Supplementary Figure 1. The concentration of $^2H_2$-palmitate in the plasma TG fraction in lean (black circles) and obese men (open circles). Intravenous infusion of $^2H_2$-palmitate started approximately 60-90 minutes before time 0. It is assumed that the incorporation of $^2H_2$-palmitate into the TG fraction in plasma takes place in the liver through secretion of VLDL. The extraction of 2H2-palmitate labelled TG across adipose tissue as shown in Fig 4 (main text) is therefore considered specific for VLDL-TG. Dotted perpendicular lines reflect the times of meal intake. The concentration is of $^2H_2$-palmitate is significantly higher in obese men compared with lean men (p>0.003, RM-ANOVA).

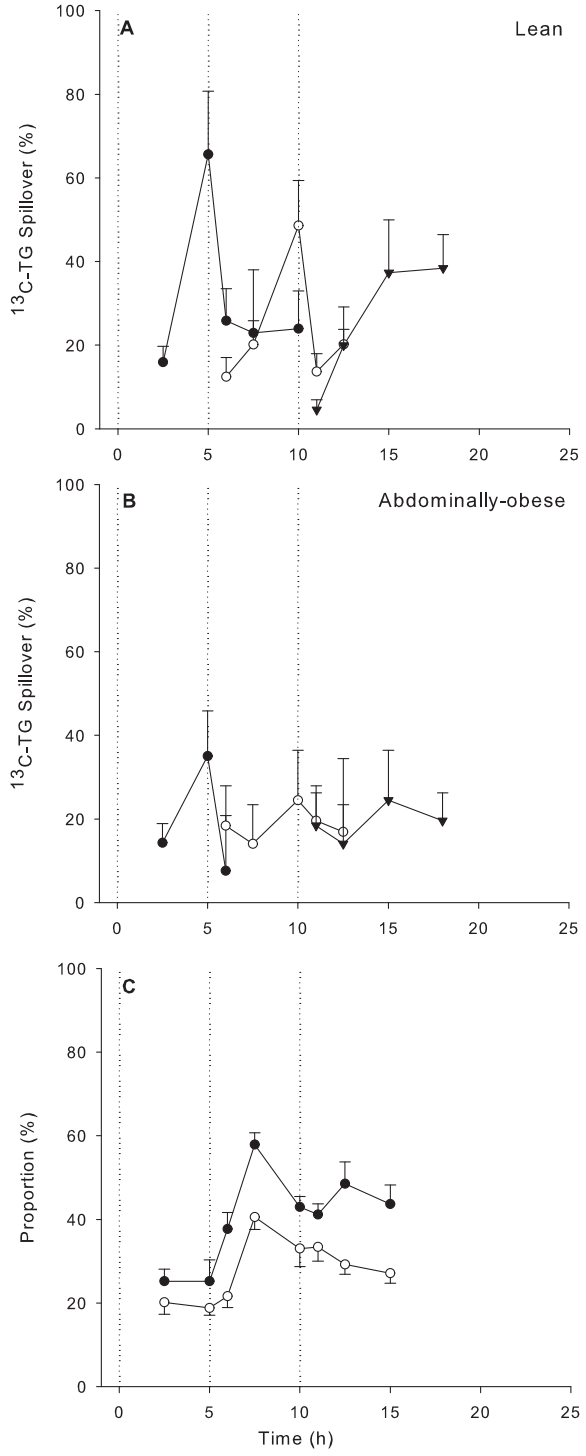
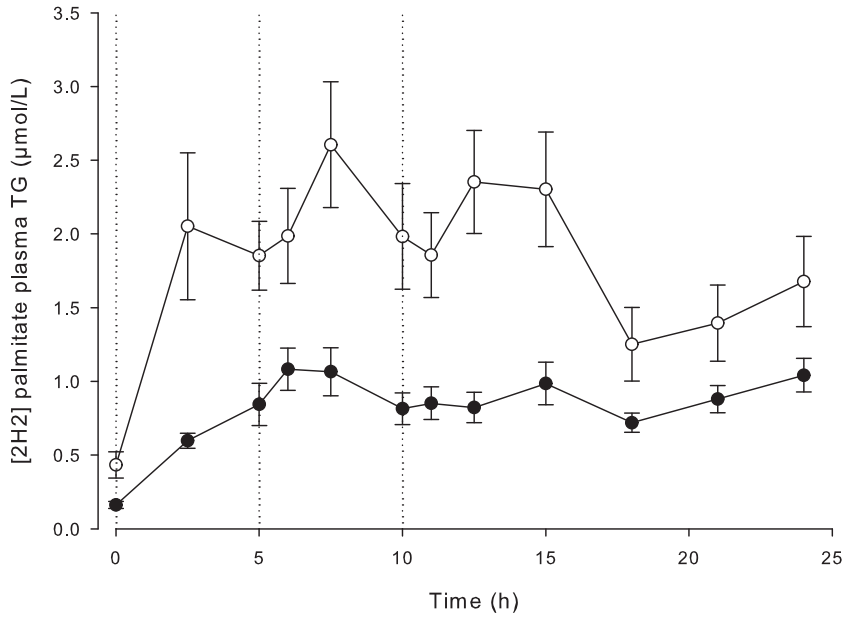


