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Metabolism Clinical and Experimental 58 (2009) 1649 – 1656
Metabolism
Clinical and Experimental
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# The synthetic rate of muscle triglyceride but not phospholipid is increased in obese rabbits

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Received 3 April 2009; accepted 21 May 2009

**Abstract**

Fat is a major energy source for skeletal muscle, and disruption of normal trafficking of fatty acids in muscle is linked to insulin resistance. We quantified muscle triglyceride (TG) and phospholipid (PL) synthesis in lean and obese rabbits by means of L-[U-$^{13}C_{16}$] palmitate infusion. Intramyocellular palmitoyl–coenzyme A was used as the precursor for rates of TG and PL synthesis, which were compared with the rates calculated using plasma nonesterified palmitate as the precursor. The muscle of obese rabbits had a greater ($P < .05$) combined pool of fatty acyl–coenzyme A plus fatty acyl–carnitine than lean rabbits (40.9 ± 3.7 vs 28.6 ± 5.3 nmol/g). Although the fractional synthetic rates of muscle TG were almost identical (0.095%/h ± 0.016%/h vs 0.092%/h ± 0.019%/h), the absolute synthetic rates were greater ($P < .01$) in the obese than in lean rabbits (39.7 ± 9.5 vs 10.1 ± 2.5 nmol $g^{-1}$ $h^{-1}$) because of greater TG content in the muscle of obese rabbits. Plasma nonesterified fatty acids and TG accounted for 51% to 55% of the true precursor pool for muscle lipid synthesis in both groups, and the rest was derived from fatty acids recycled within the muscle. In contrast, the fractional and absolute synthetic rates of muscle PL as well as PL contents were comparable in the 2 groups. In conclusion, the content and synthetic rate of muscle TG rather than PL were increased in obese rabbits, which might be linked to insulin resistance. Plasma lipids and muscle lipolysis were the 2 predominate contributors to the intramyocellular fatty acyl–coenzyme A pool for lipid synthesis.


## 1. Introduction

Insulin resistance, which is characterized by an impaired responsiveness to the normal action of insulin to stimulate glucose uptake, has recently been linked to disruptions in the normal trafficking of fatty acids in the muscle, leading to storage of increased amounts of triglyceride (TG) within the muscle cells [1-5]. Although the importance of disruptions in intracellular fat metabolism in the etiology and pathology of insulin resistance is becoming well recognized, quantitative measurement of lipid synthesis in the muscle has not been adequately described. Previous experiments used plasma nonesterified fatty acids (NEFA) as precursor for intracellular lipid synthesis [6-8]. However, TG synthesis from fatty acids derived from intracellular lipolysis (ie, intracellular recycling of fatty acids) is not included with this technique. Fatty acids are rapidly converted to fatty acyl–coenzyme A (CoA) upon entering the cell. Fatty acyl–CoA is also produced from fatty acids released in the process of intracellular lipolysis and potentially from de novo synthesized fatty acids, if any de novo synthesis is occurring. Fatty acyl–CoA is in turn a precursor for either synthesis of intracellular lipids such as TG and phospholipid (PL) or oxidation via the tricarboxylic acid cycle. Fatty acyl–carnitine must be produced from the fatty acyl–CoA for transport into the mitochondria for subsequent oxidation to occur. Thus, fatty acyl–CoA is the true precursor for intracellular production of TG, PL, and other lipids; and fatty acyl–carnitine is the precursor for intramitochondrial oxidation of fatty acids. Measurement of either synthesis or oxidation requires accurate measurement of the true precursor enrichment. Therefore, we have developed the methods of measuring the isotopic enrichment of fatty acyl–CoA [9] and fatty acyl–carnitine [10] to accurately measure

This manuscript has no potential conflict of interest for all authors.

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doi:10.1016/j.metabol.2009.05.021

the rates of synthesis of TG and PL and oxidation of fatty acids, respectively.

In a previous experiment, we reported that lipid metabolism in diet-induced obese rabbits is similar to human obesity [11]. In that experiment, we quantified plasma lipid kinetics including hepatic TG secretion. The present experiment further investigated the alterations of lipid metabolism in the skeletal muscle of this obese rabbit model. We have measured the isotopic enrichment of intramyocellular palmitoyl-CoA as well as plasma nonesterified palmitate as precursors for the calculation of the rates of intramuscular synthesis of TG and PL. Palmitoyl-carnitine enrichment was also measured to determine the relation to palmitoyl-CoA enrichment. By comparing obese and lean rabbits, we have used these approaches to gain an insight into the alterations in myocellular lipid trafficking that occurs in obese as compared with lean rabbits.

## 2. Materials and methods

### 2.1. Animals

Female New Zealand White rabbits (Myrtle's Rabbitry; Thompson Station, TN), weighing approximately 3.5 kg, were used for this study. The rabbits were housed in individual cages and were given 100 to 120 g/d of unpurified diet (Lab Rabbit Chow 5326; Purina Mills; St Louis, MO) for 1 week for acclimation. This diet provided 203 to 244 kcal/d (850-1022 kJ/d), with 23% from protein, 7.9% from fat, and 69% from carbohydrate. The rabbits were then randomly assigned to 2 groups: those to be lean and those to be obese. This protocol complied with the Public Health Service Policy on Humane Care and Use of Laboratory Animals, incorporated in the Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals, and was approved by the Animal Care and Use Committee of The University of Texas Medical Branch at Galveston.

