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Measurement of Myocardial Fatty Acid Esterification Using [1-¹¹C] Palmitate and PET: Comparison with Direct Measurements of Myocardial Triglyceride Synthesis

Andrew R. Coggan, PhD, Zulfia Kisrieva-Ware, MD, PhD, Carmen S. Dence, PhD, Paul Eisenbeis, Robert J. Gropler, MD, and Pilar Herrero, ME, MS

Division of Radiological Sciences, Edward Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO 63110

Abstract

The purpose of the present study was to assess the accuracy of non-invasive estimates of the rate of myocardial fatty acid esterification (MFAE) obtained using positron emission tomography (PET).

Methods—Sixteen dogs were studied after an overnight fast (FAST), during a euglycemic hyperinsulinemic clamp (CLAMP), or during infusion of Intralipid (IL) or IL plus dobutamine (IL/DOB) (n=4/group). The rate of MFAE was quantified using a bolus injection of [1-¹¹C]palmitate and compartmental modeling as described by Bergmann et al.³ and compared to the rate of triglyceride (TG) synthesis measured directly using a continuous infusion of [1-¹³C]palmitate and tissue sampling.

Results—Across groups, mean plasma free fatty acid (FFA) concentration varied ~20-fold, with this variation in FFA availability accompanied by a ~20-fold range in directly-measured TG synthesis (i.e., from 7±1 nmol/min/g in CLAMP to 128±75 nmol/min/g in IL). PET-based estimates of MFAE varied to a similar degree (i.e., from 22±9 nmol/min/g in CLAMP to 543±551 nmol/min/g in IL), and were significantly correlated with TG synthesis (i.e., R=0.77, P<0.001). MFAE, however, was 3- to 4-fold higher than TG synthesis in FAST, CLAMP, and IL, but comparable when cardiac work was increased in IL/DOB, suggesting that MFAE reflects, in part, the incorporation of label into amino acids via TCA cycle exchange reactions (e.g., α-ketoglutarate ↔ glutamate) as well as the synthesis of TG and other lipids.

Conclusions—Changes in the rate of MFAE as determined using PET and the compartmental model of Bergmann et al.³ parallel changes in the rate of TG synthesis, at least in the basal state. Although such measurements are not as robust as rates of myocardial FFA uptake and oxidation as estimated by the model, this method should still be useful for quantifying acute changes in FFA storage by the heart in various pathophysiological states.

Keywords

PET; triglycerides; kinetic modeling; [1-¹¹C]palmitate; [1-¹³C]palmitate

INTRODUCTION

There is now considerable evidence that an imbalance between the uptake and oxidation of free fatty acids (FFA) may lead to deleterious changes in cellular metabolism and function, a process frequently referred to as lipotoxicity. This is true as well in the heart, where the accumulation of FFA, triglycerides (TG), and/or their metabolites is thought to contribute to the development of cardiomyopathy in obesity, diabetes, and the metabolic syndrome.^{cf. 1,2} Indeed, because the vast majority of FFA taken up by the heart are normally oxidized, even a small change in their relative intracellular fate (i.e., oxidation vs. esterification to form TG, other neutral lipids, and/or phospholipids) could have important metabolic, and hence functional, consequences. Accordingly, there is a need for the ability to non-invasively quantify the rate of myocardial FFA esterification (MFAE), so as to assess the impact of such diseases on this process, determine the efficacy of various therapies, etc. In this context, Bergmann et al.³ have previously evaluated the use of [1-¹¹C]palmitate (¹¹C-P) in conjunction with positron emission tomography (PET) and compartmental modeling to quantify myocardial FFA metabolism, with the results validated against direct arterial-coronary sinus balance measurements. This approach was found to accurately predict overall rates of myocardial FFA utilization (MFAU) and oxidation (MFAO) during interventions designed to alter plasma FFA levels and the pathways of FFA metabolism within the physiological range. Our laboratory has therefore successfully employed this method in a number of clinical studies to determine the effects of hypertension,⁴ aging,⁵ obesity,⁶ hormone replacement therapy,⁷ type 1 diabetes,^{8,9} etc., on myocardial FFA metabolism. However, while in the study of Bergmann et al.³ changes in MFAE were consistent with expected changes in response to inhibition of β -oxidation by oxfenicine or hyperglycemia, this aspect of the model was not validated against direct measurements of myocardial TG synthesis.

