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Methyl-β-cyclodextrin: an alternative carrier for intravenous infusion of palmitate during tracer studies in swine (*Sus scrofa domestica*) ☆

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Abstract

Fatty acid-free albumin has been the standard carrier for intravenous infusion of fatty acids to study in vivo lipid metabolism. However, subjects can have adverse reactions to infusion of albumin. We sought an alternative to albumin as a carrier for intravenous infusion of fatty acids, using the pig as a model. Cyclodextrins are naturally occurring water-soluble molecules that can serve as carriers for lipid-soluble compounds. ^{13}C-palmitate was complexed to either 20% methyl-β-cyclodextrin, 20% 2-hydroxypropyl-β-cyclodextrin, or 5% porcine albumin (isotopic purity of infusates: 99.22 ± 0.06%). ^{13}C-palmitate–albumin was infused under fed conditions and ^{13}C-palmitate–methyl-β-cyclodextrin was infused under fasted and fed conditions in 50-kg pigs. Palmitate remained in solution at 4°C in methyl-β-cyclodextrin, but precipitated at 25–30°C in 2-hydroxypropyl-β-cyclodextrin. Pigs infused with ^{13}C-palmitate–methyl-β-cyclodextrin maintained normal body temperature and appetite; those infused with ^{13}C-palmitate–albumin became anorexic and exhibited other negative side effects to albumin. Palmitate oxidation rates under fed conditions were similar using either ^{13}C-palmitate–methyl-β-cyclodextrin or ^{13}C-palmitate–albumin complexes. Fasting increased ^{13}C-palmitate–methyl-β-cyclodextrin oxidation by approximately eight-fold. These data suggest that methyl-β-cyclodextrin may be a suitable substitute for albumin in fatty acid metabolism studies in swine. © 2001 Published by Elsevier Science Inc.

Keywords: Albumin; Fasted; Fatty acid oxidation; Fed; Methyl-β-cyclodextrin; Palmitate; Swine; Tracer methodology

1. Introduction

Fatty acid-free serum albumin has been the tracer carrier of choice for lipid metabolism studies (Wolfe et al., 1980; Wolfe, 1992) and human albumin has been used in numerous stable isotopic studies, in both humans and dogs, with no apparent ill effects (Wolfe, 1992). However, not all subjects tolerate high infusion rates of albumin, limiting the amount of stable isotopic tracer that can be infused. Although negative reactions to 5% albumin infusions are relatively rare (McClelland, 1990), albumin infusion can cause pyrogenic and anaphylactic reactions in humans (Stafford et al., 1988; Gales and Erstad, 1993; Pool and McLeod, 1995) and in animals (Ring et al., 1977). The safety of the common practice of infusion of human albumin in the

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Table 1
Feed intake (FI), palmitate (PA) rate of appearance [R_a (PA)], CO_2 production rate [R_a (CO_2)] and palmitate oxidation for individual animals when albumin (ALB) was used as a carrier for ^{13}C-palmitate tracer in growing pigs

Pig[a]	Body wt. (kg)	ALB conc. (%)	FI (kg)		Adverse response(s) to of ALB-PA infusion	R_a (μmol min^{-1} kg^{-1})		PA oxidation (μmol min^{-1} kg^{-1})	PA uptake oxidized (%)
			Before start of ALB-PA infusion[b]	After start of ALB-PA infusion[c]		PA	CO_2		
A	55.1	7[e]	1.44	0	Vomited after 60 and 105 min, shaking	3.63	768	0.646	17.8
B	53.7	5[e]	1.38	0	Vomited after 50 and 135 min, shaking	1.53	1100	0.078	5.1
C	58.4	5[e]	1.44	0	Vomited after 50 min	1.86	630	0.111	6.0
D	59.4	5[e]	1.44	0	None observed	2.17	824	0.195	9.0
E	63.3	5[f]	1.50	0	Listless, shaking	5.69	841	0.554	9.7
F	54.9	5[f]	1.44	0.72	Listless, full FI	3.87	ND[h]	0.327[d]	8.4[d]
G	49.6	5[g]	1.32	0.22	Listless, reduced FI	4.41	744	0.136	3.1