### 2.2. Experimental design

The rabbits in the lean group were given the same diet as in the acclimation phase. The rabbits in the obese group were given a high-fat diet, ad libitum, consisting of the same unpurified solid grain diet mixed with additional 10% corn oil and 8% lard; its energy sources consisted of 13% from protein, 49% from fat, and 38% from carbohydrate. After 10 weeks of feeding, the rabbits were food deprived for 24 hours before tracer infusion to ensure that there was no exogenous fat entry.

### 2.3. Isotope infusion protocol

The tracer infusion was performed under general anesthesia of ketamine and xylazine. The initial doses were 35 mg/kg of ketamine and 5 mg/kg of xylazine for intramuscular injection, which was followed by continuous intravenous infusion of a mixture containing 17 mg/mL of ketamine and 0.664 mg/mL of xylazine; the infusion rate was adjusted to maintain a desired depth of anesthesia [12]. Surgical procedures were performed to insert catheters into the carotid artery and jugular vein [12]. The arterial line was used for drawing blood and monitoring arterial blood pressure and heart rate; the venous line was used for infusing a stable isotope tracer and anesthetics. Tracheotomy was performed for placement of a tracheal tube, which was connected to a hood filled with oxygen-enriched room air. U-$^{13}C_{16}$-palmitate (99% enriched; Cambridge Isotope Laboratories, Andover, MA), bound to albumin in a 5% solution, was infused at 0.11 $\mu$mol kg$^{-1}$ min$^{-1}$ for 7 hours. Blood and muscle samples were taken before the start of the tracer infusion and every hour during the tracer infusion. The blood samples were centrifuged, and plasma was separated and stored at −20°C. The muscle samples were taken from the adductor muscle of both legs in the sequence from the distal to the proximal site of the muscle. The muscle samples were briefly washed with ice-cold saline, immediately frozen in liquid nitrogen, and stored at −80°C for later analysis.

During the tracer infusion, heart rate, mean arterial blood pressure, and rectal temperature were maintained stable by adjusting the infusion rates of anesthetics and physiologic saline and by using heating lamps. At the end of tracer infusion, the rabbits were euthanized.

### 2.4. Sample analysis

Plasma NEFA and TG were processed for palmitate enrichment on a gas chromatograph–mass spectrometer (MSD System; Agilent Technologies, Palo Alto, CA) [11,13]. Ions were selectively monitored at mass-to-charge ratios of 270, 285, and 286 for palmitate. Seven fatty acids in plasma NEFA were measured using a gas chromatograph system with flame ionization detection (model 6890, Agilent); their individual contributions to the total 7 fatty acids were expressed as percentages [11]. The 7 fatty acids were myristate (14:0), palmitate (16:0), palmitoleate [16:1 (n-1)], stearate (18:0), oleate [C18:1(n-9)], linoleate [18:2 (n-6)], and linolenate [(18:3(n-3)].

Muscle samples were pulverized into powder in liquid nitrogen. Fatty acyl–CoA and fatty acyl–carnitine were processed and measured on an Agilent 1100 series liquid chromatograph–1956B SL single quadrupole mass spectrometer as was previously reported [9,10]. Heptadecanoyl–CoA (Sigma Chemical, St Louis, MO) and $d_3$-palmitoyl-carnitine (Cambridge Isotope Laboratories) were added as internal standards for calculating fatty acyl–CoA and fatty acyl–carnitine contents [9,10]. Muscle TG, PL, and diacylglycerol (DAG) were extracted from 30 to 50 mg of wet muscle overnight at 4°C in 1:2 (vol/vol) methanol-chloroform solution containing 0.05 mg/mL butylated hydroxytoluene. Internal standards added for quantification of lipids were triheptadecanoin (118 nmol) for TG; 1,3-dipentadecanoin (3.7 nmol) for DAG; and L-α-phosphatidylcholine, diheptadecanoyl (65.6 nmol) for PL. These

internal standards were also loaded on the thin-layer chromatography (TLC) plate (Partisil LK5D, Silica Gel 150 Å; Schleicher & Schuell, Maidstone, England) for identification of corresponding lipids. After overnight extraction, the samples were centrifuged; the supernatant was dried under nitrogen gas. The samples were reconstituted with 50 $\mu$L chloroform for TLC isolation of TG and DAG; the isolation was processed in a tank with a mixture of hexane–ethyl ether–acetic acid (70:30:1 by volume). Because the PL fraction does not respond to the isolation condition, the sample spots were recovered from the TLC plate and reextracted with methanol-chloroform solution by 30 minutes of shaking on a shaker (Eberbach, Ann Arbor, MI). After centrifugation, the supernatant was loaded on the TLC plate again; PL was isolated in a tank with chloroform-methanol-water (65:30:5 in milliliters) for 20 to 30 minutes and another tank with heptane–ethyl ether–glacial acid (80:20:2 in milliliters) for 45 minutes. The isolated PL fraction was recovered from the TLC plate.

