Lipid metabolism during fasting

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Jensen, Michael D., Karin Ekberg, and Bernard R. Landau. Lipid metabolism during fasting. *Am J Physiol Endocrinol Metab* 281: E789–E793, 2001.— These studies were conducted to understand the relationship between measures of systemic free fatty acid (FFA) reesterification and regional FFA, glycerol, and triglyceride metabolism during fasting. Indirect calorimetry was used to measure fatty acid oxidation in six men after a 60-h fast. Systemic and regional (splanchnic, renal, and leg) FFA ($[^3H]$palmitate) and glycerol ($[^3H]$glycerol) kinetics, as well as splanchnic triglyceride release, were measured. The rate of systemic FFA reesterification was 366 ± 93 μmol/min, which was greater ($P < 0.05$) than splanchnic triglyceride fatty acid output (64 ± 6 μmol/min), a measure of VLDL triglyceride fatty acid export. The majority of glycerol uptake occurred in the splanchnic and renal beds, although some leg glycerol uptake was detected. Systemic FFA release was approximately double that usually present in overnight postabsorptive men, yet the regional FFA release rates were of the same proportions previously observed in overnight postabsorptive men. In conclusion, FFA reesterification at rest during fasting far exceeds splanchnic triglyceride fatty acid output. This indicates that nonhepatic sites of FFA reesterification are important, and that peripheral reesterification of FFA exceeds the rate of simultaneous intracellular triglyceride fatty acid oxidation.

lipolysis; free fatty acids; glycerol; triglycerides

ADIPOSE TISSUE LIPOLYSIS is an important fuel source, providing free fatty acids (FFA) and gluconeogenic carbons in the form of glycerol. The glycerol derived from adipose tissue lipolysis may become more important when humans fast for more than 24 h, because virtually all glucose must then be derived by gluconeogenesis. Recent studies have documented that splanchnic and renal glycerol uptake in 60-h-fasted humans is largely converted to glucose (22). Intrahepatic glycerol-3-phosphate (G-3-*P*), which is partially derived from circulating free glycerol, can also serve another physiological need, i.e., reesterification of fatty acids for export as very low density lipoprotein (VLDL) triglyceride. The quantitative need for G-3-*P* for this purpose has rarely been considered in the context of hepatic fuel metabolism during fasting.

In overnight postabsorptive adults, triglyceride recycling, a measure of lipolysis in excess of oxidative needs, is reported to be ~ 1.2 μmol·kg^{-1}·min^{-1} (~86 μmol/min) (21). This triglyceride recycling rate is similar to the rate of splanchnic triglyceride release that we have observed (14). These concordant data are consistent with the suggestion that FFA released in excess of fatty acid oxidation (fatty acid recycling) primarily represents hepatic reesterification of FFA and their subsequent export as VLDL triglyceride (12), although recent data suggest that this may not be the case (14). During prolonged fasting, FFA reesterification is reported to increase as much as sixfold (9). If so, and if VLDL triglyceride export is the major means of dealing with "excess" lipolysis, this process could consume a significant portion of hepatic G-3-*P*. Therefore, it is possible that, during fasting, increased rates of both gluconeogenesis and fatty acid reesterification compete for a limited hepatic G-3-*P* pool.

Recently, we observed that estimated FFA reesterification (lipolysis minus fatty acid oxidation as determined by indirect calorimetry) was substantially in excess of splanchnic FFA uptake in men fasted for 60 h (22). In that study, FFA release rates were estimated as $3\times$ glycerol rate of appearance (R_a), which could be misleading if the ratio of FFA to glycerol R_a is <3:1 (25). In addition, splanchnic FFA kinetic studies were not performed (22). If visceral adipose tissue lipolysis (and therefore splanchnic FFA release) is increased substantially during fasting, hepatic FFA uptake could be much greater than predicted from net splanchnic FFA uptake.

These issues caused us to question the extent of fatty acid reesterification in the liver during fasting. This study was conducted to directly measure systemic and regional FFA uptake and release in fasting humans and to assess net splanchnic triglyceride export as a minimum estimate of hepatic G-3-*P* requirements for fatty acid reesterification. To place the splanchnic data into context, measures of regional substrate kinetics across the leg and kidney bed were also performed.

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MATERIALS AND METHODS

Subjects

Six men participated in these studies, which were performed in the Division of Clinical Physiology, Karolinska Hospital, Stockholm, Sweden. All volunteers were healthy and took no medications on a regular basis. Their characteristics are provided in Table 1. They were informed of the nature, purpose, and possible risks of the study before consenting to participate. The Institutional Human Ethics and the Isotope Radiation Committees approved the experimental protocol.

