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ScienceDirect
Metabolism Clinical and Experimental 55 (2006) 1481–1487
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Fasting decreases free fatty acid turnover in mice overexpressing skeletal muscle lipoprotein lipase

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Received 8 March 2006; accepted 22 June 2006

Abstract

Skeletal muscle lipoprotein lipase (LPL) overexpression in mice results in whole-body insulin resistance and increased intramuscular triglyceride stores, but decreased plasma triglyceride concentration and unchanged plasma free fatty acid (FFA) concentration. The effects of skeletal muscle LPL overexpression and fasting duration on FFA kinetics are unknown. Transgenic mice with muscle-specific LPL overexpression (MCKhLPL) and control mice (Con) were studied at rest during a 50-minute constant infusion of [9,10-^{3}H]palmitate to determine FFA kinetics after both 4 and 16 hours of fasting. FFA concentration was not different between groups after the 4-hour (Con, 0.80 ± 0.06 mmol/L; MCKhLPL, 0.83 ± 0.07 mmol/L) and 16-hour (Con, 0.83 ± 0.04 mmol/L; MCKhLPL, 0.80 ± 0.07 mmol/L) fast. FFA turnover (R_a) was not significantly different between MCKhLPL and Con groups after the 4-hour fast (Con R_a = 2.52 ± 0.36 μmol/min; MCKhLPL R_a = 2.37 ± 0.27 μmol/min). However, FFA turnover was significantly decreased after the 16-hour fast in MCKhLPL mice vs controls (Con R_a = 2.89 ± 0.52 μmol/min; MCKhLPL R_a = 1.64 ± 0.17 μmol/min; $P < .05$). The significantly lower FFA R_a in MCKhLPL vs control mice was due to a decrease in MCKhLPL FFA turnover from the 4- to 16-hour fast, whereas FFA turnover was unchanged in controls. The changes in FFA appearance after the 16-hour fast in MCKhLPL mice are most likely explained by increased reliance by skeletal muscle on plasma triglyceride as a fuel. These data suggest increased skeletal muscle LPL expression decreases dependence on plasma FFA during prolonged fasting in mice.

1. Introduction

Lipoprotein lipase (LPL) is an enzyme that functions on the luminal surface of endothelial cells to hydrolyze chylomicron and very low density lipoprotein triglycerides to monoglyceride and free fatty acids (FFAs) [1]. Overexpression of skeletal muscle–specific LPL (MCKhLPL) consistently decreases plasma triglycerides [2-4] and results in increased whole-body insulin resistance in some [2,4] but not all studies [5]. Fasting insulin concentrations have been shown not to change [2,4] or increase [3,5] in LPL overexpressers. In some studies, skeletal muscle LPL overexpression decreased glucose rate of appearance [2], whereas others reported no change in basal glucose turnover [5]. Increased intramuscular triglyceride stores relative to age-matched control mice have been a consistent finding in skeletal muscle LPL overexpression [2,5]. Thus, although MCKhLPL mice are not diabetic, insulin resistance and type 2 diabetes mellitus in humans share many of the metabolic phenotypes of MCKhLPL mice [6]. Because alterations in triglyceride partitioning may be involved in development of insulin resistance and type 2 diabetes mellitus in both mice and humans [7], MCKhLPL mice are a useful tool to understand mechanisms promoting type 2 diabetes mellitus in humans.

Increased utilization of triglyceride as a fuel in MCKhLPL mice would be expected to decrease FFA uptake and potentially increase FFA concentration compared with controls. However, plasma FFA concentration was reported as unchanged [4] or decreased [3] in mice overexpressing skeletal muscle LPL after a 4-hour fast. Others have

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doi:10.1016/j.metabol.2006.06.018

reported similar FFA concentration after a longer overnight fast in both young [2,3] and old [5] MCKhLPL mice compared with age-matched controls. Thus, despite increased skeletal muscle LPL content and greater lipoprotein uptake [8], MCKhLPL mice exhibit unchanged or decreased FFA concentration compared with controls. Although FFA concentration is similar in transgenic and control mice, it is not known if skeletal muscle LPL overexpression alters whole-body FFA kinetics.

The purpose of this study was to determine if skeletal muscle LPL overexpression influences plasma FFA turnover and if prolonged fasting alters FFA kinetics in MCKhLPL vs control mice. Given plasma triglyceride concentration is consistently decreased in MCKhLPL mice, likely due to increased triglyceride uptake in skeletal muscle, we expected a similar decrease in FFA turnover. Thus, we hypothesized FFA turnover would be decreased after both 4 and 16 hours of fasting in MCKhLPL mice because of increased reliance on lipoprotein-derived triglyceride as a fuel.