The purpose of the present study was therefore to assess whether PET-based estimates of MFAE accurately reflect the rate of myocardial TG synthesis. To do so, we compared results obtained using PET to direct measurements of the rate of myocardial TG synthesis made using [1-¹³C]palmitate (¹³C-P) in dogs pharmacologically treated to achieve a wide range in myocardial FFA metabolism.

MATERIALS AND METHODS

Experimental Protocol

Purpose-bred mongrel dogs weighing 20–25 kg were fasted overnight, anesthetized, and instrumented as previously described.^{10,11} Briefly, catheters were placed in the thoracic aorta (via a femoral artery) for monitoring of blood pressure and for sampling of arterial blood and in both femoral veins for tracer and drug administration, respectively. All experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals.

Sixteen dogs were studied during various interventions initiated 60–90 min prior to imaging. Specifically, four dogs were studied in the fasted state (FAST), while another four were studied during a euglycemic hyperinsulinemic clamp (CLAMP), which entailed a continuous infusion of insulin (70 mU/h/kg) with an adjustable infusion of 20% dextrose to maintain euglycemia. The other eight dogs received a continuous infusion of 20% Intralipid (IL; Fresenius Kabi Clayton, LP; 1 mL/min) supplemented with heparin (10,000 U bolus followed by 1500 U every 90 min) to raise FFA levels. Four of these dogs were infused with IL alone (IL), while the other four received a concomitant infusion of dobutamine (20 μ g/min/kg) starting 10 min prior to ¹¹C-P injection to increase cardiac work (IL/DOB). The electrocardiogram, systolic (SBP) and diastolic (DBP) blood pressure, heart rate (HR), and blood gases were monitored throughout the study.

All PET studies were performed on a Siemens ECAT 962 HR+. A transmission scan was first performed to correct for photon attenuation. To measure myocardial blood flow (MBF), 740–925 MBq of H_2^{15}O was administered as a bolus, and dynamic PET data were acquired as previously described.^{12,13} After allowing for decay of ^{15}O , a bolus of ^{11}C -P (740–925 MBq) was administered followed by 60 min of dynamic PET data acquisition. To determine the accuracy of the PET estimates of MFAE, ^{13}C -P (Cambridge Isotope Laboratories, Inc., USA) bound to human albumin was infused at a rate of 0.1 $\mu\text{mol}/\text{min}/\text{kg}$ starting 10 min prior to ^{11}C -P injection and continuing until the end of the study. Arterial blood samples were obtained immediately before and 5, 10, 15, 20, 25, 30, 40, 50, and 60 min after ^{11}C -P administration. After the imaging protocol was completed, the dog was transported back to the surgical suite, where the chest was opened and myocardial tissue samples were obtained as previously described.¹¹ Animals were then euthanized with an overdose of sodium thiopental (≥ 60 mg/kg) followed by 60 mL of saturated KCl.

Analysis of Blood and Tissue Samples

A small portion of each blood sample was immediately spun in a microcentrifuge to obtain plasma, which was heated at 60° C for ≥ 15 min to destroy lipoprotein lipase activity¹⁴ before being frozen in liquid N_2 and stored at -80°C until subsequent analysis. These samples were used to determine FFA concentrations via an enzymatic colorimetric method (NEFA C kit, Wako Chemicals USA, Richmond, VA) and ^{13}C -P enrichment via gas chromatography mass spectrometry (Agilent Technologies 5973N, Santa Clara, CA) as previously described.¹⁵ The remainder of each blood sample was centrifuged for 10 min at 3500 g to obtain plasma, which was immediately processed and counted to determine $^{11}\text{CO}_2$ and ^{11}C -P concentrations as previously described.³ Small aliquots of plasma obtained immediately before ^{11}C -P injection and after 30 and 60 min were frozen at -80°C until analyzed for insulin concentration via radioimmunoassay (Linco Research Co., St. Charles, MO) and glucose and lactate levels using an enzymatic method (2300 STAT Plus Analyzer, YSI Life Sciences, Yellow Springs, OH).