Experimental Design

Protocol. Each volunteer fasted for 60 h as an outpatient before reporting to the laboratory, as in our previous studies (22). Oxygen consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) (indirect calorimetry) were measured with the subject resting quietly in bed. A series of indwelling catheters was then placed for infusions and blood sampling. A forearm intravenous catheter was used for the infusion of isotopic tracers of palmitate and glycerol. The same catheter was used to infuse para-aminohippurate (PAH) to measure renal and indocyanine green (ICG) to measure splanchnic blood flow. Catheters were placed using standard techniques in the brachial artery and femoral, hepatic, and renal veins for blood sampling while the subjects rested quietly. Infusions of [9,10-³H]palmitate (0.3 μCi/min) and [2-³H]glycerol (0.3 μCi/min) were begun, and after 60 min, for isotopic equilibration, a series of blood samples was obtained from each catheter at 10-min intervals over 30 min. The catheters were then removed, and the subjects were allowed to eat.

Materials and assays. [9,10-³H]palmitate was purchased from NEN Research Products (Wilmington, DE) and bound to human albumin for infusion. [2-³H]glycerol was purchased from Amersham (Arlington Heights, IL). ICG (Pulsion Medical System, Munich, Germany) and PAH (Merck, West Point, PA) were used in these studies.

Plasma palmitate and FFA concentrations and specific activity (SA) were determined by a modification (19) of a published HPLC technique (24). Femoral, renal, and hepatic venous and arterial glycerol and plasma triglyceride concentrations were measured twice in triplicate using a microfluorometric method (13). Plasma free glycerol SA was measured using HPLC (20). Plasma ICG (6) and PAH (5) concentrations were measured on the day of the study. Blood glucose concentrations were measured with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA).

Calculations

Systemic FFA and glycerol fluxes were measured using the mean steady-state plasma FFA and glycerol SA (dpm/μmol)

Table 1. *Subject characteristics*

Age, yr	32 ± 2
Height, cm	183 ± 3
Weight, kg	81.0 ± 2.7
$\dot{V}O_2$, ml/min	262 ± 7
RQ	0.72 ± 0.01
Arterial glucose, mmol/l	3.36 ± 0.17
Arterial insulin, pmol/l	8 ± 1
Arterial β-hydroxybutyrate, μmol/l	$1,726 \pm 234$

Values are means $\pm$ SE. $\dot{V}O_2$, oxygen consumption; RQ, respiratory quotient.

and the tracer infusion rates (dpm/min) (15); because steady-state conditions were present for all studies, R_a and rates of disappearance (R_d) are equal (20). Net fatty acid oxidation rates were calculated using each individual's $\dot{V}O_2$ and $\dot{V}CO_2$; nitrogen losses were estimated using values from previous studies (18). Net fatty acid "reesterification" rates were calculated using the formula: fatty acid reesterification = FFA disappearance − fatty acid oxidation.

Splanchnic plasma flow (SPF) (4) was measured using the arterial and hepatic venous plasma ICG concentrations. Renal plasma flow was measured using the arterial and renal venous concentrations of PAH (8). Splanchnic and renal blood flow were calculated as plasma flow divided by (1 − hematocrit). We estimated leg plasma flow and leg blood flow in this study by use of the mean values for men from previous studies (17), because we could not justify placing an additional catheter into the femoral artery for dye infusion purposes.

Net balance of FFA and glycerol across the splanchnic, renal, and leg tissue beds was calculated using the venous-arterial concentration gradients together with plasma or blood flow values. Plasma FFA and glycerol concentrations and SA were constant over the sampling interval, as were splanchnic and renal blood flow; therefore, steady-state plasma FFA SA and concentration were used together with measures of renal and hepatic plasma flow to measure regional (leg and splanchnic) FFA uptake and release (15, 23). Regional glycerol kinetics were similarly measured, except that because glycerol is distributed in whole blood (3) (glycerol equilibrates freely between plasma and erythrocytes), regional glycerol kinetics were performed using blood flow values. The formulas used to determine regional glycerol kinetics have been published (15).

Arterial plasma triglyceride concentrations were subtracted from hepatic venous concentrations, and the difference was multiplied by splanchnic plasma flow to determine net splanchnic triglyceride release.

Statistics

All results are expressed as means $\pm$ SE. Comparisons between substrate concentrations in arterial, femoral venous, hepatic venous, and renal venous blood were made using a Student's paired *t*-test.