2. Methods

2.1. Mice

Adult male transgenic (MCKhLPL) and nontransgenic FVB control mice (Con) were caged at approximately 20°C on a 12:12-hour light/dark cycle. MCKhLPL mice constitutively overexpress skeletal muscle LPL and have been previously described by our laboratory [3]. Adult mice with an average age of 31.9 ± 0.1 weeks were used for these studies. Mice were given unrestricted access to standard chow (Diet 8640; Harlan Teklad, Madison, WI) and water before the experiment. A total of 9 control and 9 transgenic mice were tested after a 4-hour fast, whereas 12 control and 10 transgenic mice were tested after the 16-hour fast. Studies were conducted in accordance with protocols approved by the animal care and use committee at the University of Colorado at Denver and Health Sciences Center.

2.2. Isotope study

Before the isotope study, food was removed for either 4 or 16 hours before the experiments, which were conducted between 10 AM and 2 PM. Mice were anesthetized with Avertin (2,2,2-tribromoethanol, 250 mg/kg; Aldrich, Milwaukee, WI). A jugular vein catheter was then placed, immediately followed by constant infusion of radioactive palmitate. Based on a recipe originally described by Baker et al [9], 4 μCi of tritiated palmitate ([9,10-^{3}H(N)]palmitic acid; American Radiolabeled Chemicals, St Louis, MO) was mixed with 0.6 mL mouse serum (Sigma-Aldrich, Milwaukee, WI) and 0.3 mL saline for each animal. Tracer palmitate was infused at 0.5 mL/h throughout the experiment by using a 1-mL syringe (Becton-Dickinson, Franklin Lakes, NJ) attached to a syringe pump (Harvard Apparatus, Holliston, MA). Blood was sampled via the ocular capillary bed at minutes 30, 40, and 50 of isotope infusion. Approximately 150 μL of blood was taken during each blood draw. The goal was to maintain isovolemia during the experiment by infusing (420 μL of the palmitate-serum-saline mixture) approximately the volume of blood we were sampling (450 μL of blood).

2.3. Extraction of lipids

Blood was spun in a microcentrifuge and plasma frozen at −20°C until analysis. Lipid was extracted from 50 μL of plasma as described by Dole [10]. Free fatty acids were then separated from other blood lipids by using solid-phase extraction on aminopropyl columns as initially described by Kaluzny et al [11]. Isolated FFA (see below) were then dried under flowing nitrogen in an aluminum bead bath heated to 40°C to 50°C, and resuspended in 5 mL scintillation fluid (Ready Safe, Beckman Coulter, Fullerton, CA). To minimize chemiluminescence, samples were left in the dark for at least 2 weeks before counting in a liquid scintillation counter (Beckman LS 6000TA).

2.4. FFA isolation

After 10 minutes of palmitate infusion, it was assumed that the radioactive palmitate tracer would be incorporated into other lipid moieties, such as triglyceride and lipoproteins [12]. Therefore, FFAs were separated from other labeled lipid fractions by solid-phase extraction before counting for radioactivity by using a modified protocol as originally described by Kaluzny et al [11]. Aminopropyl NH_2 solid-phase extraction columns (Prepsep Amino 500 mg/3 mL, Fisher Scientific) were added to the vacuum apparatus (Visiprep DL, Supelco, Bellefonte, PA) and rinsed with 6 mL hexane under vacuum, with care being taken never to let the column run dry. Lipid isolated from plasma using the Dole extraction was dried under N_2 at 40°C, resuspended in chloroform and loaded onto the column. Plasma FFAs from transgenic and nontransgenic mice were isolated simultaneously.

Solvents were added to the aminopropyl solid-phase extraction columns and pulled through the column matrix under vacuum (≥10 kPa). After the plasma lipid isolation was loaded onto the columns, 8 mL of chloroform-isopropanol (2:1) was passed through the column to elute neutral lipids. Free fatty acids were then eluted with 8 mL of 2% acetic acid in diethyl ether and dried down for counting as described above.

2.5. FFA concentration

Plasma FFA concentration was measured by using 5 μL of plasma in duplicate with a commercially available kit (Wako Chemicals, Richmond, VA). Samples for all mice were run with a quality control standard in triplicate and adjusted based on the internal standard concentration to account for interday assay variation. Samples were read on a Dynatech MRX 96-well microplate reader at 550 nm (Dynatech Laboratories, Chantilly, VA).

2.6. Calculations

Rate of appearance was calculated by using standard equations as initially described by Steele [13] for steady-state conditions.

$$R_a\ (\mu mol/min) = F - \{V[(C_2 + C_1)/2] \times [(SA_{t_2} - SA_{t_1})/(t_2 - t_1)]\}/((SA_{t_2} + SA_{t_1})/2)$$

$$R_d\ (\mu mol/min) = R_a\ (\mu mol/min)$$

$$MCR\ (ml/min) = R_d/[(C_2 + C_1)/2]$$

where F is the infusion rate in counts per minute, V is the estimated volume distribution for palmitate in milliliters per kilogram, C_1 is [tracee] at t_1, C_2 is [tracee] at t_2, SA is the specific activity in counts per minute per micromole, t_1 is time 1 of sampling, t_2 is time 2 of sampling, R_d is the FFA rate of disappearance, and MCR is the metabolic clearance rate.