Frozen tissue samples weighing ~ 0.5 g were powdered under liquid N_2 , transferred into pre-weighed tubes cooled with dry ice, and extracted using chloroform:methanol (2:1).¹⁶ The organic phase was removed and stored at -80°C for subsequent analysis, after which the tubes were dried in a rotary evaporator and re-weighed to determine the exact mass of tissue extracted (in grams of lipid-free solids). A small portion of the crude lipid extract was used to measure total TG content (L-Type TG H kit, Wako Chemicals USA, Richmond, VA). The remainder was purified using solid phase extraction¹⁷ and the $^{13}\text{C}:^{12}\text{C}$ ratio of palmitate in the triglyceride fraction was determined using gas chromatography combustion isotope ratio mass spectrometry (Finnigan MAT Delta+ XL, Thermo Fisher Scientific, Waltham, MA). Since a baseline tissue sample was not available, the $^{13}\text{C}:^{12}\text{C}$ ratio of stearate was also monitored and used to calculate ^{13}C -P enrichment under the assumption that at natural abundance the $^{13}\text{C}:^{12}\text{C}$ ratios of palmitate and stearate are essentially identical.¹⁸

PET Image Analysis and Modeling

Myocardial H_2^{15}O and ^{11}C -P images were analyzed as previously described^{3,12,13} to determine blood and myocardial time-activity curves (TAC). MBF and myocardial FFA metabolism were then quantified using compartmental models developed and validated by our group.^{3,12,13} Because the details of these models have been published previously, only the FFA model is briefly presented here. This model describes the kinetics of ^{11}C -P in the myocardium with four compartments (i.e., Q_{1-4} ; mL/g) and six rates/rate constants (i.e., MBF and k_{1-5} ; mL/g/min and min^{-1} , respectively), representing the movement of ^{11}C -P from large vessels into the myocardial microvasculature, into and out of the interstitial/intracellular space, into and out of neutral lipids (and/or amino acids), and into mitochondria for β oxidation, respectively (Fig. 1). The rate constants as well as the spillover correction coefficient are estimated by

numerically solving the system of differential equations describing the model using a non-linear least squares approach. Absolute rates (in nmol/min/g) of MFAU, MFAO, and MFAE are then calculated by assuming that the tracee (i.e., FFA) system is in dynamic steady-state and that degradation of triglyceride synthesized from exogenous ^{11}C -P is negligible (i.e., $k_4=0$) during the study. The latter assumption is justified because of the limited labeling of the TG pools that would be expected to occur after bolus injection of ^{11}C -P. Although PET data were acquired over a 60 min period, only data collected during the first 30 min were used in these analyses, so as to be consistent with the original investigation of Bergmann et al.³ Inclusion of data obtained between 30 and 60 min following injection of ^{11}C -P did not significantly alter the results (data not shown).

Calculation of Myocardial TG Synthesis Rate from Tissue Measurements

The fractional synthesis rate (FSR) of myocardial TG was calculated as:

$$FSR (\text{min}^{-1}) = \frac{E_B(t) - E_B(0)}{\int_0^t E_F(t) dt - \int_0^t E_B(t) dt} \quad 1)$$

where E_F and E_B are the plasma non-esterified (i.e., free) and tissue esterified (i.e., bound) ^{13}C -P enrichments (measured in mol % excess, MPE), respectively. Note that this equation, which is based on Eq. 18 of Zilversmit,¹⁹ differs from the usual equation given for FSR (which excludes the second term of the denominator) in that it does not assume unidirectional transfer of tracer from the precursor to the product pool during the time course of an experiment. This variation of the FSR equation was chosen because, unlike with the bolus injection of ^{11}C -P, the continuous infusion of ^{13}C -P combined with the high rate of TG synthesis in some animals resulted in significant accumulation of the latter tracer in the TG pool (see *Discussion*). The absolute rate of TG synthesis (in nmol FFA/min/g) was then calculated by multiplying the FSR by the tissue TG content and by 23.25 ($=1/(0.215 \times 0.20)$), under the assumption that palmitate accounted for 21.5% of all FFA in TG (20) and that lipid-free solids constituted 20% of wet weight.