Palmitate methyl ester enrichments in muscle TG, PL, and DAG were measured on a gas chromatograph–combustion–isotope ratio mass spectrometer (Finnigan MAT; Thermo Electron, Bremen, Germany). The measured $^{13}CO_2$ enrichment was multiplied by 17/16 to convert to palmitate enrichment because, in the palmitate methyl ester, 16 of the total 17 carbons have a chance to be labeled. The concentrations of the lipids were measured on the gas chromatograph system with flame ionization detection.

### 2.5. Calculations

The concentrations (or contents) of palmitate in plasma (or muscle) lipids were calculated by the internal standard method [13]. Palmitate concentrations (or contents) were divided by percentage palmitate in the total of 7 fatty acids to obtain total fatty acid concentrations (or contents).

Fractional synthetic rates (FSRs) of muscle TG and PL were calculated by the tracer incorporation method, which is based on the precursor-product principle [13]. The general equation was:

$$\mathrm{FSR} = (E_{t2} - E_{t1})/[E_P(t_2 - t_1) \times (t2 - t1)], \quad (1)$$

where $(E_{t2} - E_{t1})$ is the increment of product enrichment, expressed as mole percentage excess (MPE), from $t_1$ to $t_2$; $E_P(t_2 - t_1)$ is the average precursor enrichment from $t_1$ to $t_2$. When intramyocellular palmitoyl-CoA was used as precursor, the FSR represents total lipid synthesis from both plasma NEFA and fatty acids from muscle lipolysis; the latter may include a portion of plasma TG breakdown through the action of lipoprotein lipase. When plasma nonesterified palmitate is used as precursor, the FSR represents lipid synthesis from plasma NEFA. The difference between the total FSR and FSR from plasma NEFA reflects recycling of fatty acids, whereby fatty acids from TG breakdown are reused for synthesis of TG and PL.

The equation to calculate the absolute synthetic rate of TG or PL was:

$$\text{Absolute synthetic rate} = \text{FRS (percentage per hour)} \times \text{pool size (nanomoles per gram of wet muscle)}. \quad (2)$$

The percentage contributions from plasma NEFA, plasma TG, and intramuscular TG to intramyocellular fatty acyl–CoA were calculated using the following equations:

$$\text{Percentage contribution from plasma NEFA} = (\mathrm{ECoA_0}/\mathrm{EPal_0}) \times 100\%, \quad (3)$$

where $\mathrm{ECoA_0}$ and $\mathrm{EPal_0}$ are MPE values of muscle palmitoyl-CoA (represented by palmitoyl-carnitine) and plasma nonesterified palmitate, respectively, at the beginning of tracer infusion when plasma TG-bound palmitate MPE is zero.

$$\text{Percentage contribution from plasma TG} = [(\mathrm{ECoA_p}/\mathrm{EPal_p}) - (\mathrm{ECoA_0}/\mathrm{EPal_0})] \times 100\%, \quad (4)$$

where $\mathrm{ECoA_p}$ and $\mathrm{EPal_p}$ are MPE values of muscle palmitoyl-CoA (represented by palmitoyl-carnitine) and plasma nonesterified palmitate at the plateau; $(\mathrm{ECoA_p}/\mathrm{EPal_p})$ represents percentage contribution from plasma NEFA and TG.

$$\text{Percentage contribution from intramuscular TG} = [1 - (\mathrm{ECoA_p}/\mathrm{EPal_p})] \times 100\%. \quad (5)$$

### 2.6. Statistical analysis

Values are expressed as means ± SEM. Differences comparing 2 parameters were tested by Student $t$ test. A $P$ value less than .05 was considered as statistically significant.

## 3. Results

The initial body weights did not differ between the lean (3.54 ± 0.05 kg) and obese (3.48 ± 0.07 kg) rabbits. After 10 weeks of feeding, body weights increased significantly ($P < .001$) in both groups and were greater ($P < .001$) in the obese (5.36 ± 0.05 kg) than in the lean (3.89 ± 0.07 kg) rabbits. Because we found that some of the lean rabbits were sensitive to ketamine and xylazine, we reduced the initial doses to 65% to 85% of the routine doses (35 mg/kg of ketamine and 5 mg/kg of xylazine) in 4 of the 5 rabbits. The total doses of ketamine and xylazine used (initial doses plus continuous infusion for 7 hours) were 321 ± 33 and 15.5 ± 1.7 mg/kg in the lean group, which were not significantly different ($P = .17$) from those of 359 ± 18 and 17.5 ± 0.7 mg/kg in the obese group. Plasma NEFA and TG were greater ($P < .01$-$.05$) in the obese group than in the lean