RESULTS

Subject Characteristics

Data regarding the characteristics, indirect calorimetry, and substrate concentrations are provided in Table 1. The expected adaptations to fasting were observed: moderate hypoglycemia, elevated plasma β-hydroxybutyrate concentrations, and low respiratory quotients (RQ). The renal vein catheter became displaced in one of the studies, and it was not possible to introduce a femoral venous catheter in one other subject. Therefore, renal and leg values are the results from five studies.

Systemic Lipid Kinetics

The arterial plasma FFA and glycerol concentrations for the subjects are provided in Table 2. Concentrations and SA of palmitate and glycerol were stable over the sampling interval (Fig. 1). Free fatty acid flux was 739 ± 85 μmol/min, and systemic glycerol flux was

Table 2. *Plasma FFA, glycerol, and triglyceride concentrations*

	FFA, μmol/l	Glycerol, μmol/l	Triglyceride, μmol/l
Arterial	1,178 ± 97	140 ± 12	696 ± 89
Hepatic vein	860 ± 42*	50 ± 6*	734 ± 91*
Renal vein	1,166 ± 112	90 ± 7*	689 ± 120
Femoral vein	1,557 ± 175*	242 ± 36*	678 ± 90

Values are means ± SE; $n = 6$, except $n = 5$ for renal vein and femoral vein values. FFA, free fatty acids. *$P < 0.05$ vs. arterial concentrations.

301 ± 38 μmol/min. Fatty acid oxidation rates measured using indirect calorimetry were 373 ± 22 μmol/min, resulting in a calculated fatty acid reesterification rates of 366 ± 93 μmol/min.

Regional Substrate Kinetics

Splanchnic blood flow was 1.22 ± 0.04 l/min, and plasma flow was 0.67 ± 0.02 l/min. Renal blood flow was 1.64 ± 0.07 l/min, and plasma flow was 0.88 ± 0.03 l/min, respectively. Leg blood and plasma flows were estimated to be 0.42 and 0.23 l/min.

Concentrations of FFA, glycerol, and triglycerides in the hepatic, renal, and femoral veins are provided in Table 2. The net balances as well as regional uptake and release rates of FFA and glycerol are provided in Table 3. The fractional uptake of systemic FFA by the splanchnic bed was 38 ± 1%. Leg FFA uptake and release were calculated using estimated leg plasma flow, and glycerol uptake and release were calculated using estimated blood flow. Splanchnic glycerol uptake accounted for 54 ± 5%, and renal glycerol uptake accounted for 40 ± 8% of systemic glycerol removal.

Splanchnic FFA release was 15 ± 3% of systemic R_a, whereas renal FFA release was not significantly different from zero. Estimated leg FFA release (both legs) accounted for 22 ± 3% of FFA release. Free glycerol

Table 3. *Regional FFA and glycerol balance data*

	Net Balance	Uptake	Release
FFA, μmol/min			
Splanchnic	−214 ± 44	297 ± 29	102 ± 12
Leg	78 ± 33	40 ± 16	96 ± 15
Kidney	5 ± 39	26 ± 10	18 ± 18
Glycerol, μmol/min			
Splanchnic	−118 ± 19	159 ± 19	42 ± 2
Leg	44 ± 16	14 ± 6	58 ± 14
Kidney	−71 ± 16	113 ± 22	43 ± 8

Values are means ± SE; $n = 6$, except $n = 5$ for renal vein and femoral vein values. Negative net balance reflects net uptake of substrate, and positive net balance reflects net release of substrate. Leg values are calculated for a single leg, and kidney values represent contributions from both kidneys.

was released from both the splanchnic and the renal beds.

Net splanchnic glucose release was 0.40 ± 0.01 mmol/min, and renal glucose release was 0.22 ± 0.04 mmol/min. The net splanchnic triglyceride release was 21 ± 2 μmol/min (64 ± 6 μmol/min of fatty acids), and the arteriovenous triglyceride concentration difference across the renal bed was not significantly different from zero.