2.7. FFA time course study

In a separate experiment, 5 MCKhLPL and 4 Con mice (11-14 weeks old) were used to determine the time course for changes in FFA concentration during fasting. Mice were placed in cages that contained no food, and approximately 50 μL of blood was drawn via ocular bleed under inhalation anesthesia (isoflurane for less than 3 minutes) at hours 1, 2, 3, 4, 6, 8, 12, and 24 of fasting. FFA concentration was determined by using a Wako FFA kit (Wako Chemicals, Richmond, VA) and spectrophotometric absorption at 550 nm.

2.8. Statistics

Results are displayed as means ± SEM. Overall differences between groups were determined by using two-sided unpaired Student t tests. Changes in specific activity over time were determined by using a repeated-measures analysis of variance. Changes in FFA concentration in the FFA time course experiment were determined by using a 2-way repeated-measures analysis of variance. An α level of .05 was used throughout for statistical significance.

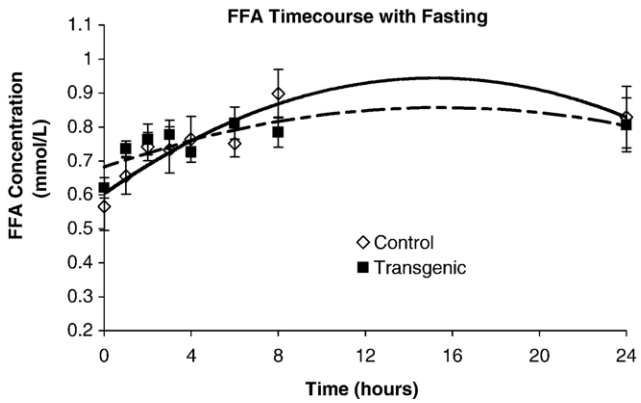

Fig. 1. Free fatty acid concentration after 1 to 24 hours of fasting in transgenic and control mice. Samples are taken from individual mice over time. Values are means ± SEM.

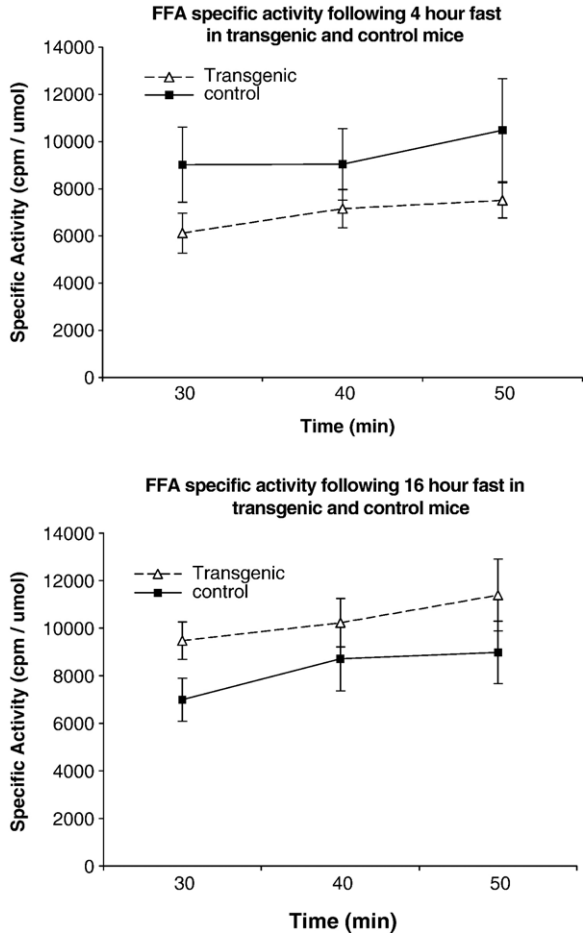

Fig. 2. Palmitate specific activity in transgenic and control mice during a 50-minute isotope infusion after a 4- and 16-hour fast. Values are means ± SEM.

3. Results

Transgenic and control mice for this study were on average 31.9 weeks old, with no significant difference in age between the 2 groups (MCKhLPL, 32.1 ± 0.18 weeks; Con, 31.5 ± 0.29 weeks). Body weight was also not different between the 2 groups after 4-hour (MCKhLPL, 31.6 ± 0.9 g; Con, 35.0 ± 1.2 g, P = .10) and 16-hour fasts (MCKhLPL, 29.8 ± 1.4 g; Con, 32.0 ± 1.2 g; P = .18). We have previously reported no differences in percent body fat between MCKhLPL and control mice on a high-carbohydrate diet [3].