Figure 2. The proportion of fatty acids derived from chylomicron TG being lipolysed, but not taken up, by adipose tissue (spillover fraction). The fraction of FA released from chylomicron-TG that appears in the venous effluent in adipose tissue; 'the spillover fraction' in lean (panel A) and abdominally-obese males (panel B). Meals were given at time 0 (labelled with [U-^{13}C]linoleic acid), 5h ([U-^{13}C]oleic acid) and 10 h ([U-^{13}C]palmitic acid). Measurement of spillover fractions was not possible before a significant quantity of chylomicron-TG had appeared in plasma, which for the first meal did not happen until 150 min after food intake.
The chylomicron-TG derived [U-^{13}C]-TG spillover fraction in lean males was 16% 2.5 h after the first meal. This increased to 65% at the end of this first
postprandial period. The proportion of chylomicron-derived spillover was reduced back to under 12% one hour after ingestion of the second meal and increased to 48% at the end of this postprandial period. The third meal intake, one hour after ingestion, reduced the fraction to 4% and by the end of this postprandial period increased to 37%. There was progressive decrease in chylomicron-derived spillover across meal periods (p=0.006).
Panel B shows the chylomicron-TG derived [U-^{13}C]-TG) spillover fraction in abdominally-obese males was 14% 2.5 h after the first meal. This increased to 35% by the end of this postprandial period. The proportion of chylomicron-derived spillover was reduced to back to under 18% one hour after ingestion of the second meal and increased to 24% by the end of this postprandial period. The third meal intake, one hour after ingestion, reduced the fraction to 18% which increased to 24% by the end of the third postprandial period. There was no difference in chylomicron-TG derived spillover fraction across meal periods (p=0.13).
Panel C shows the percentage NEFA that originates from the spillover process from chylomicrons in the systemic circulation. Abdominally-obese men had significantly lower proportion of spillover fatty acids in the NEFA fraction than lean men (repeated measures ANOVA, p=0.01).



Supplementary Table 1. Relative mRNA expression of genes involved in fatty acid trafficking in abdominal adipose biopsies from lean and obese men.

Gene	Protein/Enzyme	Lean (n=10)		Obese (n=10)		Fold change	
		Mean	SD	Mean	SD		*P*
LPL	Lipoprotein Lipase	1.14	0.26	0.82	0.18	0.71	<0.01
GPIHBP1	GPI anchored HDL binding protein 1	0.70	0.22	0.42	0.16	0.60	<0.01
CD36	CD36 molecule	1.01	0.16	0.90	0.12	0.89	ns
ACLS1	Acyl-CoA synthetase long-chain family member 1	0.84	0.18	0.54	0.14	0.65	<0.01
AGPAT9	Glycerol-3-phosphate acyltransferase 3	0.64	0.29	0.23	0.09	0.45	<0.05
GPAM	Glycerol-3-phosphate acyltransferase 3, mitochondrial	0.90	0.31	0.60	0.15	0.67	<0.05
DGAT1	Diacylglycerol acyltransferase 1	1.17	0.27	0.71	0.16	0.61	<0.001
DGAT2	Diacylglycerol acyltransferase 2	0.51	0.18	0.31	0.12	0.62	<0.01
PNPL2	Adipose tissue triglyceride lipase	1.03	0.19	0.62	0.19	0.61	<0.001
HSL	Hormone sensitive lipase	1.17	0.19	0.77	0.17	0.66	<0.001
PLIN	Perilipin	1.17	0.17	0.83	0.18	0.71	<0.001
ADRB2	Beta-adrenoreceptor 2	1.05	0.16	0.68	0.15	0.65	<0.001
LEP	Leptin	0.59	0.15	0.62	0.14	1.04	ns
CD68	CD68 molecule	0.38	0.11	0.67	0.25	1.76	<0.01
CD11b	CD11b molecule	0.44	0.19	0.74	0.26	1.66	<0.001
CD163	CD163 molecule	0.20	0.08	0.39	0.18	1.98	<0.001

Additional supplemental materials

Three isoenergetic meals with a total caloric content corresponding to the estimated individual metabolic expenditure over 24 h and with similar macronutrient composition were fed 5 h apart, at time points 0, 5 h and 10 h as described in detail before (1). The meals each contained 685-943 kcal (lean subjects) and 742-1008 kcal (abdominally-obese). Subjects remained semi-recumbent during the day and lay flat during the night. They had three scheduled non-strenuous, 10 min walks, before the second and third meals and the night sleep.