Table 1
Plasma and muscle lipid contents

| | Plasma lipids | | Muscle lipids | | | | | |
|---|---|---|---|---|---|---|---|---|
| | NEFA (mmol/L) | TG (μmol/L) | Acyl-CoA (nmol/g) | Acyl-CNT (nmol/g) | CoA + CNT (nmol/g) | TG (μmol/g) | PL (μmol/g) | DAG (μmol/g) |
| Lean | 0.32 ± 0.03 | 297 ± 46 | 21.1 ± 5.5 | 7.5 ± 0.5 | 28.6 ± 5.3 | 11.6 ± 2.4 | 3.5 ± 0.8 | 0.73 ± 0.16 |
| Obese | 0.59 ± 0.06* | 532 ± 80† | 30.1 ± 4.0 | 10.8 ± 1.2 | 40.9 ± 3.7† | 64.4 ± 12.9* | 4.0 ± 1.1 | 0.75 ± 0.11 |

Data are means ± SEM; n = 5 in each group. Plasma and muscle lipid concentrations are averages of 1-, 3-, 5-, and 7-hour measurements. CoA + CNT stands for sum of fatty acyl–CoA and fatty acyl-carnitine. CNT indicates carnitine.

* Different from lean, $P < .01$.

† Different from lean, $P < .05$.

rabbits (Table 1). The skeletal muscle of obese rabbits had greater ($P < .05$) amounts of TG and combined pool of fatty acyl–CoA plus fatty acyl–carnitine, whereas the contents of PL and DAG were comparable with that of lean rabbits (Table 1).

Plasma nonesterified palmitate enrichments were basically stable during the 7-hour tracer infusion in the 2 groups (Fig. 1A, B). Plasma TG-bound palmitate enrichments increased over time and then reached plateaus in both groups (Fig. 1A, B), which reflected the time course for hepatic TG synthesis and secretion. The different enrichment patterns of plasma lipids between the lean and obese rabbits reflected the different fatty acid sources for hepatic TG synthesis as was addressed in our previous publication [11]. Muscle TG-bound palmitate enrichment increased over time; the increase was linear during the first 3 hours and deviated from linear incorporation thereafter (Fig. 2A). The enrichments of muscle PL-bound palmitate basically followed linear increases; the first 3 hours showed better linear incorporation than later hours (Fig. 2B). Thus, muscle TG and PL FSRs were calculated from the first 3 hours of tracer infusion using both plasma nonesterified palmitate and

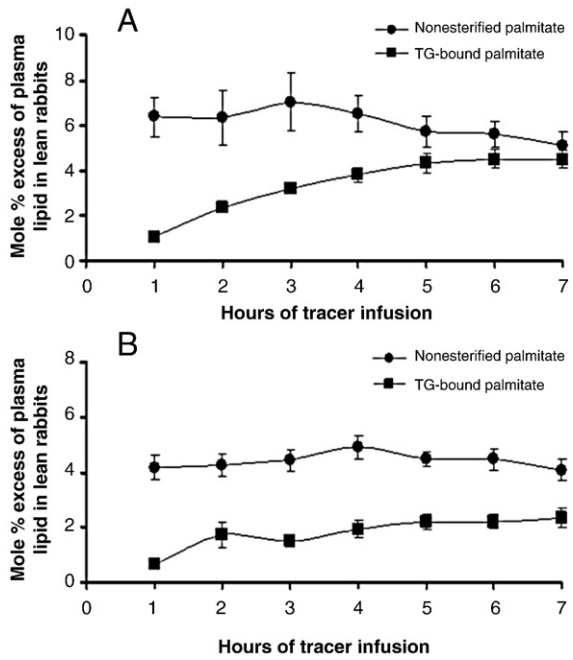


Fig. 1. Plasma nonesterified palmitate and TG-bound palmitate enrichment. A, Plasma nonesterified and TG-bound palmitate enrichments in lean rabbits during the 7 hours of tracer infusion. Plasma nonesterified palmitate enrichment was basically constant; plasma TG-bound palmitate enrichment increased over time and reached a comparable level as nonesterified palmitate by the end of tracer infusion. B, Plasma nonesterified and TG-bound palmitate enrichments in obese rabbits during the 7 hours of tracer infusion. Plasma nonesterified palmitate enrichment was also constant. Plasma TG-bound palmitate enrichments increased during the first 2 hours and reached a plateau thereafter. The graduated increases in plasma TG-bound palmitate enrichment reflected the time course for hepatic uptake of plasma NEFA, synthesis of TG, and secretion as very low-density lipoprotein. The different enrichment patterns between the lean and obese rabbits reflected the different sources of fatty acids used for TG synthesis, which were addressed in our previous publication [11]. Values are means ± SEM; n = 5 in each group.

