adipose tissue metabolism in vivo. Glucose was chosen due to the potency with which it stimulates insulin secretion and because there is no accompanying rise in plasma chylomicron concentration which would complicate the separation of VLDL from plasma. The major aims were therefore to investigate acute changes in secretion of VLDL from the splanchnic bed and how these integrate with changes in adipose tissue metabolism brought about by insulin acting on intracellular lipolysis and on extraction of VLDL mediated by increased lipoprotein lipase activity (15). Finally, the experimental protocol also provided us with the opportunity to examine the effects of glucose on the metabolism of fatty acids and ketone bodies across the splanchnic bed. This aspect is of particular importance in view of the current interest in the mechanisms underlying the interaction between hepatic fatty acid and glucose metabolism (16).

## METHODS

### Oral glucose experiments

Seven healthy subjects (two females, five males) mean age 28 years (range 23–33), mean weight 76.6 kg (range 68–83), and mean height 177.1 cm (range 171–188) were studied. The average body mass index was 24.4 $kg \cdot m^{-2}$ (range 22.1–27.1). The subjects gave their informed consent to participate in the study, which was approved by the local ethical committee of Copenhagen.

The experiment started at 8 am when the subjects arrived in the laboratory after an overnight fast. During the experiment, the subjects rested supine at a room temperature of 22°–24°C. A polyethylene catheter (1.2 mm outer diameter) was inserted percutaneously into a subcutaneous vein on the anterior abdominal wall draining mainly adipose tissue. The catheterization was performed by ultrasound Colour-Doppler guidance, because this enables catheterization of veins deep in the subcutaneous adipose tissue which are not visible through the skin. This catheterization was performed in 5 of the 7 experimental subjects.

The right femoral vein was catheterized during local analgesia (Lidocain 1%, 5–10 ml) and a polyethylene catheter (outer diameter 2.0 mm) was advanced to a right-sided hepatic vein and left in situ during the rest of the experiment. The catheterization was done during fluoroscopic control, and the position of the catheter was regularly controlled by fluoroscopy during the experiment. A catheter was inserted into the radial artery of the non-dominant arm and a cubital vein was also catheterized for infusion of indocyanine green (ICG). After the catherization procedures, a primed/constant infusion of ICG was started and 60 min after, baseline measurements were performed. The experiment consisted of an initial 30 min basal control period in the fasting state in which three determinations of metabolite and hormone concentration were made. Simultaneously splanchnic blood flow and adipose tissue blood flow were determined and whole body energy expenditure was measured. After the control period, 75 g glucose dissolved in tap water with addition of a little lime flavor was given and the measurements were continued for 5 h with blood sampling from the artery, hepatic vein, and adipose vein every 30 min.

### Insulin clamp experiments

Six healthy subjects (one female, five males) were studied in this series. The mean age was 24.5 years (range 22–27), mean height 186.8 cm (range 176–195), and mean weight 85.8 kg (range 63–114). The average body mass index was 24.5 $kg \cdot m^{-2}$ (range 20.3–31.2).

The experiment began at 8:00 am after an overnight fast. The subjects were catheterized as described above, except with respect to the subcutaneous adipose tissue which was not examined in this series. When steady state arterial concentrations of ICG had been established, a 60-min basal control period was begun, in which four determinations of metabolite and hormone concentrations were made. Thereafter a primed/constant infusion of insulin was begun at a rate of 4 $mU \cdot kg^{-1}/50\ mU \cdot m^{-2} \cdot min^{-1}$. Arterial glucose concentrations were measured every 5 min during the insulin infusion, and an infusion of glucose (500 g glucose monohydrate$\cdot l^{-1}$) in the femoral vein was adjusted accordingly, to keep the blood glucose concentration constant at the fasting control level. When a new steady state had been reached with respect to a constant glucose disposal rate, a 60-min period was begun in which another four determinations of metabolite and hormone concentrations were done.

### Measurements

*Splanchnic blood flow* was measured by continuous infusion of ICG (13). Immediately after the catheterization a priming dose of ICG was given followed by a continuous infusion for the rest of the experiment. After 45–60 min a steady arterial ICG concentration is normally achieved, and the control period was then begun. Blood samples were then drawn every 30 min for determination of splanchnic blood flow during the rest of the experiment. At each time point three samples were drawn within 10 min in order to enable correction for possible quasi-steady state in the arterial concentration of ICG. The plasma ICG concentration was determined by spectrophotometry at 805 and 904 nm. The coefficient of variation of the plasma flow measurements was 8%.

*Adipose tissue blood flow* was determined by the $^{133}$Xe-washout method as previously described (14). About 0.5–1 MBq $^{133}$Xe dissolved in 0.1 ml isotonic sodium chloride was injected into the subcutaneous adipose tissue region drained by the vein that was catheterized. The washout rate of $^{133}$Xe was measured continuously during the experiment by a scintillation counter system strapped to the skin surface above the $^{133}$Xe depot (Oakfield Instruments, Oxford, UK). The adipose tissue blood flow was calculated from the washout rate constant determined in the particular experimental period assuming a tissue/blood partition coefficient for Xe of 8 $ml \cdot ml^{-1}$ in these subjects (15).

*Whole body energy expenditure.* Whole body oxygen uptake and carbon dioxide output were measured continuously by an open circuit ventilated-hood system (Oxycon, Jaeger) during all experiments (16). Energy expenditure was calculated as described previously (17).