Statistical Analysis

Differences between interventions were compared using one-way or two-way analysis of variance (ANOVA) for repeated measures, as appropriate. A P value of ≤ 0.05 was considered statistically significant. Significant differences identified by ANOVA were localized by *post hoc* tests. Data are presented as mean $\pm$ SD.

RESULTS

Hemodynamics

SBP and DBP were not different between interventions, but as expected HR (beats/min) was higher in IL/DOB (175 ± 32) than in the other three trials (pooled value 86 ± 25 ; $P<0.05$). The rate-pressure product (RPP; beats/min mmHg) was therefore also higher in IL/DOB (21085 ± 4652) than in CLAMP (9244 ± 2476 ; $P<0.05$) and IL (6234 ± 1336 ; $P<0.01$), and tended to be higher than in FAST (9484 ± 2712 ; $P<0.07$). Consequently, MBF (mL/min/g) was higher in IL/DOB (2.30 ± 0.24) compared to the other three trials (pooled value 0.80 ± 0.26 , $P<0.0001$). There were, however, no differences in HR, RPP, or MBF between FAST, CLAMP, and IL.

Plasma Concentrations of FFA, Glucose, Lactate, and Insulin

Table 1 shows the arterial concentrations of FFA, glucose, lactate, and insulin resulting from the study interventions. By design, plasma FFA levels were lowest in CLAMP, intermediate in FAST, and highest in IL studies (i.e., IL and IL/DOB), with the lipolytic effect of dobutamine resulting in IL/DOB having the highest FFA concentration, more than 20-fold higher than in CLAMP. Plasma glucose and lactate concentrations, on the other hand, were comparable across interventions. As intended, plasma insulin concentration was significantly higher in CLAMP than in the other three groups. Plasma insulin concentrations also tended to be higher in IL/DOB vs. FAST and CLAMP, but this difference was not significant.

PET Measurements of Myocardial FFA Metabolism

Absolute (i.e., in nmol/min/g) and relative (i.e., as a percentage) rates of MFAU, MFAO, and MFAE as determined using the PET modeling approach are shown in Table 2. In absolute terms, MFAU was lowest in CLAMP, intermediate in FAST, and highest in IL and especially IL/DOB, roughly paralleling the difference in FFA availability. MFAO followed a similar pattern, although due to the increase in cardiac work the difference in MFAO between IL/DOB vs. IL (i.e., +170%) was much greater than for MFAU (i.e., +20%). In contrast, MFAE was 6-fold higher in IL than in IL/DOB, with much lower values observed in FAST and CLAMP. These differences in MFAE, however, only approached statistical significance ($P=0.06$), due primarily to high variability in the IL group.

In relative terms, MFAO was highest in FAST and IL/DOB, lower in CLAMP, and lowest of all in IL. Differences in MFAE naturally mirrored these results, with esterification apparently accounting for a majority of FFA disposal in IL experiments but <10% in IL/DOB. The interventions selected were therefore successful in creating a wide range in absolute and relative rates of MFAE not only between groups but also within each group, such that the accuracy of this aspect of the PET model could be assessed.

Direct Measurement of the Rate of Myocardial TG Synthesis

Table 3 shows the data used to directly determine the rate of myocardial TG synthesis. On average, tissue TG content was lowest in FAST and highest in IL/DOB, but with large variation between animals/within each group. Inverse to the plasma FFA levels, the plasma ^{13}C -P (i.e., precursor) enrichment was highest in CLAMP, intermediate in FAST, and lowest in IL studies, i.e., IL and especially IL/DOB. On the other hand, the ^{13}C -P enrichment of tissue TG (i.e., the product enrichment) did not differ significantly across interventions, although it tended to be higher in CLAMP and lower in IL/DOB than in the other two groups. The FSR calculated from these enrichment data was therefore approximately 15-fold higher in IL than in CLAMP or FAST, but not significantly different from IL/DOB. The absolute rate of TG synthesis was also approximately 15-fold higher in IL than in CLAMP or FAST and 50% higher than in IL/DOB, but these differences were not significant due to the large variation between animals in TG content.