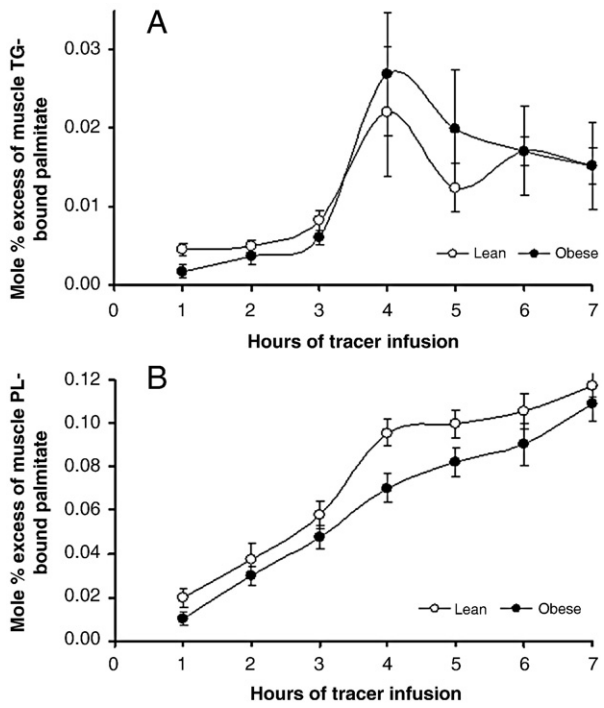


Fig. 2. Increases of intramuscular TG- and PL-bound palmitate enrichments. A, Increases of intramuscular TG-bound palmitate enrichment during the 7 hours of tracer infusion. The increases of enrichments followed linear lines during the first 3 hours of tracer infusion and deviated from the linear lines during the later hours. B, Increases of intramuscular PL-bound palmitate enrichment during the same period. The first 3 hours had better linear label incorporation than the later 4 hours. Therefore, muscle PL FSRs were also calculated from the first 3 hours of tracer infusion. Values are means ± SEM; n = 5 in each group.

intramyocellular palmitoyl-CoA as precursor. The deviation of linear incorporation during the later hours of tracer infusion might have been due to a leakage of plasma TG into the interstitial fluid in the muscle samples, as is addressed in "Discussion." Muscle DAG constituted a small pool, and its synthesis was not calculated.

Measured palmitoyl-CoA enrichment was unexpectedly lower than that of palmitoyl-carnitine (Fig. 3A, B). Because palmitoyl-CoA is the upstream metabolite of palmitoyl-carnitine, the greater enrichment of palmitoyl-carnitine than palmitoyl-CoA might be caused by analytic errors. We therefore infused U-$^{13}C_{16}$-palmitate in 3 additional normal rabbits after 24 hours of food deprivation. Muscle samples were taken from the adductor muscle of both legs at 5, 10, 20, 40, and 60 minutes of tracer infusion. We eliminated the step of washing in cold saline and froze the muscle samples immediately after excision. The results showed that the palmitoyl-CoA and carnitine enrichments were not significantly different (Fig. 3C). Intramyocellular palmitoyl-CoA and carnitine enrichments were approximately 50% of the corresponding plasma nonesterified palmitate enrichment (Fig. 3C). In another rabbit, we used the freeze clamp technique to obtain in situ frozen muscle at 0.5, 1.0, 1.5, and 2.0 hours of tracer infusion; the enrichments of palmitoyl-CoA and carnitine were almost identical (2.89% ± 0.23% vs 2.79% ± 0.33%, $P$ = .80). Thus, we concluded that the enrichments of fatty acyl–CoA and fatty acyl–carnitine were equilibrated in vivo. The lower enrichment of palmitoyl-CoA when samples were washed with saline was attributable to the seconds of time delay before freezing. When muscle samples were obtained, the transport of labeled palmitate from plasma was stopped, whereas muscle lipid breakdown still released unlabeled palmitate to dilute the palmitoyl-CoA pool. Fatty acyl–carnitine is a downstream metabolite of acyl-CoA, so it was less affected. We therefore used palmitoyl-carnitine enrichment to represent palmitoyl-CoA enrichment for calculation of total lipid synthesis.

The true precursor enrichments for muscle lipid synthesis were substantially lower than the plasma nonesterified palmitate enrichments, indicating that the fatty acids released from intramuscular lipid breakdown were a major source of precursor other than the arterial fatty acids inflow (Table 2). The enrichments of intramuscular TG- and PL-bound palmitate were very low in comparison with the precursor enrichments. Thus, the potential contribution of tracer recycle to the true precursor was negligible.

Muscle PL FSRs were greater ($P$ < .01-0.001) than TG FSR in both groups (Table 3). There were no significant differences in either TG or PL FSR between the lean and obese groups. However, because of the greater content of muscle TG in the obese group, the absolute synthetic rate of muscle TG was greater ($P$ < .01-.05) in the obese group, which included synthesis from plasma NEFA and from recycled fatty acids (Table 3). Because the rabbits were studied after 24 hours of food deprivation, the contribution from de novo fat synthesis was considered to be zero. In contrast, the synthetic rates of muscle PL were comparable between the 2 groups (Table 3) because of the comparable PL contents in the muscle (Table 1).

