

## SUPPLEMENTARY DATA

### Calculations

We averaged the hemoglobin measurements for each time point across the three sites.

We calculated the oxygen content as described by Frayn et al (1).

$O_2$  delivery ( $\mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ ) = arterial  $O_2$  content (mmol/l) x ATBF ( $\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ )

Tissue  $O_2$  consumption ( $\mu\text{mol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ ) = [arterial  $O_2$  content (mmol/l) – tissue venous  $O_2$  content (mmol/l)] x ATBF ( $\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ )

We calculated the  $CO_2$  content of blood using the equation of Douglas et al (2). We then determined the  $CO_2$  content and tissue  $CO_2$  production for the individuals with complete data over time. We plotted these over time and found there were changes with feeding, but baseline and end of study (24 h) were similar. We interpolated data over the postprandial period around the missing time points. If a baseline time point was missing and we had an end of study (24 h) time point we used that, and vice versa.

The tissue-specific respiratory quotient (RQ) was calculated as  $CO_2$  (mmol/l) produced /  $O_2$  (mmol/l) consumed.

AT FA reestification was calculated from the net exchange of FAs and glycerol (3). The provision of glycerol 3-phosphate for reesterification as a percentage of the glucose taken up into the tissue, and the potential contribution of complete oxidation of glucose to oxygen consumption, were calculated as described by Frayn et al (1). The proportion of glucose taken up and released as lactate and pyruvate was calculated as in Frayn et al (4).

### Results

In the fasting state, plasma insulin concentrations are typically at their nadir, therefore intracellular lipolysis within adipose tissue is high, and the fatty acids liberated are released into systemic circulation. Not all of the fatty acids liberated during intracellular lipolysis enter systemic circulation, with approx. 20% ( $21.5 \pm 11.6\%$ ) being reesterified within the tissue (Supplementary Figure 2). During the postprandial period there was a dramatic increase, with a maximum of approx. 80% (but on average over the three postprandial periods approx. 60%) of fatty acids being reesterified. Over the late postprandial (3-5 h) period reesterification started to decline and was back to the baseline levels, at 17% by 24 h (Supplementary Figure 2,  $P < 0.001$  for change over time).

Blood 3-hydroxybutyrate (3OHB) concentrations are a marker of hepatic fatty acid oxidation. In the fasting state there was net uptake of 3OHB from blood into skeletal muscle and adipose tissue (Supplementary Figure 3A). With feeding there was a significant decrease in 3OHB concentrations across the postprandial period in all three sites ( $P < 0.05$ , time x site) (Supplementary Figure 3B). In a subset (n=18) of the group we had measured blood acetoacetate concentrations for arterial and venous blood draining subcutaneous adipose tissue. We calculated the 3OHB to acetoacetate ratio as a marker of the mitochondrial redox state (5), but found no association with BMI, percentage body fat, or waist circumference.

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**Supplementary Table 1.** Subject characteristics

	Lean	Overweight	Obese
Males/Females	15/5	13/1	11/3
Age (y)	39 ± 2	40 ± 3	40 ± 2
BMI (kg/m <sup>2</sup> )	22.6 ± 0.3 <sup>*†</sup>	27.1 ± 0.4 <sup>‡</sup>	37.1 ± 2.0
Body fat (%)	20.1 ± 1.4 <sup>*†</sup>	25.8 ± 1.8 <sup>‡</sup>	33.7 ± 2.5
<b><i>Fasting biochemical parameters</i></b>			
Arterial blood O <sub>2</sub> saturation (%)	98 ± 0.2 <sup>†</sup>	98 ± 0.3	97 ± 0.3
Plasma glucose (mmol/l)	4.8 ± 0.1 <sup>*†</sup>	5.2 ± 0.1	5.3 ± 0.2
Plasma insulin (mU/l)	9.6 ± 0.9 <sup>†</sup>	12.1 ± 0.9	21.2 ± 4.6
Plasma TG (μmol/l)	899 ± 94 <sup>†</sup>	1061 ± 85	1484 ± 150
Plasma NEFA (μmol/l)	529 ± 42	579 ± 40	577 ± 56
Whole blood 3OHB (μmol/l)	131 ± 26	109 ± 14	123 ± 22
Adipose tissue blood flow (ml.100g <sup>-1</sup> .min <sup>-1</sup> )	4.7 ± 0.9	3.0 ± 0.7	3.2 ± 0.7
Skeletal muscle blood flow (ml.100ml <sup>-1</sup> .min <sup>-1</sup> )	2.4 ± 0.3	1.7 ± 0.3	1.8 ± 0.3