*Metabolic measurements.* Insulin concentrations in plasma were determined in duplicates using a commercial radioimmunoassay

(RIA) kit (NOVO Nordic, Bagsvaerd, Denmark). Glycerol, glucose, and lactate were measured in duplicates in neutralized perchloric extracts of whole blood and NEFA, total TAG and 3-hydroxybutyrate (3-OHB) in duplicates in heparinized plasma with enzymatic methods as previously described (18–20). VLDL was separated by ultracentrifugation. Approximately 0.5 ml of plasma (in triplicate at each time point and each site in the glucose experiments and in duplicate in the insulin clamp experiments) was layered under a solution of density 1.006 g/ml in pre-weighed Beckman 6 ml polyallomer bell-top tubes. The tubes were re-weighed and the volume of plasma was obtained by difference. The filled tubes were heat-sealed and ultracentrifuged for 16 h at 40,000 rpm in a 44-place Beckman 50.4 Ti rotor in two concentric rings, the inner ring giving 145,000 *g* on average and the other ring giving 172,000 *g* on average. The VLDL was isolated by removing the top layer (approx. 1.3 ml) after slicing. All fractions were weighed to estimate their volume and, thus, to allow calculation of the corresponding concentrations of VLDL-TAG and apoB in the original plasma. ApoB was determined by an ELISA method (21). VLDL-TAG was determined as described previously (20). The coefficient of variation of the VLDL-TAG measurements was 1.7%. Each of the duplicate plasma samples was analyzed in triplicate for VLDL apoB.

### Calculations

Splanchnic blood flow and subcutaneous adipose tissue blood flow were calculated as previously described (14, 22). Net fluxes of the different metabolites across the splanchnic and subcutaneous adipose tissues were calculated by multiplication of the appropriate flow value by the a-v concentration difference of the metabolite (whole blood flow for calculation of glycerol, glucose, and lactate fluxes and plasma flow for calculation of NEFA, TAG, 3-OHB, VLDL-TAG, and apoB fluxes).

The activities of the hormone-sensitive lipase (HSL) and the lipoprotein lipase (LPL) were estimated by comparing the glycerol output from the subcutaneous adipose tissue with the TAG clearance. The HSL activity was taken to be reflected in the difference between the glycerol output and the TAG clearance, while the TAG clearance reflected the LPL activity (23). The latter assumption is, however, only partly correct, as it has been found that a small fraction of lipoprotein remnant particles may be removed by whole particle uptake in adipose tissue (24).

### Statistics

Data were analyzed using SPSS for Windows Release 8.0 (SPSS Inc., Chicago, IL). All the data are presented as mean ± SEM. The results from the glucose experiments were analyzed by repeated-measures ANOVA with time as a within-subject factor. Areas-under-curves (AUCs) were calculated by the trapezoid method for splanchnic output and extraction of metabolites over the whole experiment. Kendall's rank correlation method was used to assess relationships between AUCs or fasting values. The significance of net substrate uptake or release was analyzed by comparing either mean values or AUCs with zero using a paired *t*-test. Analysis of covariance with subject as a factor was used to analyze relationships between variables, each of which changed with time (25). In the insulin clamp experiments the values given are the mean of four measurements performed during basal conditions and during the clamp. A paired *t*-test was used to compare basal values with clamp values.

## RESULTS

### Oral glucose experiments

*Whole body response.* The arterial concentrations of different metabolites are given in **Table 1**. The glucose con-

TABLE 1. Whole energy expenditure, RQ, and arterial concentration of different metabolites

| | Time in Minutes after the Glucose Load | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Basal | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 |
| Energy expend., kJ/min[b] | 4.63 ± 0.24 | 4.93 ± 0.22 | 5.04 ± 0.22 | 4.97 ± 0.24 | 4.90 ± 0.24 | 4.86 ± 0.22 | 4.78 ± 0.20 | 4.73 ± 0.24 | 4.66 ± 0.21 | 4.67 ± 0.21 | 4.75 ± 0.25 |
| RQ[b] | 0.83 ± 0.02 | 0.81 ± 0.03 | 0.87 ± 0.02 | 0.89 ± 0.02 | 0.88 ± 0.01 | 0.89 ± 0.01 | 0.90 ± 0.01 | 0.88 ± 0.02 | 0.86 ± 0.01 | 0.81 ± 0.02 | 0.80 ± 0.03 |
| Concentration | | | | | | | | | | | |
| Glucagon, ng/l[b] | 33 ± 5 | 33 ± 5 | 30 ± 6 | 29 ± 6 | 27 ± 5 | 28 ± 6 | 36 ± 5 | 42 ± 6 | 43 ± 7 | 42 ± 5 | 45 ± 4 |
| Glucose, mmol/l[b] | 5.21 ± 0.07 | 8.12 ± 0.74 | 8.88 ± 0.65 | 8.03 ± 0.42 | 7.11 ± 0.65 | 6.45 ± 0.65 | 4.96 ± 0.31 | 5.04 ± 0.54 | 5.12 ± 0.35 | 5.07 ± 0.32 | 5.05 ± 0.26 |
| Glycerol, μmol/l[b] | 57 ± 10 | 39 ± 7 | 32 ± 6 | 33 ± 8 | 30 ± 7 | 36 ± 5 | 43 ± 6 | 68 ± 13 | 77 ± 14 | 74 ± 10 | 118 ± 9 |
| NEFA, μmol/l[b] | 669 ± 126 | 495 ± 121 | 321 ± 119 | 272 ± 95 | 232 ± 89 | 208 ± 77 | 209 ± 51 | 462 ± 81 | 509 ± 107 | 686 ± 118 | 911 ± 149 |
| Hydroxybutyrate, μmol/l[b] | 47 ± 8 | 32 ± 10 | 24 ± 9 | 21 ± 9 | 16 ± 6 | 14 ± 4 | 13 ± 4 | 24 ± 14 | 80 ± 23 | 116 ± 38 | 231 ± 67 |
| VLDL TAG, μmol/l[a] | 259 ± 53 | 262 ± 51 | 271 ± 63 | 260 ± 61 | 271 ± 61 | 299 ± 76 | 308 ± 68 | 326 ± 82 | 316 ± 74 | 342 ± 77 | 369 ± 66 |
| VLDL apoB, mg/l[b] | 18.8 ± 3.6 | 17.7 ± 3.6 | 17.8 ± 5.3 | 17.9 ± 4.4 | 18.3 ± 5.9 | 17.0 ± 3.8 | 18.6 ± 3.3 | 18.2 ± 3.9 | 22.4 ± 2.9 | 23.4 ± 3.8 | 28.0 ± 4.5 |