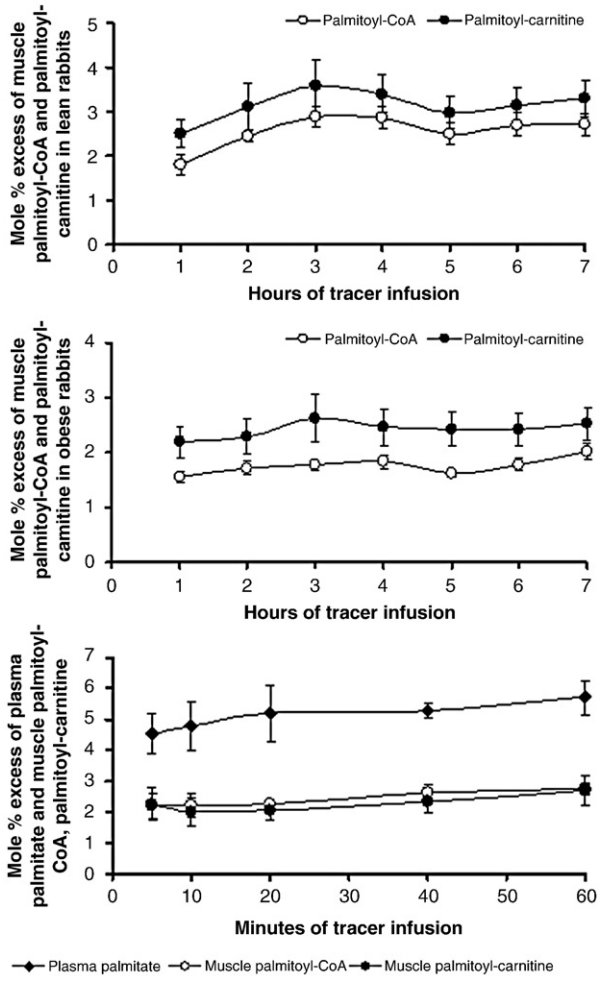


Fig. 3. Intramuscular palmitoyl-CoA and palmitoyl-carnitine enrichments. A, Changes of intramuscular palmitoyl-CoA and palmitoyl-carnitine enrichments during the 7 hours of tracer infusion in lean rabbits. Palmitoyl-CoA enrichment was consistently lower than that of palmitoylcarnitine, which was considered to be the effect of lipolysis after excision. Because we washed the muscle samples after excision before freezing in liquid nitrogen, several seconds of time course for washing the samples caused dilution of palmitoyl-CoA enrichment by the fatty acids released from lipolysis. The enrichments increased apparently during the first 3 hours, indicating active lipoprotein lipase in the lean rabbits. B, Changes of intramuscular palmitoyl-CoA and palmitoyl-carnitine enrichment in obese rabbits. There were similar parallel enrichments as in the lean rabbits. The increases in enrichment during the first 3 hours were less than those in the lean rabbits, indicating that the lipoprotein lipase was not active in obese rabbits. C, Enrichments of intramuscular palmitoyl-CoA and palmitoyl-carnitine and plasma nonesterified palmitate in additional rabbits in which the muscle samples were immediately frozen in liquid nitrogen without washing in cold saline. The enrichments of palmitoyl-CoA and carnitine were comparable, indicating rapid equilibrium between these 2 metabolites. Values are means ± SEM; n = 5 in lean and obese groups, and n = 3 in the additional group.

Table 2
Precursor and product enrichments for intramuscular lipid synthesis

| | Precursor MPE | | Product MPE | |
|---|---|---|---|---|
| | Plasma palmitate | Muscle palmitoyl-CoA | Muscle TG-bound palmitate | Muscle PL-bound palmitate |
| Lean | 6.05% ± 0.60% | 3.06% ± 0.46% | 0.0083% ± 0.0012% | 0.0629% ± 0.0071% |
| Obese | 4.29% ± 0.32%* | 2.37% ± 0.34% | 0.0061% ± 0.0009% | 0.0476% ± 0.0052% |

Data are means ± SEM; n = 5 in each group. The MPE values for plasma palmitate (ie, plasma nonesterified palmitate) were means from the first 3 hours of tracer infusion; the MPE values for muscle palmitoyl-CoA (represented by palmitoyl-carnitine) and muscle TG- and PL-bound palmitate were values measured at hour 3 time point.

* Different from lean, $P < .01$.

The percentage contributions of plasma NEFA, plasma TG, and intramuscular TG breakdown to the intramuscular true precursor were calculated using Eqs. 3 to 5. Because the percentage of intramuscular true precursor MPE to plasma nonesterified palmitate MPE reached plateaus after 2 hours of tracer infusion in both the lean and obese groups (Fig. 4), the percentage value at 2 hours reflected contribution from plasma NEFA and TG. The percentage value at hour 0, which reflected percentage contribution from plasma NEFA alone, was estimated from the changes from hour 1 to hour 2. In the lean group, the percentage value increased from 39.8% ± 3.8% at hour 1 to 50.7% ± 9.2% at hour 2, an increment of 10.8% ± 3.8% (27.4% of the percentage value at hour 1). We therefore extrapolated that the percentage value at time 0 was 31%, which was the contribution of plasma NEFA alone to the true precursor pool. According to Eqs. 4 and 5, the percentage contributions from plasma TG and intramuscular TG breakdown were 20% and 49%, respectively. In the obese rabbits, this percentage value increased from 53.6% ± 7.8% to 54.5% ± 6.9%, an increment of 0.8% ± 1.8% (1.5% of the percentage value at hour 1). When extrapolated to hour 0, the percentage value was 53%, which was the contribution from plasma NEFA in the obese group. According to Eqs. 4 and 5, percentage contributions from plasma TG and intramuscular TG breakdown were 2% and 45%, respectively. Thus, in the obese rabbits, the contribution from plasma TG was very small. Theoretically, the amount of tracer recycle that was released from newly synthesized muscle TG might also contribute to the increment of this percentage value, which might overestimate the plasma TG contribution. However, in the present study, the MPE values in the muscle lipids were very low in comparison with the true precursor MPE (Table 2); thus, this source of error was negligible.