Biochemical and isotopic enrichment analyses: Samples were analyzed enzymatically for plasma glucose, TG (with glycerol blank removed) and NEFA concentrations as described before (1). Insulin concentrations were determined by radioimmunoassay (Linco Research, St. Charles, MO). FA compositions (µmol/100 µmol total FAs) in NEFA and TG fractions were determined by gas chromatography (GC) (2), and palmitate concentrations were calculated by multiplying the proportion of palmitate by the corresponding plasma concentrations of plasma NEFA and TG determined enzymatically. [$^{2}H_{2}$]Palmitate enrichments in the FA methyl ester derivatives of plasma NEFA and VLDL-TG were determined by gas chromatography-mass spectrometry (GC-MS) as previously described (3). [U-^{13}C]FA enrichments were measured in plasma NEFA and TG using a GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS) as reported previously (4).

Calculations and statistical analysis: Plasma concentrations were converted to whole blood concentrations by multiplication with (1-hematocrit) for lipids and with 1–(0.3*hematocrit) (5) for glucose. Tissue uptake or release of a substrate was calculated as the product of the A-V or V-A difference and blood flow. A positive A-V

difference implies net uptake or extraction across a tissue, whereas a positive V-A difference implies net release from a tissue. Fractional TG extraction was calculated as the A-V difference divided by the respective arterial concentration. Absolute (unidirectional) uptake of NEFA by forearm muscle was taken as the arterial NEFA concentrations multiplied by the forearm fractional extraction of plasma [$^2H_2$]palmitate. Whole body rate of appearance (R_a) of NEFA was calculated using the non-steady state calculation of isotopic enrichment in plasma during intravenous infusion of [$^2H_2$]palmitate (6). The time resolution of this is inevitably lower than in the larger number of NEFA plasma concentration measurements and may yield the impression of smoother curves. The net 'transcapillary flux' of FAs in tissues was calculated as described before, with a negative value implying FA release and a positive value indicating fat storage (7). Chylomicron-derived spillover was calculated as outlined in Ruge et al. (1) and the effect on whole body NEFA concentrations was calculated by quantifying the dilution from the meal:

$$\frac{[[^{13}C]\text{specific fatty acid in plasma NEFA pool}]}{\text{Proportion of the specific fatty acid in the test meal} * \text{meal TTR}}$$

This equation assumes there is no discrimination by LPL for individual fatty acids fatty acids. In the plasma NEFA pool, total fatty acids arising from spillover were calculated as the sum of the values from each meal.

Adipose tissue data were calculated per unit mass of tissue (per 100 g or ml). To calculate the whole body effect, the per unit tissue mass was multiplied by the total body fat mass, as described previously (1).

As serial measurements are made of arterio-venous concentrations differences it is important to consider implications of non-steady state. A principal problem with arterio-venous measurements is the lack of knowledge of transit time across the tissue. This effect may have larger implications for substances that equilibrate in the interstitial space and less implications for substances contained in the plasma compartment, such as triglycerides, compared with for example glucose (8). One can also overcome some of these problems by studying responses over longer periods of time, such as summarizing data by calculation of area under curve (AUC).

In the tissue-specific study, individual between-meal comparisons were made in 5 h blocks (0-5 h, 5-10 h and 10-15 h). AUCs were calculated using the trapezoid rule and are presented as time-averaged values (AUC/divided by the relevant time period). Differences in AUCs between groups were analysed with an independent t-test or Wilcoxon-signed rank test and within-group differences by a paired t-test or Mann Whitney U test. Repeated measures analysis of variance with time as a within- and group as a between-subject factor was used to test for time and group effects. For ease of presentation, graphical data are presented as mean±SEM unless otherwise stated.

Data were analysed using SPSS for Windows v15 (SPSS, UK, Chertsey, UK). All data sets were tested for normality according to the Shapiro-Wilk test. In the cohort study, the data are presented as median (range) and differences between groups were tested with Mann-Whitney U test.

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