Changes with time significant by repeated measures ANOVA: [a] $P < 0.05$; [b] $P < 0.01$.

centration increased immediately after the glucose load. The concentrations of glycerol, NEFA, and 3-OHB decreased just after the glucose load and then increased significantly at the end of the experiment. The arterial VLDL-TAG concentration was constant at the basal level during the first 2–3 h after the glucose intake and then it increased significantly. The arterial VLDL apoB concentration was on average constant during the first 4 h of the study and increased during the last hour.

The arterial and hepatic vein insulin concentrations are shown in **Fig. 1**. The arterial concentration increased from 20 ± 3 pmol·l$^{-1}$ to 155 ± 26 pmol·l$^{-1}$ 60 min after the glucose intake and then decreased steadily to reach the pre-glucose level 180 min post-glucose. The hepatic venous concentration had a similar course, reaching a maximum of 245 ± 47 pmol·l$^{-1}$ 60 min post-glucose.

Both energy expenditure and RQ increased from the basal level and then declined to pre-glucose level in the end of the study.

*Splanchnic tissues.* In the fasting state, there was significant splanchnic extraction of NEFA ($P = 0.02$), glycerol ($P < 0.001$), and glucose ($P = 0.023$) with release of VLDL-TAG ($P = 0.006$) and 3-OHB ($P = 0.003$). There were significant positive correlations between fasting spanchnic extraction of NEFA and release of VLDL-TAG ($P = 0.015$), and between NEFA extraction and VLDL-apoB output ($P = 0.039$).

The splanchnic vascular and metabolic response to the oral glucose load is given in **Table 2**, **Fig. 2** and **Fig. 3**. The splanchnic plasma flow increased 30 and 60 min after the glucose intake and decreased again to the pre-glucose level at 150 min. Splanchnic glucose output increased 30 min after the glucose intake to decrease again to the pre-glucose level after 90 min, and it stayed at this level during the rest of the experiment.

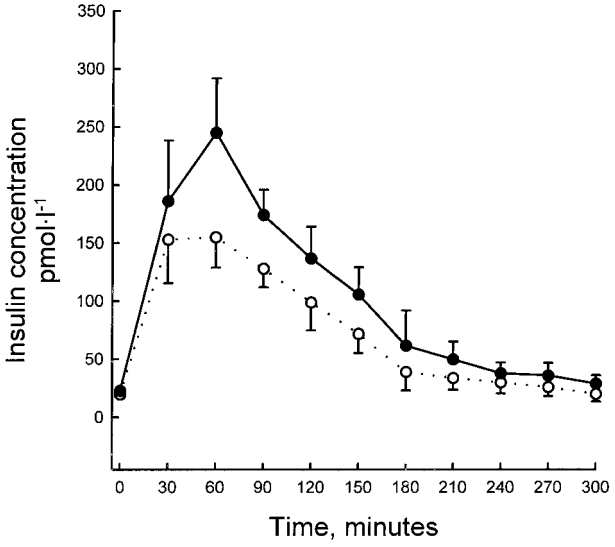


**Fig. 1.** Insulin concentrations in arterial (open points) and hepatic vein (solid points) plasma before (time 0) and after a 75 g oral glucose load. Results of repeated-measures ANOVA: changes with time significant at $P < 0.001$.

TABLE 2. Splanchnic plasma flow and net substrate fluxes across the splanchnic bed

| | Time in Minutes after the Glucose Load | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Basal | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 |
| Plasma flow ml/min[a] | 839 ± 63 | 1003 ± 34 | 1001 ± 36 | 893 ± 34 | 929 ± 53 | 8 53 ± 46 | 872 ± 64 | 873 ± 70 | 920 ± 59 | 916 ± 53 | 930 ± 57 |
| Glucose output, mmol/min[a] | 0.95 ± 0.31 | 2.83 ± 0.76 | 1.73 ± 0.38 | 1.10 ± 0.23 | 1.00 ± 0.23 | 0.97 ± 0.28 | 1.18 ± 0.46 | 1.06 ± 0.34 | 1.06 ± 0.27 | 1.07 ± 0.40 | 0.83 ± 0.33 |
| Glycerol uptake, μmol/min[b] | 47 ± 4 | 29 ± 6 | 21 ± 4 | 10 ± 9 | 14 ± 2 | 15 ± 4 | 31 ± 6 | 54 ± 8 | 64 ± 12 | 67 ± 9 | 109 ± 19 |
| VLDL-apoB output mg/min | 1.8 ± 1.0 | 3.9 ± 2.7 | 2.0 ± 1.6 | 3.0 ± 1.6 | 0.6 ± 0.9 | 2.1 ± 1.7 | 1.7 ± 0.9 | 4.9 ± 2.2 | 2.9 ± 1.3 | 2.1 ± 0.9 | −1.4 ± 1.4 |
| Hydroxybutyrate output, μmol/min[b] | 29 ± 8 | 17 ± 8 | 8 ± 6 | 3 ± 1 | 4 ± 2 | 6 ± 2 | 10 ± 3 | 23 ± 11 | 54 ± 23 | 57 ± 24 | 121 ± 52 |

Changes with time significant by repeated measures ANOVA.
[a] $P < 0.05$.
[b] $P < 0.01$.