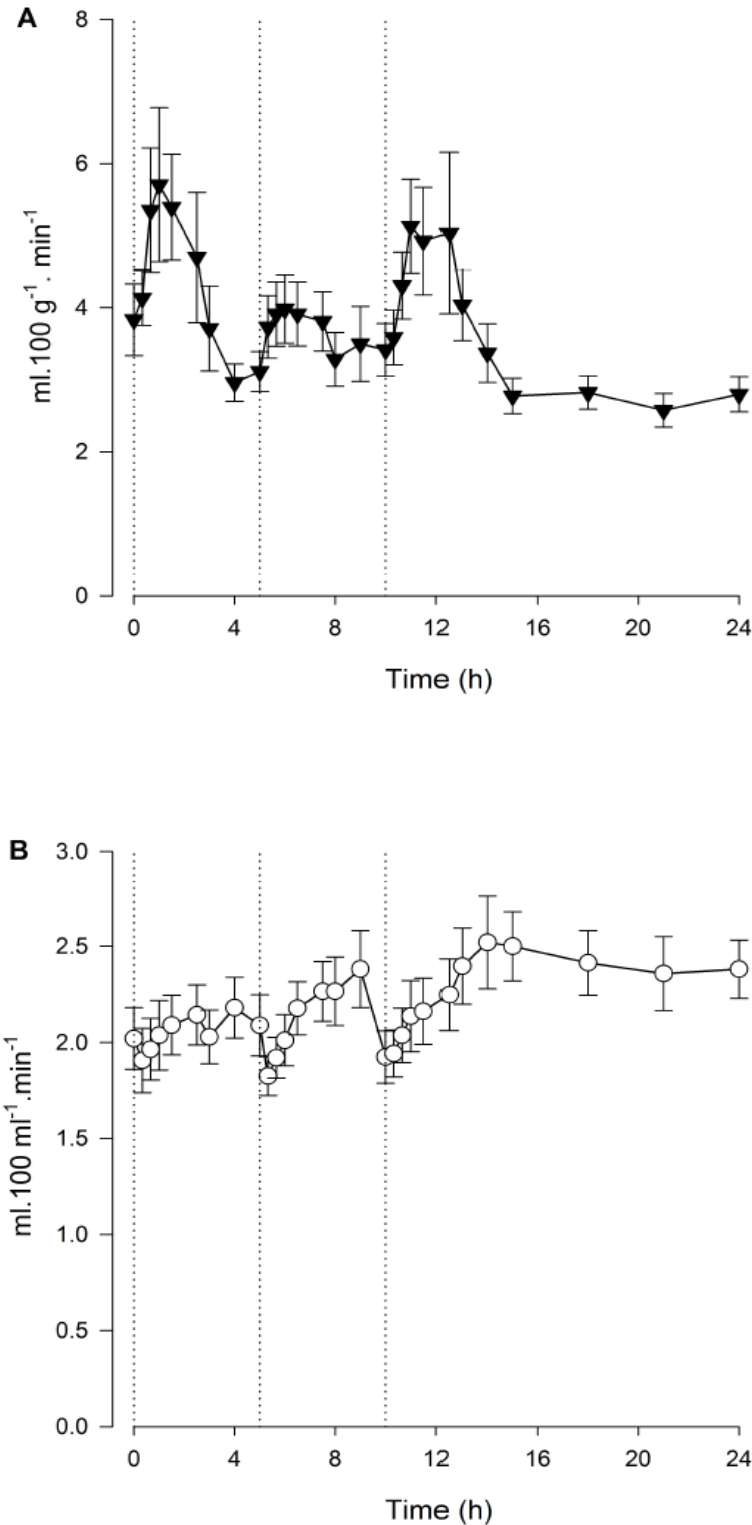
Data presented as mean ± sem

\**P*<0.05 lean vs overweight; †*P*<0.05 lean vs obese; ‡*P*<0.05 overweight vs obese

Abbreviations: BMI, Body mass index; TG, triacylglycerol; NEFA, non-esterified fatty acids; 3OHB, 3-hydroxybutyrate