DISCUSSION

These studies were designed to address unresolved issues regarding the physiology of lipid substrate metabolism in fasting, namely the relationship between splanchnic uptake of lipid derived substrates and estimates of fatty acid reesterification. We combined indirect calorimetry with measures of regional and systemic substrate kinetics to assess the contribution of leg, splanchnic, and renal tissues to FFA and glycerol utilization in relationship to systemic fatty acid oxidation and reesterification. As expected, the FFA rees-
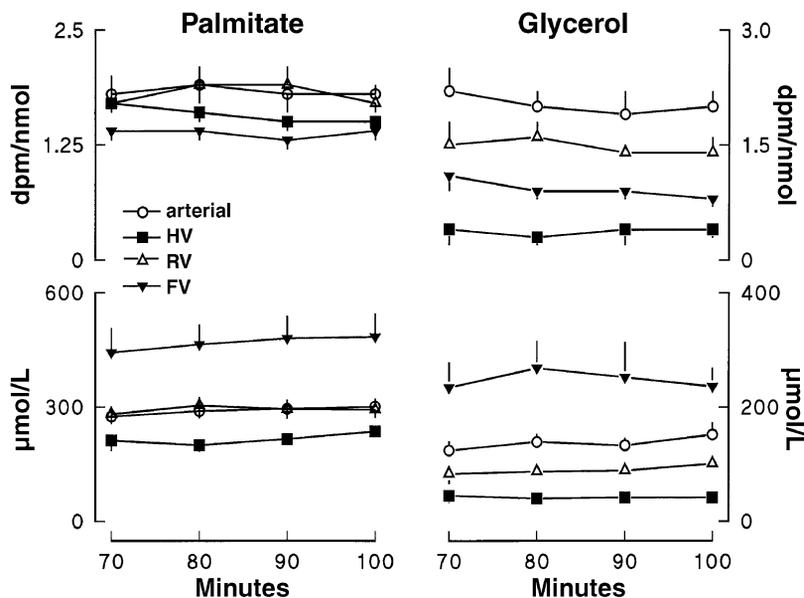

Fig. 1. Plasma concentrations (*bottom*) and specific activity (SA; *top*) of palmitate and glycerol in arterial, femoral venous (FV), hepatic venous (HV), and renal vein (RV) plasma. Steady-state conditions were present.

terification rates with fasting were quite high. There was no commensurately elevated splanchnic triglyceride release; in fact, splanchnic triglyceride release was less than one-half of what we previously found in overnight, postabsorptive adults (14). Thus the estimates of systemic FFA reesterification obtained by combining indirect calorimetry and tracer techniques do not appear to reflect hepatic export of fatty acids in triglyceride.

A previous study (22) of systemic and regional glycerol kinetics during fasting suggested that FFA reesterification should be occurring at the rate of ~820 μmol/min. If all FFA are reesterified in the liver, that would require ~270 μmol/min of intrahepatic G-3-P, substantially more than the splanchnic glycerol uptake (120 μmol/min) observed (22). We reasoned that if the liver were the only site of FFA reesterification during fasting, hepatic triglyceride export would result in net utilization of gluconeogenic carbons in amounts greater than those derived/delivered from lipolysis. This was inconsistent with the finding that ~90% of glycerol uptake was converted to glucose (22). In the present study, splanchnic glycerol uptake alone was about sixfold the amount of G-3-P needed for the splanchnic triglyceride released.

We also observed high rates (366 $\pm$ 93 μmol/min) of FFA reesterification, as assessed by subtracting fatty acid oxidation (indirect calorimetry) from FFA R_d. Net splanchnic triglyceride export (an index of hepatic VLDL triglyceride secretion in fasting humans) was 64 $\pm$ 6 μmol/min, accounting for ~17% of FFA reesterification. The relationships among FFA flux, fatty acid oxidation and reesterification, and splanchnic triglyceride fatty acid output are depicted in Fig. 2. The presence of extrahepatic sites of FFA reesterification is the most likely explanation for the difference between systemic FFA reesterification rates and splanchnic triglyceride fatty acid output. We have recently reported direct evidence that muscle is capable of reesterifying significant amounts of FFA in humans (11), suggesting that muscle could be an important site of FFA reesteri-
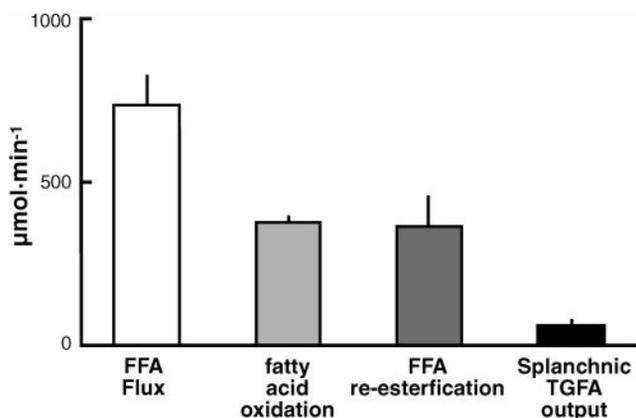

Fig. 2. Free fatty acid (FFA) flux, fatty acid oxidation (indirect calorimetry), FFA reesterification (FFA flux − fatty acid oxidation), and splanchnic triglyceride fatty acid (TGFA) output in men fasted for 60 h.

fication after 60 h of fasting. It should be noted, however, that the current findings apply to resting conditions. During ambulation, muscle lipid oxidation likely increases immediately and substantially, whereas FFA availability increases only gradually (11). Thus modest net utilization of intramyocellular triglyceride fatty acids during physical activity likely offsets the net accumulation that occurs at rest.