In the FFA time course study, FFA concentration increased significantly in MCKhLPL mice from 0 to 1 hour of fasting from 0.62 ± 0.3 to 0.74 ± 0.2 mmol/L, respectively (Fig. 1). There were no significant differences in FFA concentration from 1 to 24 hours of fasting in MCKhLPL mice, with the final FFA concentration of 0.81 ± 0.8 mmol/L. Similarly, FFA concentration increased in control mice from 0 to 1 (0.57 ± 0.7 to 0.66 ±

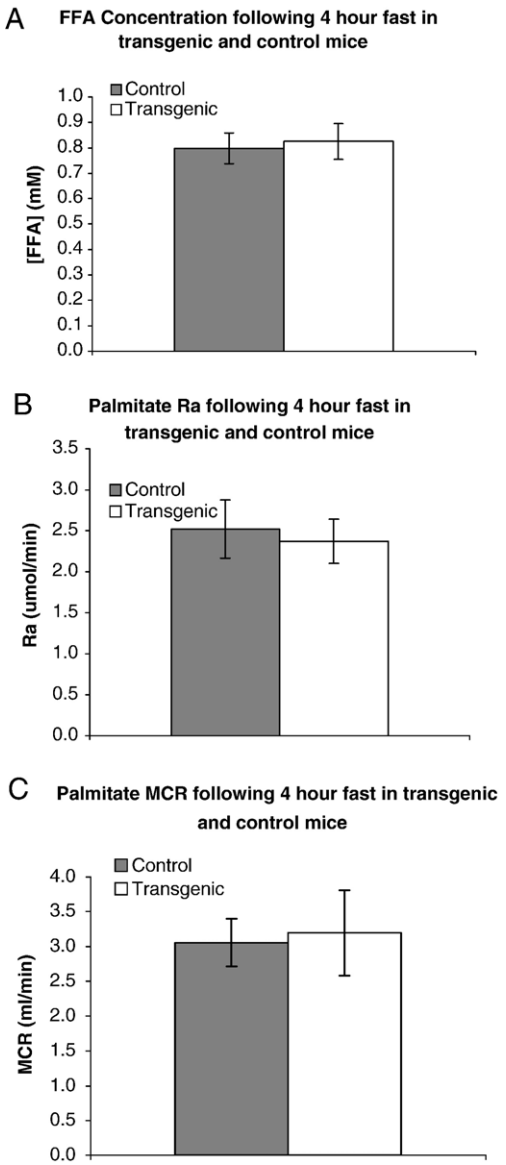

Fig. 3. Free fatty acid concentration (A), palmitate R_a (B), and MCR (C) in transgenic and control mice during a 50-minute isotope infusion after a 4-hour fast. Values are means ± SEM.

0.5 mmol/L) and 1 to 2 (0.66 ± 0.5 to 0.74 ± 0.4 mmol/L) hours of fasting, respectively. There were no significant changes from 2 to 24 hours of fasting in control mice, with the final FFA concentration of 0.83 ± 0.9 mmol/L.

Specific activity increased significantly from 30 to 50 minutes in both control and transgenic mice after 4- and 16-hour fasts (Fig. 2A, B). Therefore, we used non–steady-state equations for the calculation of FFA turnover. Mean specific activity after the 4-hour fast was not significantly different between groups at 9513 ± 1705 cpm/mL for Con and 7551 ± 877 cpm/mL for MCKhLPL. After the 16-hour fast, mean specific activity was also not different at 8228 ± 1137 cpm/mL for Con and 10,367 ± 935 cpm/mL for MCKhLPL. The relative change in specific activity can explain the changes in FFA turnover in MCKhLPL, as specific activity increased 37% in transgenic mice after the 16-hour fast compared with a 13% decrease in controls. Isotope infusion rates were also similar between groups and fasts. During the 4-hour fast, mean palmitate infusion rates tended to be lower for MCKhLPL compared with controls (16,290 ± 1414 for MCKhLPL and 20,022 ± 1582 cpm/min for Con, $P = .10$). During the 16-hour fast, mean palmitate infusion rates were not significantly different between groups (15,805 ± 1434 for MCKhLPL and 18,417 ± 1630 cpm/min for Con).