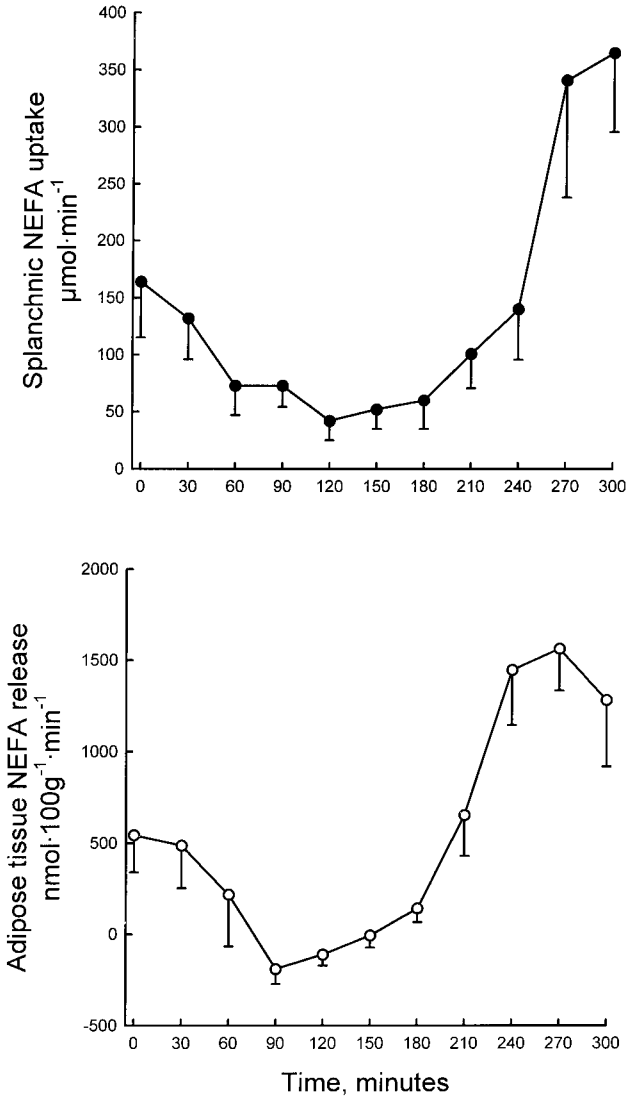


**Fig. 2.** Splanchnic NEFA output (solid points) and adipose tissue NEFA release (open points) before (time 0) and after a 75 g oral glucose load. Results of repeated-measures ANOVA: changes with time significant at $P < 0.001$ for both adipose tissue NEFA output and splanchnic NEFA ouput. Adipose tissue NEFA output and splanchnic uptake were highly related within subjects ($P < 0.001$ by analysis of covariance: see text for more details).

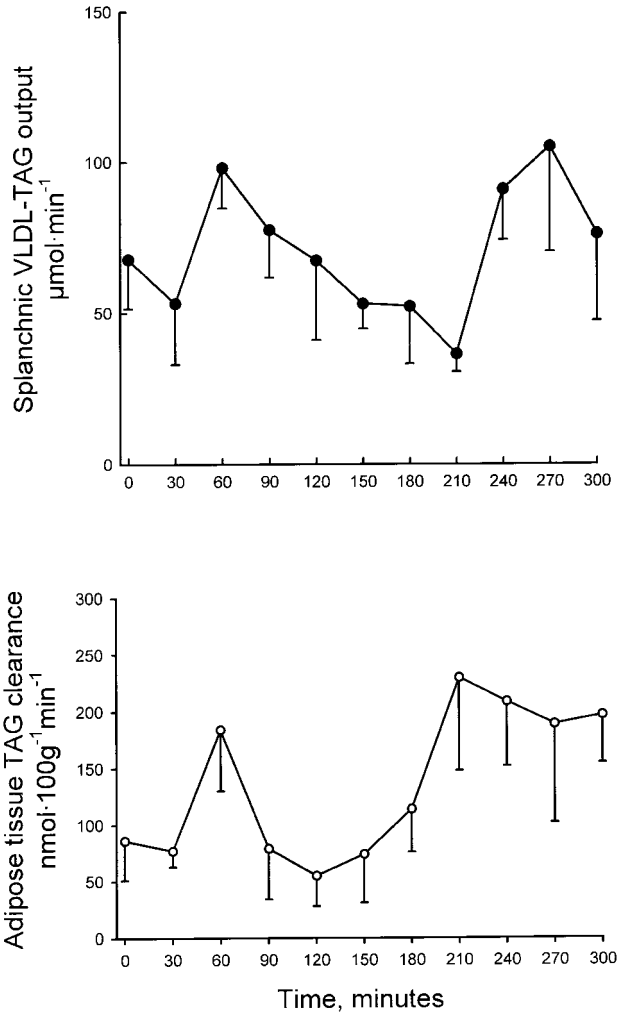


**Fig. 3.** Splanchnic VLDL-TAG output (solid points) and adipose tissue TAG clearance (open points) before (time 0) and after a 75 g oral glucose load. Results of repeated-measures ANOVA: changes with time significant for splanchnic VLDL output at $P = 0.017$, and for adipose tissue TAG clearance at $P = 0.042$.