Comparison of PET and Direct Measurements

Mean values for TG synthesis and MFAE in each group are compared in Figure 2, whereas data from individual experiments are presented in Figure 3. When the data were considered on a group mean basis (Fig. 2), changes in MFAE generally paralleled changes in TG synthesis, although MFAE was 3- to 4-fold higher than TG synthesis in all but the IL/DOB group. MFAE and TG synthesis were also significantly correlated on an individual basis (Fig. 3), albeit with a slope much greater than unity. However, this correlation was much stronger (i.e., $R=0.91$; $P<0.001$) when just the FAST, CLAMP, and IL animals were considered, as there was no relationship between MFAE and TG synthesis in IL/DOB animals (Fig. 3, open circles).

DISCUSSION

The purpose of the present study was to assess the accuracy of PET-based estimates of MFAE obtained using the model of Bergmann et al.³ To do so, we compared such data to direct measurements of the rate of myocardial TG synthesis made using ^{13}C -P in dogs pharmacologically treated to create a very wide range in FFA metabolism. We found that the PET modeling approach provided a value for MFAE that was proportional to the directly-measured rate of TG synthesis on both a group mean and an individual basis, at least in the FAST, CLAMP, and IL groups. In absolute terms, however, the values derived via PET were several-fold greater than those obtained via direct measurement, and there was no relationship between MFAE and the rate of TG synthesis in the IL/DOB group, suggesting that the exact relationship between the two measurements is dependent on the level of myocardial work (and hence, presumably, tricarboxylic acid (TCA) cycle activity—see below). We therefore conclude that while MFAE can provide a reasonable indication of the rate of storage of FFA as TG by the heart, at least in the basal state, such data need to be interpreted more cautiously than other parameters of the PET model, i.e., MFAU and MFAO, which have previously been demonstrated to be quite robust.³

As indicated above, although under basal conditions the rate of MFAE determined via PET correlated with the rate of TG synthesis measured using ^{13}C -P, the former was, on average, three- to four-fold higher than the latter. There are several possible explanations for this discrepancy. The first, of course, is simply that the stable isotopic measurements somehow underestimated the true rate of TG synthesis. Indeed, this may have been true to some degree in the IL and IL/DOB groups, as due to the length of the experiments and the high rate of TG synthesis in these animals the plasma and tissue enrichments were close to (and in two cases essentially at) equilibrium. Because of this, we calculated FSR using an alternative to the standard equation that acknowledges bi-directional exchange of tracer between precursor and product pools. Even so, TG synthesis may still have been slightly underestimated in IL-infused animals because of uncertainty regarding the exact time course of changes in enrichment of the product pool (i.e., tissue was only sampled once, at the end of each experiment). MFAE, however, was also much higher than TG synthesis in the F and C groups, in which the plasma and tissue enrichments were far from equilibrium. Thus, underestimation of the true rate of TG synthesis due to excessive equilibration of the precursor and product pools would not explain the much higher values obtained for MFAE in these two groups. At the same time, the fact that such equilibration was achievable when IL was infused argues against underestimation of the rate of TG synthesis due to compartmentation of TG in the cardiac myocytes of healthy dogs, as has been hypothesized to occur in the hearts of streptozotocin-treated diabetic rats.²¹

A second possibility is that MFAE was greater than TG synthesis because MFAE should include synthesis of all lipid species, not just TG. In fact, previous studies have demonstrated that although the rate of TG synthesis is 3- to 9-fold higher than the rate of phospholipid (PL) synthesis in perfused rat hearts^{22–24} and *in situ* in swine,²⁵ in non-ischemic dog hearts the rate of PL synthesis is equal to²⁶ or much greater than²⁷ the rate of TG synthesis. We therefore quantified the rate of PL synthesis in 2–3 animals/group using the same approach as described for TG. In FAST and CLAMP dogs, the rate of PL synthesis averaged 17.7 ± 11.5 nmol/min/g ($n=5$), or approximately equal to the difference between MFAE and TG synthesis in these two groups. Hence, the fact that MFAE should reflect the synthesis of PL (as well as diglycerides, etc.) in addition to TG could, at least theoretically, account for the difference between the model-derived and directly-measured values in the FAST and CLAMP groups. However, the rate of PL synthesis in IL-infused animals (i.e., IL and IL/DOB, $n=4$) was 14.8 ± 11.7 nmol/min/g, i.e., essentially the same as in FAST or CLAMP dogs. The latter results are in keeping with prior studies demonstrating that, unlike myocardial TG synthesis, myocardial PL synthesis is relatively unresponsive to acute or chronic changes in substrate or hormone concentrations.