## 4. Discussion

We found that obese rabbits had larger amounts of intramuscular TG and fatty acid metabolites than their lean counterparts. In contrast, intramuscular PL contents were not different between the lean and obese rabbits. Therefore, the intramuscular metabolism of TG but not PL was disrupted in obesity. Generally, a net TG deposition requires increased synthesis, decreased degradation, or both. The greater rate of intramuscular TG synthesis accounted for at least in part the accumulation of TG and its precursor. The findings of larger amounts of intramuscular TG and its precursor may be linked to insulin resistance [14-18]-. Although the responsiveness to insulin action was not measured, the obese rabbit model used in the present experiment has been characterized by hyperglycemia and hyperinsulinemia [19], which are typical clinical signs of insulin resistance. Because PL is a major component of biological membranes, the constant content

Table 3
Fractional synthetic rates and absolute synthetic rates of muscle TG and PL

| Group | TG | | | PL | | |
|---|---|---|---|---|---|---|
| | Using plasma precursor | Using true precursor | Recycle | Using plasma precursor | Using true precursor | Recycle |
| *Lean* | | | | | | |
| FSR (%/h) | 0.047 ± 0.008 | 0.095 ± 0.016 | 0.048 ± 0.009 | 0.35 ± 0.04§ | 0.76 ± 0.15§ | 0.40 ± 0.13§ |
| Synthesis (nmol/[g h]) | 4.6 ± 1.0 | 10.0 ± 2.5 | 5.4 ± 1.7 | 11.6 ± 1.8 | 24.5 ± 4.7 | 12.8 ± 3.5 |
| *Obese* | | | | | | |
| FSR (%/h) | 0.047 ± 0.006 | 0.092 ± 0.019 | 0.045 ± 0.013 | 0.37 ± 0.02§ | 0.70 ± 0.07§ | 0.33 ± 0.07§ |
| Synthesis (nmol/[g h]) | 20.0 ± 4.1* | 39.7 ± 9.5* | 19.6 ± 5.8† | 14.1 ± 3.3 | 25.7 ± 5.9 | 11.7 ± 2.9 |

Data are means ± SEM; n = 5 in each group. Plasma precursor means plasma nonesterified palmitate; true precursor means intramyocellular palmitoyl-CoA (reflected by palmitoyl-carnitine). Recycle means the incorporation of fatty acids released from muscle lipid breakdown into muscle lipids. Synthesis rate is calculated by FSR × pool size.

* $P < .01$.

† $P < .05$ vs lean.

§ $P < .01$ vs corresponding values for calculating muscle TG FSR.

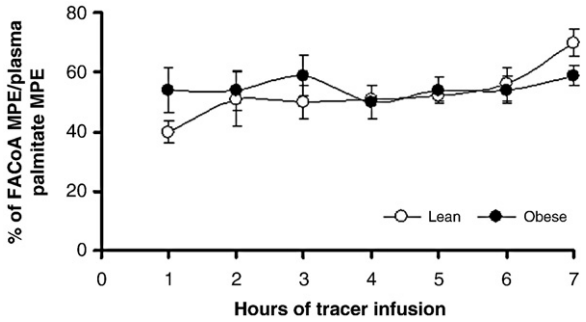


Fig. 4. Percentages of intramyocellular palmitoyl-CoA (reflected by palmitoyl-carnitine enrichment) to plasma nonesterified palmitate enrichment. In obese rabbits, the percentage of contribution was basically constant over time, indicating that plasma NEFA was the predominate contributor to the true precursor pool for muscle lipid synthesis and that the contribution from plasma TG was negligible. In lean rabbits, this percentage increased during the first 2 hours of tracer infusion, which was reasonably explained by plasma TG contribution to the true precursor pool via the action of lipoprotein lipase.

and synthesis of muscle PL indicate that the density and turnover of the membranes are regulated and not altered in obesity.

The present experiment demonstrated that both plasma lipids and muscle lipolysis contributed to the precursor pool for muscle TG synthesis (Table 2). When plasma nonesterified palmitate was used as precursor, the calculated muscle lipid synthetic rates were approximately 50% of the true synthetic rates that used intramyocellular palmitoyl-CoA as precursor. Because the use of fatty acids from muscle lipolysis for TG synthesis reflects a recycling of fatty acids, which should not change net TG balance, the increased uptake of fatty acids from the elevated concentrations in plasma in the obese rabbits was responsible for the net accumulation of muscle TG. Therefore, reducing hyperlipidemia should be an effective therapy for muscle TG accumulation. This notion is consistent with the current knowledge that hyperlipidemia is an important link between obesity and insulin resistance [20].