The splanchnic glycerol uptake was reduced by the glucose intake, decreasing from 47 ± 4 μmol·min$^{-1}$ to 10 ± 9 μmol·min$^{-1}$ at 90 min, and it then increased to reach 109 ± 19 μmol·min$^{-1}$ at 300 min. Similarly, the NEFA uptake decreased from the pre-glucose value of 164 ± 49 μmol·min$^{-1}$ to a minimum of 42 ± 17 μmol·min$^{-1}$ 120 min post-glucose (Fig. 2). In the last part of the experiment, from 270–300 min, the NEFA uptake increased to around 350 μmol·min$^{-1}$. The splanchnic 3-OHB output was reduced by the glucose intake, decreasing from 29 ± 8 μmol·min$^{-1}$ to 3 ± 1 μmol·min$^{-1}$ at 90 min, and it then increased to reach 121 ± 52 μmol·min$^{-1}$ at 300 min.

VLDL-TAG output (Fig. 3) increased at 60 min post-glucose, then decreased to a value of 36 ± 6 μmol·min$^{-1}$ at 210 min, and increased again at the end of the experiment. There was a significant output of VLDL-apoB at about 2 mg·min$^{-1}$ during the whole experiment ($P = 0.005$). It was not possible to demonstrate any significant change in this.

The mean AUC for NEFA extraction was 36.8 mmol, that for VLDL-TAG output was 21.6 mmol, and that for 3-OHB output was 7.3 mmol. If it is assumed that 3 moles of NEFA may form 1 mole of TAG or 3 × 8 of 3-OHB, then VLDL-TAG output (equivalent to 64.9 mmol NEFA) exceeded NEFA uptake ($P = 0.004$ by paired $t$-test), and 3-OHB output (equivalent to 0.90 mmol NEFA) was very small in this predominantly post-glucose experiment. As in the fasting data, there was a significant positive correlation between the AUCs for splanchnic NEFA extraction and VLDL-TAG output ($P = 0.039$).

The mean AUC for VLDL-apoB output was about 700 mg (SEM 160 mg). Assuming a relative molecular mass of 513 kDa for apoB-100, this is equivalent to 1.36 μmol apoB-100. The ratio of AUCs for splanchnic VLDL-TAG

and apoB output suggests that, on average, during this experiment particles released from the splanchnic bed contained 18,700 (SEM 3,600) molecules of TAG. In contrast, the average arterial VLDL particle composition over the period of the experiment based on the ratio of AUCs for arterial VLDL-TAG and VLDL-apoB was 9,100 molecules of TAG per apoB (SEM 1,900), significantly lower than the value for splanchnic output ($P = 0.01$ by paired *t*-test). However, it must be emphasized that the above calculations are approximate estimates. Thus, the numbers should only be interpreted as showing tendencies in the changes occurring in the particle size.

*Adipose tissue.* The subcutaneous adipose tissue vascular and metabolic response to the oral glucose load is given in **Table 3**.

Adipose tissue blood flow increased after the glucose intake from 1.6 ± 0.3 ml·100 $g^{-1}$·$min^{-1}$ pre-glucose to a maximum of 3.8 ± 0.6 ml·100 $g^{-1}$·$min^{-1}$ post-glucose. The blood flow stayed increased during the rest of the experiment.

Adipose tissue glucose uptake did not change significantly during the course of the study. Adipose tissue glycerol output was 224 ± 56 μmol·100 $g^{-1}$·$min^{-1}$ in the fasting period. After glucose it was reduced to half the basal value to increase again 210 min post-glucose. At the end of the study, the output increased to reach a value 2- to 3-fold the pre-glucose value.

The NEFA output was 543 ± 202 μmol·100 $g^{-1}$·$min^{-1}$ in the fasting control period (Fig. 2). It decreased to zero after the glucose intake and had a tendency to be a NEFA uptake, with a value 90 min post-glucose at −190 ± 75 μmol·100 $g^{-1}$·$min^{-1}$ ($P = 0.057$). It then increased to reach values 2- to 3-fold the fasting control value at the end of the experiment. The mean AUC for NEFA release was 161 μmol·100 $g^{-1}$. There was a striking parallel between adipose tissue NEFA release and splanchnic NEFA uptake (Fig. 2). The relationship between adipose tissue NEFA release and splanchnic NEFA uptake was highly significant by analysis of covariance ($P < 0.001$) with subject as a factor (25).

The proportion of fatty acids re-esterified in adipose tissue was calculated from the ratio of NEFA to glycerol release. It peaked at 90 min at a value greater than 100%, reflecting the tendency to net uptake of plasma NEFA, and towards the end of the study returned towards zero (**Fig. 4**). This implies that almost all the fatty acids released by HSL and LPL action were delivered into the circulation. The hydrolysis of plasma TAG by LPL would then have been sufficient to provide around one-third of the NEFA released by adipose tissue (average over 240–300 min 34%, 95% confidence intervals 10–59%).

Adipose tissue showed a small but significant ($P = 0.016$) uptake of 3-OHB in the fasting state. It decreased to zero after the glucose intake and then increased at the end of the experiment.