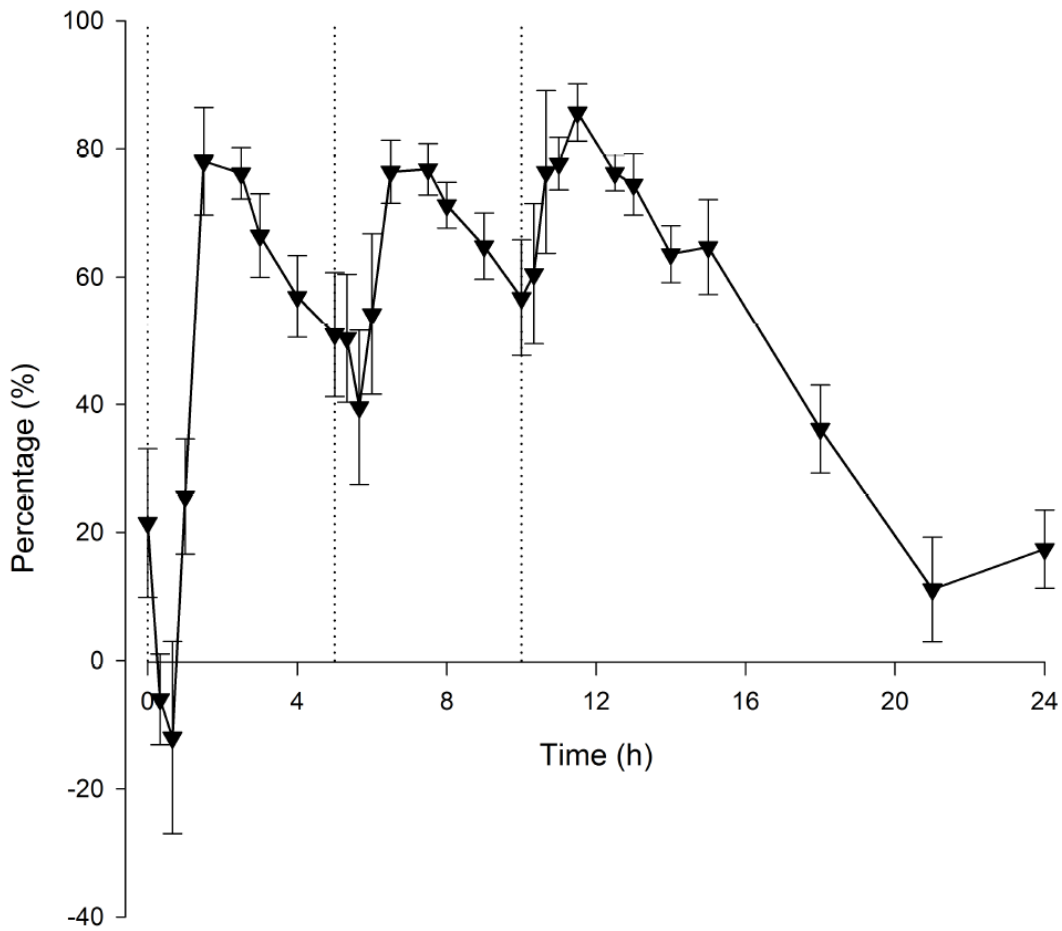
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**Supplementary Figure 1A-B.** Fasting and postprandial adipose tissue blood flow (ATBF) and skeletal muscle blood flow. **Figure 1A.** Fasting and postprandial ATBF increase in response to the consumption of meals ( $P=0.008$ , repeated measures over time). **Figure 2B.** Fasting and postprandial skeletal muscle blood flow did not change in response to consumption of meals ( $P=NS$ , repeated measures over time). Meals of similar energy and macronutrient composition were fed at 0, 5 and 10 h (..... lines).



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**Supplementary Figure 2.** The proportion of fatty acids undergoing reesterification in subcutaneous adipose tissue over time,  $P < 0.001$  repeated measures over time. Meals of similar energy and macronutrient composition were fed at 0, 5 and 10 h (..... lines).



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**Supplementary Figure 3A-B.** The fasting and postprandial concentrations of 3-hydroxybutyrate (3OHB) in arterial (ART), skeletal muscle venous (SM\_V), and subcutaneous adipose tissue venous (SCAT\_V) blood. **Figure 3A.** Fasting blood 3OHB concentrations ( $\mu\text{mol/l}$ ) in ART (black bar), SM\_V (light grey bar) and SCAT\_V (dark grey bar),  $^*P < 0.001$  ART vs SM\_V;  $^\dagger P < 0.001$  ART vs SCAT\_V;  $^\ddagger P < 0.001$  SM\_V vs SCAT\_V. **Figure 3B.** The postprandial concentrations ( $\mu\text{mol/l}$ ) of 3OHB in ART ( $\bullet$ ), SM\_V ( $\circ$ ), and SCAT\_V ( $\blacktriangledown$ ) blood. Meals of similar energy and macronutrient composition were fed at 0, 5 and 10 h (··· lines),  $P < 0.05$  repeated measures over time for all sites and  $P < 0.001$  time x site interaction for all sites.