Free fatty acid concentration was not significantly different between control and transgenic mice after the 4-hour (Con, 0.80 ± 0.06 mmol/L; MCKhLPL, 0.83 ± 0.07 mmol/L; Fig. 3A) or 16-hour (Con, 0.83 ± 0.04 mmol/L; MCKhLPL, 0.80 ± 0.07 mmol/L; Fig. 4A)

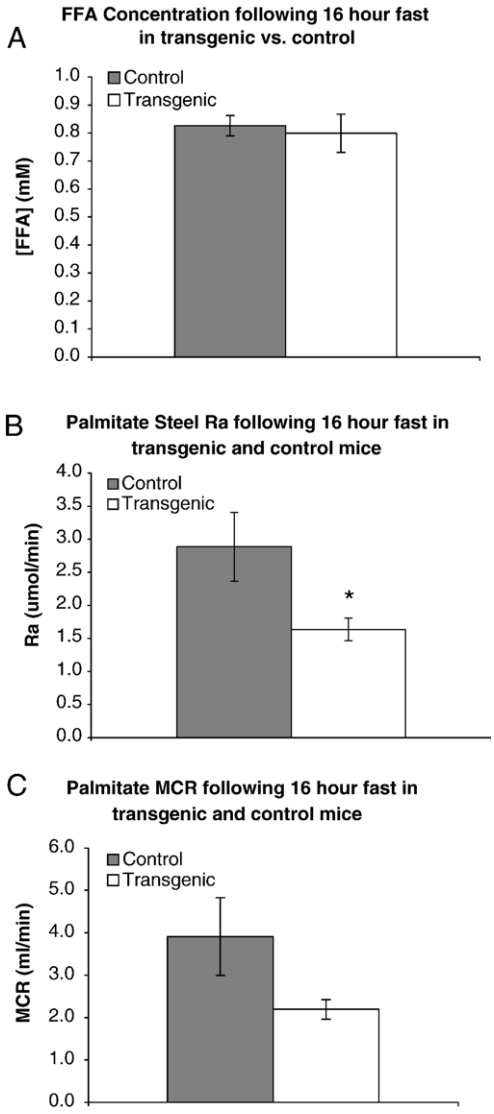

Fig. 4. Free fatty acid concentration (A), palmitate R_a (B), and MCR (C) in transgenic and control mice during a 50-minute isotope infusion after a 16-hour fast. Values are means ± SEM. *$P < .05$, significantly different than control.

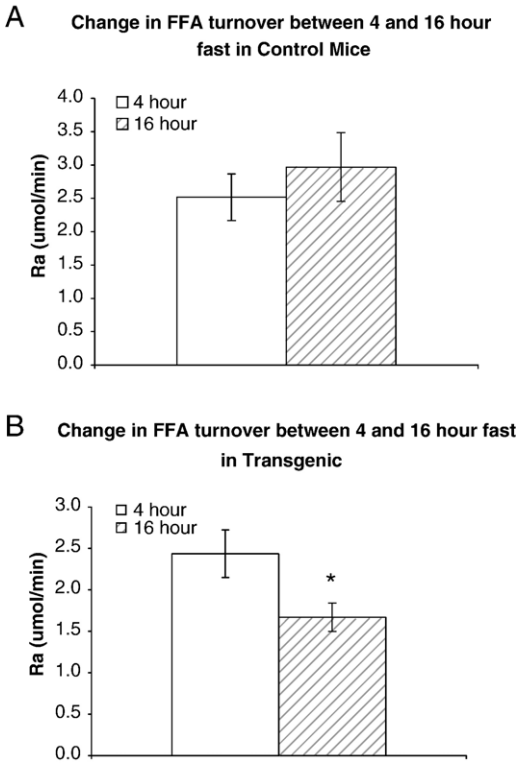

Fig. 5. Free fatty acid turnover in control (A) and transgenic (B) mice after both 4- and 16-hour fasts. Values are means ± SEM. *$P < .05$, significantly different than 4-hour fast.

fasts. FFA concentration did not change from 4 to 16 hours of fasting in either group.

Free fatty acid rate of appearance (R_a) and disappearance (R_d) were identical during both 4 and 16-hour fasts because values were calculated in steady state without changes in FFA concentration. Therefore, only FFA R_a is presented, which represents FFA turnover in the steady state (R_a and R_d). After the 4-hour fast, FFA rate of appearance (R_a) was not different between Con and MCKhLPL mice (Fig. 3B) (Con R_a = 2.52 ± 0.36 μmol/min, MCKhLPL R_a = 2.37 ± 0.27 μmol/min). FFA MCR (Fig. 3C) (Con, 3.05 ± 0.34 mL/min; MCKhLPL, 3.19 ± 0.61 mL/min) was also not significantly different between groups.

Free fatty acid turnover was significantly lower after the 16-hour fast in MCKhLPL compared with control mice (Fig. 4B; Con R_a = 2.89 ± 0.52 μmol/min MCKhLPL R_a = 1.64 ± 0.17 μmol/min). FFA MCR (Fig. 4C; Con, 3.91 ± 0.92 mL/min; MCKhLPL, 2.2 ± 0.23 mL/min) tended ($P = .11$) to be lower in MCKhLPL vs control. FFA turnover decreased significantly ($P < .05$) from 4 to 16 hours in MCKhLPL, but not Con mice (Fig. 5A, B).