There was significant extraction both of total plasma TAG ($P = 0.01$) and of VLDL-TAG ($P = 0.027$) in adipose tissue. Extraction of plasma TAG by adipose tissue changed significantly with time ($P = 0.042$), decreasing by 2 h and then increasing again (Fig. 3). Although the

TABLE 3. Adipose tissue blood flow and substrate fluxes across the subcutaneous adipose tissue

| | Time in Minutes after the Glucose Load | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Basal | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 |
| Blood flow, ml/min[a] | 1.6 ± 0.3 | 2.2 ± 0.4 | 3.8 ± 0.6 | 3.7 ± 0.6 | 3.4 ± 0.8 | 2.7 ± 0.5 | 2.7 ± 0.5 | 2.8 ± 0.6 | 2.8 ± 0.6 | 3.0 ± 0.7 | 3.4 ± 0.7 |
| Glucose uptake μmol/100 g/min | 0.5 ± 0.3 | 1.4 ± 0.1 | 0.6 ± 1.0 | 1.1 ± 0.8 | 1.3 ± 0.7 | 1.0 ± 0.2 | −0.2 ± 0.4 | −0.0 ± 0.2 | 0.8 ± 0.7 | 0.6 ± 0.4 | 0.6 ± 0.6 |
| Glycerol output, nmol/100 g/min[b] | 224 ± 56 | 115 ± 32 | 113 ± 15 | 92 ± 11 | 98 ± 17 | 116 ± 29 | 138 ± 40 | 427 ± 74 | 579 ± 90 | 514 ± 74 | 732 ± 173 |
| VLDL-TAG uptake, nmol/100 g/min[a] | 43 ± 17 | 42 ± 30 | 116 ± 42 | 120 ± 32 | 75 ± 24 | 75 ± 23 | 37 ± 41 | 85 ± 25 | 124 ± 73 | 18 ± 36 | 144 ± 31 |
| Hydroxybutyrate uptake, nmol/100 g/min[a] | 11 ± 6 | 1 ± 3 | −1 ± 5 | 9 ± 3 | −4 ± 4 | −4 ± 10 | 1 ± 2 | 9 ± 2 | 45 ± 27 | 91 ± 60 | 74 ± 34 |

Changes with time significant by repeated measures ANOVA.
[a] $P < 0.05$.
[b] $P < 0.01$.

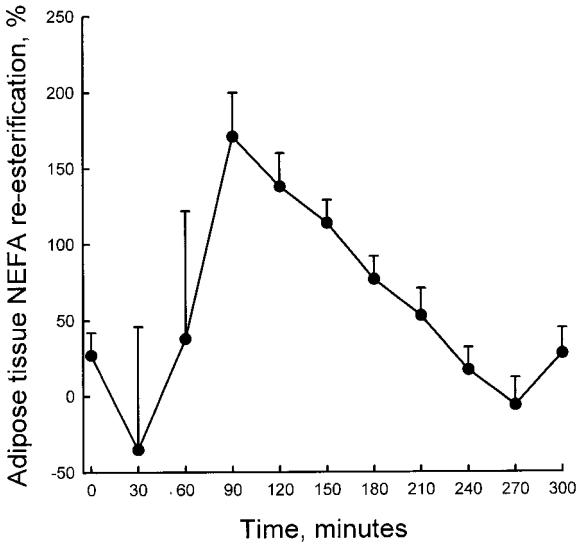


**Fig. 4.** Fractional re-esterification of fatty acids in adipose tissue before (time 0) and after a 75 g oral glucose load. Fractional re-esterification was calculated from rates of glycerol and NEFA release (see Methods). Values greater than 100% reflect net extraction of plasma NEFA by adipose tissue.

changes in VLDL-TAG uptake with time were not significant, the extraction of VLDL-TAG by adipose tissue tended to vary in time in a similar manner to splanchnic VLDL-TAG output. The two were significantly related by analysis of covariance ($P = 0.032$).

### Insulin clamp experiments

The results from the insulin clamp experiments are given in **Table 4**. On average, insulin decreased splanchnic VLDL-TAG output. However, due to large interindividual differences, the decrease could not be demonstrated to be significant. In five subjects insulin decreased the splanchnic VLDL-TAG output. In one subject the output increased during the insulin clamp. The individual VLDL-TAG output values are given in **Fig. 5**. The glucose disposal rate was on average 7.1 ± 2.4 $mg \cdot kg^{-1} \cdot min^{-1}$ (range 3.5–10.0). The arterial concentrations of VLDL-TAG, insulin, NEFA, and glucose are also given. Insulin gave rise to a decrease in the arterial VLDL-TAG and NEFA concentrations. The glucose concentration was virtually constant during the experiment. The hepatic venous insulin concentration was on average 32% of the arterial concentration implying that the liver extracted 68% of the insulin in the first passage.

## DISCUSSION

This is to our knowledge the first time the effect of an oral glucose load, with respect to lipoprotein metabolism, has been measured simultaneously across the splanchnic bed and the subcutaneous adipose tissue. The main findings of the present study are that there was no immediate inhibition of splanchnic VLDL-TAG output when plasma insulin levels increased after oral glucose, and that there is a tight co-ordination of adipose tissue and splanchnic lipid metabolism in this situation. The former finding clearly differs from that observed after direct exposure of isolated liver preparations to insulin in vitro (26–30), conditions under which there is general agreement that VLDL output is suppressed. The finding also differs from that observed after exogenous insulin infusion to human subjects in vivo (8, 9).