The contribution of plasma lipids to the intramyocellular fatty acyl–CoA pool includes not only NEFA but also TG through the action of lipoprotein lipase. The latter contribution could be estimated from the changes in percentage palmitoyl-CoA enrichment apparently derived from plasma nonesterified palmitate enrichment over time (Fig. 4). At the beginning of tracer infusion when plasma TG enrichment was zero (Fig. 1A, B), the label in the true precursor was solely derived from plasma nonesterified palmitate. As plasma TG enrichment increased over time, any contribution of circulating TG to the muscle fatty acyl–CoA pool would be reflected by a progressive increase in fatty acyl–CoA enrichment. We estimated that plasma lipids contributed to 51% to 55% of the true precursor for muscle lipid synthesis in both groups, and the remainder was derived from muscle lipid breakdown (ie, recycled fatty acids). In the obese rabbits, the plasma lipid contribution was predominately derived from NEFA, whereas in the lean rabbits, plasma NEFA and TG contributed to 31% and 20%, respectively. These findings are consistent with the notion that obesity is associated with low muscle lipoprotein lipase activity [21-24].

Fatty acyl–CoA and fatty acyl–carnitine are active metabolites of fatty acids in the intracellular compartment [13]. These 2 metabolites have small pool sizes in comparison with lipids in plasma and muscle (Table 1). The enrichments of palmitoyl-CoA and palmitoyl-carnitine were equilibrated by 5 minutes after starting the palmitate tracer infusion (Fig. 3C). Our recent experiments showed that, after a bolus palmitate tracer infusion, the enrichments of these 2 metabolites reached peaks in muscle within 2 minutes (unpublished data), indicating rapid turnover. It is proposed that a large fatty acyl–CoA pool is a marker for insulin resistance [4,25-27]. This is probably because fatty acids and their metabolites are toxic to cells and their accumulation may impair insulin signaling cascades.

The consistent pool size and synthetic rates of muscle PL in the lean and obese rabbits are striking (Table 1). Muscle cells are terminally differentiated in adulthood; there is little cell division in muscle. We may assume that the synthetic rate of muscle PL equals its breakdown rate to maintain the cell membrane constant. However, the PL in the muscle is under rapid turnover, with FSRs much greater than those of TG (Table 2). Although the physiologic implications of the rapid PL turnover in the muscle are not clear, the present experiment demonstrates that muscle PL was not involved in the lipid dysfunction in the muscle of this obese model.

Some technical issues need to be addressed. After 3 hours of palmitate tracer infusion, muscle TG enrichment deviated from linear incorporation (Fig. 2A). A reasonable explanation is that, during the later hours of tracer infusion, the permeability of capillary in muscle was increased, leading to "leak" of plasma TG into the interstitial compartment. For example, in lean rabbits, the muscle TG content was 11.6 nmol/mg (Table 1); so in a 40-mg muscle sample, there was 464 nmol TG. If the muscle contained 80% water that included 10% plasma because of possible "leakage," then there would be approximately 1 nmol plasma TG (plasma TG concentration of 0.3 nmol/$\mu$L, Table 1). After 3 hours of tracer infusion, plasma TG enrichment was approximately 4%; so there would be 0.04 nmol of labeled TG. This amount of labeled TG would increase muscle TG enrichment by 0.009%, which could overestimate muscle TG enrichment by 100% because of the low enrichment in muscle TG (Fig. 2A). The derivation of linear incorporation did not affect the results because our calculations were based on the linear increases of isotopic enrichment in muscle lipids during the first 3 hours of tracer infusion.

The measurement of intramyocellular fatty acyl–CoA and fatty acyl–carnitine represents another technical challenge. Ideally, the so-called freeze clamp technique is used to measure in situ the metabolites. Alternatively, muscle samples have to be frozen immediately after

excision to minimize dilution from intracellular lipid breakdown (Fig. 3C). If fatty acyl–CoA enrichment is suspected to be underestimated, fatty acyl–carnitine enrichment may be a better approximation of the precursor enrichment because it is less affected by the dilution from intracellular lipid breakdown.

The present study is one of a series to quantify fatty acid kinetics at whole-body and tissue levels. The results demonstrated that the muscle in obese rabbits had increased amount and synthetic rate of TG, along with increased amount of fatty acyl–CoA and fatty acyl–carnitine, which were associated with high plasma lipid concentrations and rapid recycling of fatty acids from the large muscle TG pool as 2 predominate precursor sources. Although the incorporation into TG synthesis is an important route of intramuscular fatty acid trafficking, further efforts are warranted to measure intramuscular TG breakdown and oxidation rates, which are our ongoing projects. Quantitative measurements of intramuscular fatty acid kinetics are anticipated to be a useful approach to study the mechanisms of insulin resistance in obesity.

## Acknowledgment

The authors are grateful to Yun-xia Lin, Guy Jones, Guarang Jariwala, Dr Dayong Sun, and Dr Chengyue Meng for technical assistance. We also thank The Animal Resource Center of University of Texas Medical Branch for professional care of animals.

Supported by Shriners Hospital Grants 8560, 8600, and 8490.

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