^{22–24} Accordingly, synthesis of other lipids seemingly would not account for the much higher value for MFAE than TG synthesis in the IL group, or the lack of correlation between these measurements in the IL/DOB group.

A third possible explanation for why the model-derived rate of MFAE was higher than the directly-measured rate of TG synthesis stems from the fact that the former is essentially a measure of the long(er)-term retention of ¹¹C by the heart, regardless of the exact metabolic fate of ¹¹C-P. As such, MFAE could be influenced by metabolic reactions other than just lipid synthesis, as originally recognized by Bergmann et al.³. In this context, the exchange of carbon atoms between TCA cycle intermediates and amino acids, i.e., between α -ketoglutarate (α -KG) and glutamate or between oxaloacetate and aspartate, may be particularly important. As part of the malate-aspartate shuttle, these reactions proceed at high rates relative to total TCA cycle flux in the resting heart,^{28,29} such that significant amounts of label could be temporarily “lost” to amino acids or even protein, which would be measured as esterification using the PET modeling approach. At high cardiac workloads, however, the activity of the α -KG-malate transporter apparently becomes rate-limiting,³⁰ such that the transfer of carbons out of the mitochondria via this pathway is greatly diminished.^{30,31} Indeed, when in the presence of high FFA levels cardiac work was increased via dobutamine infusion (i.e., comparing IL/DOB vs. IL), MFAE decreased much more than TG synthesis. This is consistent with the interpretation that MFAE normally reflects, at least to some degree, the sequestration of label in amino acids and/or protein as well as TG (and PL, etc.) synthesis.

Based on the above, it seems likely that multiple mechanisms are responsible for the differences between MFAE and TG synthesis observed in the present study. Nonetheless, it is important to emphasize that the two measurements paralleled one another quite closely, at least under basal conditions, i.e., in the FAST, CLAMP, and IL groups. This was true over a very broad range of FFA concentrations and/or insulin levels, spanning well beyond the variations likely to be found in healthy volunteers or patients studied in the fasted state. As such, changes in MFAE should be a reasonable indicator of changes in myocardial TG synthesis during acute interventions that alter myocardial FFA metabolism via changes in the substrate or hormone environment. e.g.,⁹. On the other hand, based on the present data this is unlikely to be true during pharmacological interventions (e.g., dobutamine infusion) that also significantly increase cardiac work and, hence, influence the rate of exchange of TCA cycle intermediates with amino acids. Finally, it remains to be determined whether MFAE is a reliable index of TG synthesis when cardiac work is reduced below normal (e.g., via beta blockade), or in chronic disease states, e.g., type 2 diabetes.

CONCLUSION

At least in the basal state, changes in the rate of MFAE as estimated using PET and the compartmental model of Bergmann et al.³ are a reliable indicator of acute changes in the rate of TG synthesis. Such measurements, however, are not as accurate as the overall rates of myocardial FFA uptake and oxidation as estimated by the model. Nonetheless, this method should still prove useful for quantifying acute changes in FFA storage by the heart under such conditions, and consequently for improving our understanding of the impact of alterations in this process on cardiac function in various pathophysiological states.

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Figure 1.

Schematic diagram of the four-compartment model of Bergmann et al.³ Compartments Q_1 – Q_4 (in mL/g) represent the vascular space, the interstitial and cytosolic spaces, neutral lipids, amino acids, and other slow turnover pools, and mitochondrial beta-oxidation, respectively. k_n (in min^{-1}) represents the turnover rate constant between compartments, MBF is myocardial blood flow (in mL/g/min), V is vascular volume (in mL/g), and $Ca(t)$ is the arterial concentration of ^{11}C -palmitate (in cpm/mL) as a function of time.