A major difference between the present experiments with oral glucose and those in which insulin is infused is that in the former the liver is exposed to high glucose and insulin levels simultaneously. In euglycemic insulin clamp experiments, the liver is only exposed to high insulin concentrations. Therefore, we performed the euglycemic clamp experiments to define the effects of insulin alone on the splanchnic production of VLDL-TAG. The circulating level of insulin aimed for in these experiments was the level that the peak portal vein reached in the glucose experiments. This concentration was estimated from the peak concentration measured in the hepatic vein assuming a 60% hepatic extraction of insulin and assuming that 25% of the splanchnic blood flow comes from the hepatic artery and 75% from the portal vein. These experiments showed that acute hyperinsulinemia generally had an inhibitory effect on splanchnic VLDL output in the majority of the subjects but that there were individual differences (Fig. 5). The arterial concentration of NEFA declined considerably during the experiments, probably as a result of the insulin-mediated suppression of adipose tissue lipolysis (19, 31). Under these conditions, splanchnic uptake of

TABLE 4. Whole body energy expenditure, respiratory quotient, splanchnic blood flow, arterial concentrations, and net splanchnic fluxes of different metabolites during the insulin experiments

| Variable | Fasting | Insulin clamp | Significance |
|---|---|---|---|
| Energy expend., kJ/min | 4.93 ± 0.32 | 5.26 ± 0.40 | $P = 0.12$ |
| Respiratory quotient | 0.81 ± 0.02 | 0.90 ± 0.02 | $P < 0.02$ |
| Splanchnic plasma flow, ml/min | 853 ± 38 | 954 ± 43 | $P < 0.02$ |
| Arterial glucose conc., mmol/l | 5.26 ± 0.15 | 5.03 ± 0.10 | $P = 0.14$ |
| Arterial NEFA conc., μmol/l | 656 ± 38 | 25 ± 12 | $P < 0.01$ |
| Arterial insulin conc., pmol/l | 32 ± 5 | 590 ± 30 | $P < 0.01$ |
| Hepatic venous insulin conc., pmol/l | 56 ± 10 | 186 ± 14 | $P < 0.01$ |
| Arterial VLDL-TAG conc., μmol/l | 538 ± 149 | 360 ± 125 | $P < 0.01$ |
| Splanchnic glucose output, mmol/min | 0.75 ± 0.09 | −0.32 ± 0.11 | $P < 0.01$ |
| Splanchnic NEFA uptake, μmol/min | 190 ± 20 | 10 ± 6 | $P < 0.01$ |
| Splanchnic VLDL-TAG output, μmol/min | 21.4 ± 4.3 | 12.9 ± 7.2 | $P = 0.21$ |

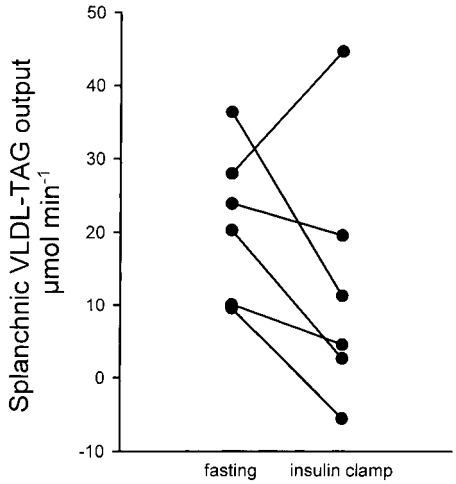


**Fig. 5.** Splanchnic VLDL-TAG output in each subject in the euglycemic hyperinsulinemic clamp experiment.

NEFA was virtually abolished (Table 4), and the reduced NEFA flux to the liver was probably a major contributor to the decline in VLDL-TAG output. Others have found that when hepatic NEFA flux is not limiting, acute exposure of the liver to increased insulin during an euglycemic clamp directly suppressed VLDL-TAG output (32). As the circulating NEFA concentrations were higher in the glucose experiments than in the insulin clamp experiments, a likely explanation of the difference of the effect on the VLDL secretion could be that the NEFA supply in the former experiments was sufficient to maintain the VLDL-TAG synthesis. An alternative explanation is the difference in glucose concentrations to which the liver is exposed. It is well established that glucose enhances VLDL output in isolated liver preparations (27), probably by stimulating bulk lipid transfer to apoB (33). This direct stimulatory effect of glucose in vitro is paralleled by experiments in vivo which have shown that acute exogenous hyperglycemia increased the splanchnic output of VLDL-TAG (34–36), even in the face of a decreased hepatic NEFA flux. This effect was ascribed, in part, to an inhibition of splanchnic NEFA oxidation (34). In the present work, the metabolic effects of the oral glucose load at the adipose tissue level resulted in a switch from a net output of NEFA to a net uptake of NEFA at 60 min post-glucose with a consequently decreased hepatic uptake (Fig. 2). This decreased availability of NEFA for VLDL-TAG production was mitigated by a decreased NEFA oxidation, as measured by splanchnic ketone body output (Table 2). Nevertheless, the decreased NEFA oxidation was not sufficient, in itself, to account for the increased VLDL-TAG secretion at 60 min post-glucose. At this time, therefore, VLDL output was sustained, in large part, either by mobilization of endogenous hepatic TAG stores or by increased utilization of fatty acids derived from splanchnic adipose tissue TAG. In this respect, we have shown that glucose directly mobilizes TAG from pre-formed lipid stores in cultured hepatocytes (33). This effect of glucose probably contributed to the negative lipid balance of the liver over the whole time course in which the AUC for VLDL-TAG output exceeded that for NEFA uptake. In the calculation of the NEFA flux across the splanchnic bed, uptake of NEFA from the visceral adipose tissue is not visible. In subjects with a body mass index between 20 and 30, the visceral adipose tissue volume is about 1.6 liter (37). However, even if the lipid mobilization from the visceral depots is assumed to be 3-fold higher than that from the abdominal, subcutaneous adipose tissue (38), it would demand a visceral fat depot of 6 liters in order to keep the liver in neutral lipid balance.