Our measures of the splanchnic uptake of FFA and glycerol should be considered minimum estimates of hepatic FFA and glycerol uptake. Splanchnic FFA release was ~100 μmol/min, which is the net effect of omental and mesenteric (visceral) adipose tissue lipolysis and hepatic uptake of FFA from the portal circulation. Given that splanchnic FFA uptake averaged 38% of systemically delivered FFA and that the majority of splanchnic FFA uptake occurs in liver (2), this suggests that visceral adipose tissue FFA release was ~165 μmol/min. Assuming that FFA released from visceral adipose tissue lipolysis are taken up by the liver in comparable proportions to the uptake of systemic FFA, hepatic FFA uptake could have been as much as 360 μmol/min. The export of 64 μmol/min as VLDL triglyceride would allow ~300 μmol/min available for hepatic energy needs and ketone body production. Considering that ketone body production rates in fasting humans can be quite high (1), this is likely an important fate for FFA taken up by the liver.

In these men fasted for 60 h, FFA concentrations and FFA flux were approximately double that usually found in overnight-postabsorptive men (16). The splanchnic FFA release into the systemic circulation accounted for 15 $\pm$ 3% of systemic FFA appearance, whereas leg adipose tissue FFA release was estimated to account for 22 $\pm$ 3% of systemic FFA. These proportions are remarkably similar to those we observed in overnight-postabsorptive men (16) and suggest that mobilization of FFA from adipose tissue in prolonged fasting is not regionally different from that which occurs after an overnight fast.

We found that splanchnic and renal glycerol uptake accounted for 86 $\pm$ 15% of systemic glycerol uptake, whereas in a previous study of 60-h-fasted men (22) these two tissue beds accounted for only ~44% of glycerol uptake. The splanchnic and renal glycerol uptake rates were reasonably comparable in the two studies. However, systemic glycerol flux was ~40% higher in the previous study (22). A possible explanation for the difference between the studies is that, at higher glycerol release rates, tissues other than kidney and liver play an increasing important role in systemic glycerol removal.

As with many studies, these experiments have limitations. We did not measure urinary ketone excretion rates in this experiment. On the basis of upon published data (1) regarding the relationship between plasma ketone body concentrations and urinary ketone losses, we could expect ~20–30 μmol/min of ketonuria in our volunteers. This would result in the consumption of oxygen without concomitant CO_2 production, resulting in a lower respiratory quotient (10). In our

subjects, the ketonuria could be expected to result in 0.6–1.2 ml/min of oxygen consumed without production of CO_2. Given the $\dot{V}O_2$ rates of these volunteers (Table 1) this error would be only an ~0.5% underestimate of the RQ, which would not materially affect our estimates of fatty acid oxidation. Another limitation is the calculation of uptake and release in the face of small arteriovenous differences in substrate concentration or SA. Despite good assay performance [coefficient of variation (CV) of concentration and SA of 1–2% (24)], the need to use arterial and venous concentration and SA data compounds the potential error. These uncertainties are then multiplied by blood or plasma flow values. Thus, despite the collection of four samples over 30 min, it is likely that uptake and release values for each individual is accurate only to within ~10%.

In summary, FFA reesterification in 60-h-fasted men are about sixfold in excess of net splanchnic triglyceride fatty acid release, which should be a reasonable estimate of hepatic VLDL triglyceride export. FFA reesterification was also greater than splanchnic FFA uptake. These data suggest that FFA are reesterified in tissues other than liver during fasting, and adds to the expressed concern (7) that VLDL production rates cannot be readily estimated using FFA reesterification rates (12). Our measures of splanchnic/hepatic FFA uptake during fasting are consistent with the needs of the liver for oxidative fuel and ketone body production, with the remainder relegated to VLDL triglyceride release. The kidney takes up a significant fraction of systemic glycerol removal in fasting adults; however, leg glycerol uptake also appears to occur.

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