4. Discussion

This is the first study to quantify FFA kinetics in mice overexpressing skeletal muscle–specific LPL. Our data suggest skeletal muscle LPL overexpression does not change FFA concentration and turnover after a 4-hour fast. However, a longer 16-hour fast decreased FFA flux in mice with skeletal muscle LPL overexpression despite unchanged FFA concentration. It is likely that decreased FFA turnover in skeletal muscle LPL overexpression resulted from increased adipocyte insulin action previously reported after an overnight fast [5], and/or increased reliance on plasma lipoproteins by skeletal muscle with prolonged fasting.

4.1. FFA concentration

Free fatty acid concentration increases rapidly during a fast in mice as shown in Fig. 1. In this separate experiment, FFA concentration significantly increased in transgenic mice within 1 hour of fasting, with no significant difference in FFA concentration from 1 to 24 hours of fasting. Similarly, FFA concentration increased in control mice for the first 2 hours of fasting, with no significant changes from 2 to 24 hours. The FFA turnover experiments corroborate this finding, as FFA concentration was not different from 4 to 16 hours of fasting. Others have found similar data showing no change in FFA concentration in mice from 4 to 16 [14] and from 12 to 48 hours of fasting [15-18]. We extend this finding to suggest both control and MCKhLPL mice reach a plateau in fasting FFA concentration within 1 to 2 hours of fasting.

After a 4-hour fast, both unchanged [4] and decreased [3] FFA concentration have been reported in MCKhLPL compared with control mice. After a 12-hour overnight fast, most have reported unchanged FFA concentration in MCKhLPL vs control mice [2,3,5]. There are no data in the literature comparing FFA concentration in MCKhLPL and control mice during a longer fast. Considering the plateau in FFA concentration observed in this and other studies after a prolonged fast [15-18], we expected to find unchanged FFA concentration from 4 to 16 hours of fasting in both transgenic and control mice. These data support the finding that within 2 hours of fasting in mice, FFA concentration increases to a level that does not change for up to 24 hours.

4.2. FFA kinetics

Most investigators reporting FFA kinetics in mice have used a bolus-decay tracer approach [9,12,19]. Only one study has been published that used a constant infusion tracer model to study FFA kinetics in mice [20]. Specifically, no experiments have been published investigating kinetics of the FFA pool in skeletal muscle LPL overexpressing mice. Skeletal muscle LPL overexpression in mice has been shown to promote skeletal muscle insulin resistance [2,4], increase whole-body fat oxidation with no change in FFA concentration [4], decrease plasma triglyceride concentration [2-4], increase intramuscular triglyceride content, and decrease glucose R_a [2]. Lower plasma triglyceride concentration and decreased glucose R_a suggested changes in substrate utilization due to skeletal muscle LPL overexpression. Therefore, we expected to find decreased FFA turnover between transgenic mice and controls after the

4-hour fast. Surprisingly, we found no significant difference in FFA turnover after a 4-hour fast (Fig. 3). Assuming adipocyte FFA reesterification was not different in transgenic and nontransgenic mice, these data suggest similar adipoycte lipolysis and FFA tissue uptake between groups after a 4-hour fast. These data suggest that any increase in utilization of plasma triglyceride as a fuel with skeletal muscle LPL overexpression was not dramatic enough to change FFA R_a after a 4-hour fast.

The response to a slightly more prolonged fast in transgenic and control mice is less well described. After an overnight fast, FFA concentration was not different in MCKhLPL vs control mice [2,5]. We extend this observation by showing unchanged FFA concentration in transgenic compared with control mice after a 24-hour fast (Fig. 1). Unlike FFA concentration, FFA turnover was significantly lower in transgenic vs control mice after a 16-hour fast. Thus, similar FFA concentrations in MCKhLPL and control mice were maintained despite decreased whole-body flux rates. The significant difference in FFA R_a between groups was due to a significant decrease in MCKhLPL R_a with prolonged fasting, as control mice did not change FFA concentration or turnover after the 16-hour fast. The significant decrease in FFA turnover in transgenic, but not control, mice after a 16-hour fast compared with a 4-hour fast is perhaps the most interesting finding of this study. Although the mechanisms responsible for decreased FFA turnover in the transgenic mice are unclear, there are several possible explanations.

One possible mechanism for decreased FFA R_a after the 16-hour fast in MCKhLPL mice is increased sensitivity to the antilipolytic effect of insulin at the level of the adipocyte. Voshol et al [5] reported significantly increased adipose tissue 2-deoxyglucose uptake during a hyperinsulinemic-euglycemic clamp after an overnight fast in skeletal muscle–specific LPL–overexpressing mice compared with controls. These data suggest adipocyte insulin action, at least with respect to glucose uptake, is increased with skeletal muscle LPL overexpression after an overnight fast. Whether increased adipocyte glucose uptake extends to increased suppression of lipolysis is not known. Enhanced adipocyte insulin action in MCKhLPL mice after an overnight fast, as suggested by the Voshol et al data, could explain why FFA R_a was only decreased after the more prolonged fast. If skeletal muscle LPL overexpression increases adipocyte insulin action during a prolonged fast, and there is no difference between insulin concentration in MCKhLPL and control mice [2-4], FFA R_a would be less than that of controls due to greater inhibition of lipolysis. Unchanged fasting insulin concentration combined with increased adipocyte insulin action suggest increased antilipolytic action of insulin may explain the decrease in FFA R_a after the 16-hour fast in mice overexpressing skeletal muscle LPL.