The results of the oral glucose study suggest that there was a decline in the output of VLDL after the peak observed at 60 min post-glucose. This pattern may reflect, at least in part, a delayed inhibitory response to the peak insulin surge which occurred at 60 min post-glucose. In this respect, Cohen and Berger (39) have suggested that the decreased lipemia observed 2–4 h after simultaneous oral consumption of fat and glucose compared to that observed after fat alone was due to an insulin-mediated decrease in the secretion of VLDL-TAG during this period. However, in the present work, the decreased splanchnic output of VLDL may also be explained by an increased clearance of TAG by the intra-abdominal adipose tissue depots.

Our simultaneous measurements of changes in adipose tissue and splanchnic lipid fluxes have also provided an opportunity to investigate the nature of the complex metabolic relationships that exist between the two tissues. It is apparent from Fig. 2 that splanchnic NEFA uptake and adipose tissue NEFA release are closely co-ordinated as are the rates of splanchnic VLDL-TAG production and adipose tissue TAG clearance (Fig. 3). Net fatty acid release from adipose tissue is sensitively regulated by a combination of LPL (acting on VLDL) and HSL (acting on TAG stores) activities and fractional fatty acid re-esterification (Fig. 4). In particular, towards the end of the experimental period, the effect of insulin waned after the peak plasma glucose, resulting in an increased HSL activity and a decreased fractional re-esterification of the product fatty acids (Fig. 4). Somewhat surprisingly, the increased adipose tissue clearance of total and VLDL-TAG during the later stages of the experiment suggested an increase in the rate of action of LPL, despite the low arterial insulin level (Table 1). Comparison of the relative efflux of glycerol and NEFA from adipose tissue and of TAG removal suggested that HSL alone was insufficient to sustain the high rate of adipose tissue NEFA efflux. This implies that most, if not all, of the NEFA released from VLDL by the relatively high LPL activity directly entered the venous effluent without uptake into the adipose tissue TAG stores. The overall increase in NEFA flux to the splanchnic region was thereby bolstered by this contribution from adipose tissue VLDL-TAG released by LPL en passant. Although splanchnic fatty acid oxidation (as measured by ketone body output) increased at this time, flux through this pathway was insufficient to clear the increased NEFA influx into the liver. Thus, hepatic lipid balance under these conditions was maintained by a stimulation of splanchnic VLDL-TAG release (Fig. 3). It would appear, there-

fore, that towards the end of the study period, the neurohormonal "counter-regulatory" responses combined to produce a large inter-tissue metabolic axis in which TAG and NEFA continuously cycled between subcutaneous adipose tissue and the splanchnic region. The arterial concentration of apoB increased significantly towards the end of the study period (Table 1). This implies (assuming no change in peripheral clearance) that hepatic VLDL-apoB output increased during this period, an increase which was accompanied by an increase in the output of VLDL-TAG (Fig. 3).

Because each particle of VLDL, irrespective of its complement of TAG, contains only one molecule of apoB, the TAG/apoB ratio of VLDL is often used as a measure of VLDL particle size (28). Using AUC data for VLDL output it may be calculated that over the whole time course the newly assembled VLDL that leave the liver are larger and more TAG-rich than those which enter the liver. Thus, systemic plasma VLDL is relatively TAG-depleted and contains, on average, a larger proportion of denser particles than does nascent hepatic VLDL. These data are supported by calculations of the absolute VLDL-TAG/apoB ratios in the arterial and hepatic venous plasma over the time course of the experiment. In general, the latter ratio was higher than the former, a change which was particularly pronounced during the early and later stages of the experiment when VLDL output rates were high.

Finally, although several previous studies have measured splanchnic VLDL-TAG and apoB synthesis and output we believe that the present work is unique in at least two important respects. First, the continuous monitoring of splanchnic lipid balance has provided information relating to the contribution of VLDL-TAG to the changing pattern of hepatic fuel selection in response to a high carbohydrate meal. We have, therefore, been able to assess dynamic changes in output in contrast to the steady state approach of many previous protocols. Previous estimates of splanchnic VLDL-TAG output in the fasting state are generally higher (45.2 ± 4.9 $\mu mol \cdot min^{-1}$ (40); 6–148 $\mu mol \cdot min^{-1}$ (41)) when measured directly as veno-arterial differences than when measured on the basis of isotope dilution (e.g., 26.6 ± 5.3 $\mu mol \cdot min^{-1}$ (34); 21.5 $\mu mol \cdot min^{-1}$ (42)). Our own measurement of 68 $\mu mol \cdot min^{-1}$ in the fasting state is similar to that observed in the former group. Our estimate of splanchnic VLDL apoB output, at approximately 2 $mg \cdot min^{-1}$ is considerably higher than that reported in previous measurements based upon stable isotope enrichment (e.g., 0.40 $mg \cdot min^{-1}$ (43); 0.68 $mg \cdot min^{-1}$ (44)) but similar to that measured directly by veno-arterial differences (2.2–8.2 $mg \cdot min^{-1}$ (45)) or by radioisotopic dilution (1.10 $mg \cdot min^{-1}$ (46)). Second, we have been able to address the question of the links through which lipid metabolism in the splanchnic region is integrated with that in subcutaneous adipose tissue and how these are affected over a period of time before and after an oral glucose load. Efficient co-ordination of lipid metabolism in these tissues avoids the chronic dyslipidemias which are associated with an increased cardiovascular risk. It should, however, be emphasized that the results from the present study may differ from what happens after a mixed meal where chylomicron production and metabolism also play a role.

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