Figure 2.

Group mean values for directly-measured myocardial triglyceride (TG) synthesis and PET estimates of myocardial fatty acid esterification (MFAE) in dogs after an overnight fast (FAST), during a euglycemic-hyperinsulinemic clamp (CLAMP), or infused with Intralipid (IL) or Intralipid plus dobutamine (IL/DOB) (n=4 per group). Values are mean $\pm$ SD.

Figure 3. Individual values for directly-measured myocardial triglyceride (TG) synthesis and PET estimates of myocardial fatty acid esterification (MFAE) in dogs after an overnight fast (FAST), during a euglycemic-hyperinsulinemic clamp (CLAMP), or infused with Intralipid (IL) (all open circles) or Intralipid plus dobutamine (IL/DOB) (closed circles). The *inset* figure displays values of ≤ 150 nmol/min/g in greater detail.

Table 1

Arterial concentrations of FFA, glucose, lactate, and insulin.

	FAST	CLAMP	IL	IL/DOB
FFA (nmol/mL)	799±352	212±66	3664±1565 [*]	4560±657 [*]
Glucose (μmol/mL)	4.89±0.58	4.90±0.60	5.26±0.17	5.37±0.64
Lactate (μmol/mL)	0.87±1.26	0.86±0.17	1.50±0.92	0.85±0.17
Insulin (μU/mL)	2±1	115±29 [†]	3±1	35±25

FFA, free fatty acids; FAST: overnight fast; CLAMP: hyperinsulinemic-euglycemic clamp; IL: Intralipid; IL/DOB: Intralipid plus dobutamine. Values are mean±SD.

^{*} P<0.05 vs. CLAMP and FAST,

[†] P<0.001 vs. all other interventions.

Table 2

PET measurements of MFAU, MFAO, and MFAE

	FAST	CLAMP	IL	IL/DOB
MFAU (nmol/min/g)	174.9±76.2	69.9±19.8	908.1±696.9 [*]	1087.5±112.2 ^{*†}
MFAO (nmol/min/g)	149.6±70.1	48.1±21.2	364.9±175.4 [*]	995.7±117.1 [‡]
MFAE (nmol/min/g)	25.3±15.9	21.8±9.1	543.2±551.0	91.9±12.7
MFAO (%)	85.4±11.0	67.5±16.1	45.7±15.5 [§]	91.4±1.8 ^{//}
MFAE (%)	14.6±11.0	32.5±16.1	54.3±15.5 [§]	8.6±1.8 ^{//}

MFAU: myocardial fatty acid utilization; MFAO: myocardial fatty acid oxidation; MFAE: myocardial fatty acid esterification; FAST: overnight fast; CLAMP: hyperinsulinemic-euglycemic clamp; IL: Intralipid; IL/DOB: Intralipid plus dobutamine. Values are mean±SD.

^{*} P<0.05 vs. CLAMP,

[†] P<0.05 vs. FAST,

[‡] P<0.001 vs. all other interventions,

[§] P<0.01 vs. FAST,

^{//} P<0.01 vs. IL.

Table 3

Tissue TG content, enrichment, and synthesis rate.

	FAST	CLAMP	IL	IL/DOB
Tissue TG (nmol/g)	4742±1565	9178±8132	11404±14013	15501±8442
Plasma enrichment (MPE)	9.43±2.80	18.57±9.60	1.81±0.58 [*]	1.17±0.32 [*]
Tissue enrichment (MPE)	1.40±0.84	2.99±2.76	1.94±1.21	0.58±0.35
TG FSR (min ⁻¹)	0.00165±0.00162	0.00119±0.00069	0.02051±0.01537 [†]	0.00592±0.00515
TG synthesis (nmol/min/g)	7.9±6.7	6.9±1.1	127.5±74.6	84.9±57.8

TG: triglyceride; FAST: overnight fast; CLAMP: hyperinsulinemic-euglycemic clamp; IL: Intralipid; IL/DOB: Intralipid and dobutamine, MPE: mol % excess; FSR: fractional synthesis rate. Values are mean±SD.

^{*} P<0.01 vs. CLAMP,

[†] P<0.05 vs. CLAMP and FAST.