Ketone body concentration increases proportionally from 2 to 24 hours of fasting in mice [14,21,22], and ketones are known to have powerful antilipolytic effects [23]. Thus, it is likely that ketone concentrations were elevated during the 16- compared with the 4-hour fast in this study and likely played a role to suppress FFA turnover. Differences in ketone concentration between MCKhLPL mice and controls after 16 hours of fasting is one potential mechanism to explain alterations in FFA R_a in this study. However, Levak-Frank et al [8] reported that low, medium, and high levels of LPL overexpression in heart and skeletal muscle after a 12-hour fast did not change ketone body concentration compared with control mice. Thus, it is unlikely that the concentration of ketone bodies were different after a 16-hour fast in MCKhLPL mice compared with controls. Therefore, alterations in lipolysis as a result of differences in ketone body concentration are not a likely explanation for differences in FFA R_a in the current study.

A change in adipocyte FFA reesterification is another potential mechanism promoting decreased FFA R_a in MCKhLPL mice after the 16-hour fast. If lipolysis was not changed by the 16-hour fast in MCKhLPL mice, increased intracellular FFA reesterification in transgenic mice could explain a decrease in FFA R_a vs controls. Adipose tissue FFA reesterification has been shown to increase with decreased adipose tissue blood flow [24], increased arterial lactate concentration [25], and increased FFA/glycerol ratios [26]. Most studies investigating differences in skeletal muscle LPL overexpression have not measured potential factors that may change FFA reesterification. Further studies are needed to determine if there are changes in FFA reesterification in MCKhLPL mice that could explain decreased R_a observed in this study.

Another potential mechanism that could affect FFA turnover in these experiments are changes in whole-body substrate utilization with skeletal muscle LPL overexpression. MCKhLPL mice, by definition, have increased skeletal muscle LPL activity. Therefore, it is likely that skeletal muscle LPL overexpression increased reliance on plasma triglyceride–derived substrate. Data from most [3-5,8] but not all studies [2] suggest skeletal muscle LPL overexpression decreases plasma triglyceride concentration, which is consistent with this idea. Therefore, it is likely that transgenic mice increase reliance on plasma lipoproteins as a fuel compared with controls. Increased whole-body fat oxidation in MCKhLPL mice during the light hours of the day has previously been reported by our laboratory [4]. Increased oxidation of lipoprotein-derived substrate was likely the source of increased fat oxidation, considering increased muscle LPL expression, but exact sources of the increased fat oxidation were not determined. Levak-Frank et al [8] reported increased skeletal muscle FFA concentration and decreased plasma triglyceride concentration proportional to the level of overexpression of skeletal muscle LPL in mice. These changes occurred without altering whole-body FFA concentration compared with controls and provide compelling data that support increased plasma triglyceride utilization decreasing FFA R_a in MCKhLPL

mice. We have found MCKhLPL mice have greater CD36 messenger RNA levels in skeletal muscle compared with controls (unpublished observations). Increased CD36 gene expression along with increased reliance on plasma lipoproteins as a fuel could suggest FFAs liberated from triglyceride hydrolysis enter the FFA pool before transport through the sarcolemma. Greater utilization of plasma lipoprotein–derived lipid with prolonged fasting may lead to increased skeletal muscle FFA concentration, which could decrease skeletal muscle FFA uptake, and may be one mechanism decreasing whole-body FFA flux in MCKhLPL mice.

4.3. Conclusions

These studies suggest FFA concentration does not change during 2 to 24 hours of fasting in control and skeletal muscle LPL–overexpressing mice. There were no differences in FFA turnover after 4 hours of fasting between groups. However, compared with a 4-hour fast, a 16-hour fast decreased FFA turnover in mice overexpressing skeletal muscle LPL but not in controls. Greater reliance on lipoprotein-derived fuels and/or increased adipoycte insulin action in skeletal muscle LPL up-regulation may be the mechanisms decreasing whole-body FFA kinetics. These data indicate skeletal muscle LPL overexpression alters the metabolic response to prolonged fasting by decreasing reliance on plasma FFA in mice.

Acknowledgment

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases grant DK-26356 to Robert H. Eckel.

References

[1] Eckel RH. Lipoprotein lipase. A multifunctional enzyme relevant to common metabolic diseases. N Engl J Med 1989;320:1060-8.
[2] Kim JK, Fillmore JJ, Chen Y, et al. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A 2001;98:7522-7.
[3] Jensen DR, Schlaepfer IR, Morin CL, et al. Prevention of diet-induced obesity in transgenic mice overexpressing skeletal muscle lipoprotein lipase. Am J Physiol 1997;273(2 Pt 2):R683-9.
[4] Ferreira LD, Pulawa LK, Jensen DR, et al. Overexpressing human lipoprotein lipase in mouse skeletal muscle is associated with insulin resistance. Diabetes 2001;50:1064-8.
[5] Voshol PJ, Jong MC, Dahlmans VE, et al. In muscle-specific lipoprotein lipase–overexpressing mice, muscle triglyceride content is increased without inhibition of insulin-stimulated whole-body and muscle-specific glucose uptake. Diabetes 2001;50:2585-890.
[6] Reaven GM. Role of insulin resistance in the pathophysiology of non–insulin dependent diabetes mellitus. Diabetes Metab Rev 1993; 9(Suppl 1):5S-12S.
[7] Kahn BB, Flier JS. Obesity and insulin resistance. J Clin Invest 2000;106:473-81.
[8] Levak-Frank S, Radner H, Walsh A, et al. Muscle-specific overexpression of lipoprotein lipase causes a severe myopathy characterized by proliferation of mitochondria and peroxisomes in transgenic mice. J Clin Invest 1995;96:976-86.
[9] Baker N, Sandborg C, Morris D, et al. Competition for host essential and nonessential fatty acids by Ehrlich ascites carcinoma in mice. Cancer Res 1977;37(7 Pt 1):2218-25.
[10] Dole VP. Fractionation of plasma nonesterified fatty acids. Proc Soc Exp Biol Med 1956;93:532-3.
[11] Kaluzny MA, Duncan LA, Merritt MV, et al. Rapid separation of lipid classes in high yield and purity using bonded phase columns. J Lipid Res 1985;26:135-40.
[12] Baker N, Gan-Elepano M, Guthrie BA, et al. Turnover and fate of plasma free fatty acids in briefly-fasted lymphoma-bearing mice. Lipids 1989;24:1028-34.
[13] Steele R. Influences of glucose loading and of injected insulin on hepatic glucose output. Ann N Y Acad Sci 1959;82:420-30.
[14] Heijboer AC, Donga E, Voshol PJ, et al. Sixteen hours of fasting differentially affects hepatic and muscle insulin sensitivity in mice. J Lipid Res 2005;46:582-8.
[15] Fielder PJ, Ogren L, Edwards D, et al. Effects of fasting on serum lactogenic hormone concentrations during mid- and late pregnancy in mice. Am J Physiol 1987;253(1 Pt 1):E40-4.
[16] Menahan LA, Sobocinski KA. Comparison of carbohydrate and lipid metabolism in mice and rats during fasting. Comp Biochem Physiol B 1983;74:859-64.
[17] Lombardo YB, Hron WT, Sobocinski KA, et al. A metabolic profile of fed and fasting genetically obese mice at 4-5 months of age. Horm Metab Res 1984;16(Suppl 1):37-42.
[18] Suzuki J, Shen WJ, Nelson BD, et al. Cardiac gene expression profile and lipid accumulation in response to starvation. Am J Physiol Endocrinol Metab 2002;283:E94-E102.
[19] Ookhtens M, Montisano D, Lyon I, et al. Transport and metabolism of extracellular free fatty acids in adipose tissue of fed and fasted mice. J Lipid Res 1987;28:528-39.
[20] Goudriaan JR, Tacken PJ, Dahlmans VE, et al. Protection from obesity in mice lacking the VLDL receptor. Arterioscler Thromb Vasc Biol 2001;21:1488-93.
[21] Schreiber RA, Yeh YY. Temporal changes in plasma levels and metabolism of ketone bodies by liver and brain after ethanol and/or starvation in C57BL/6J mice. Drug Alcohol Depend 1984;13:151-60.
[22] Rofe AM, Porter SJ, Bais R, et al. The metabolic response of tumour-bearing mice to fasting. Br J Cancer 1985;52:619-23.
[23] Coppack SW, Jensen MD, Miles JM. In vivo regulation of lipolysis in humans. J Lipid Res 1994;35:177-93.
[24] Bulow J. Subcutaneous adipose tissue blood flow and triacylglycerol-mobilization during prolonged exercise in dogs. Pflugers Arch 1982;392:230-4.
[25] Issekutz B, William A, Shaw S, et al. Effect of lactate on FFA and glycerol turnover in resting and exercising dogs. J Appl Physiol 1975;39:349-53.
[26] Madsen F, Bülow J, Nielsen N. Feed back regulation of fatty acid mobilization by arterial free fatty acid concentration. Acta Physiol Scand 1986;127:161-6.