[a] One additional pig was infused, but is not shown because its catheters shut down when ALB-PA was infused. This pig also stopped eating and had labored breathing.
[b] Feed consumed over 6 h prior to initiation of ALB-PA infusion, including FI during bicarbonate infusion. Animals offered 4.2 g h^{-1} kg^{-1}.
[c] Feed consumed over 3 h during the ALB-PA infusion. Animals offered 4.2 g h^{-1} kg^{-1}.
[d] Estimated using $R_a(CO_2)$ values from other pigs (not used in comparison with methyl-β-cyclodextrin infusion).
[e] Essentially fatty acid-free porcine albumin (Sigma, A9422).
[f] Essentially fatty acid-free bovine albumin (Sigma, A9205).
[g] Essentially fatty acid-free, essentially globulin-free porcine albumin (Sigma, A1173).
[h] ND, not determined (bicarbonate infusion line disconnected).

management of certain illnesses in patients has recently been an issue of discussion and controversy (McClelland, 1998; Offringa, 1998). It has been suggested that infusion of albumin may increase the relative risk of death in some circumstances (Cochrane Injuries Group, 1998). In addition to the possible risk of its use, human albumin for infusion can be very expensive and its availability has been limited. Infusion-quality albumin has not been readily available commercially for all species of animal models. Some researchers have used autologous serum as a carrier (Dunshea et al., 1992), but this may not always be feasible, especially if it is necessary to infuse a large amount of tracer. Using a non-blood-derived carrier for infusion of fatty acids in metabolism studies may reduce the potential risk of albumin infusion and reduce the cost of the infusate as well.

In a series of preliminary studies, we infused several pigs with a variety of fatty acid-free albumin preparations from both porcine and bovine sources (see Table 1). However, the palmitate–albumin mixtures that we infused into growing pigs consistently caused adverse reactions, including anorexia, vomiting, and elevated body temperature. In order to avoid these negative reactions, we sought a non-blood-derived alternative to serum albumin for use as a fatty acid-tracer carrier in fatty acid oxidation studies.

β-Cyclodextrins can prevent the precipitation of non-polar substances in aqueous solutions (McDonald and Muzumdar, 1998). Data from in vitro studies suggested that β-cyclodextrins may have potential as fatty acid-tracer carriers in fatty acid oxidation studies (Kato et al., 1993, 1994), and unsubstituted or natural β-cyclodextrin has been shown to behave similarly to defatted serum albumin in in vitro preparations (Ramadoss et al., 1976). Intravenously infused substituted β-cyclodextrins have also been used as carriers for a wide variety of non-water-soluble substances in humans (Irie et al., 1992; Vandewoude et al., 1997), rabbits (Irie et al., 1992), rats (Packard and Teather, 1997) and dogs (Loscher et al., 1995). The parent compound, β-cyclodextrin, is a cyclic oligosaccharide consisting of seven glucopyranose units with a hydrophobic interior. Water solubility of natural β-cyclodextrin is poor (1.8 g per 100 ml), but chemical substitutions (e.g. methyl and hydroxypropyl groups) can increase the solubility many fold (>50 g per 100 ml). The objective of the present study was to determine the feasibility of using β-cyclodextrins as non-blood-derived carriers for palmitate in fatty acid oxidation studies in growing pigs.

2. Materials and methods

2.1. Animals

We used unanesthetized crossbred female pigs [(*Sus scrofa domestica*) 52 ± 3 kg, from the Beltsville Agricultural Research Center (BARC) swine herd] to demonstrate the feasibility of using methyl-β-cyclodextrin as a substitute for albumin as a carrier for ^{13}C-palmitate in in vivo infusion studies. All animal care and use procedures were approved by the BARC Institute Animal Care and Use Committee. Pigs were restrictively fed a corn–soybean meal based diet at 80% estimated ad libitum intake based on individual body weight (diet: 18% crude protein, 3.14 Mcal kg^{-1}), and were housed in metabolism crates for at least 1 week before study. Before being placed in crates, pigs were trained to breathe into a breath collection mask.

Catheters were non-surgically placed in the jugular veins (via both medial auricular veins) under general anesthesia [2 mg kg^{-1} each of xylazine and Telazol (Fort Dodge Laboratories Inc., Fort Dodge, IA)] to allow for simultaneous sampling and infusion (Wray-Cahen et al., 1993). Measurements were taken at least 4 days after catheterization. Kinetic measurements were carried out under steady-state fed or fasted conditions.

In preliminary studies, eight pigs were infused with ^{13}C-palmitate–albumin complexes. The adverse responses to the albumin infusion are shown in Table 1. The initiation of the ^{13}C-palmitate–albumin infusion appeared to cause three pigs to vomit within 1 h and to cause the catheters of one pig to lose patency. Only two pigs infused with the ^{13}C-palmitate–albumin complex continued to consume feed throughout the infusion period (one at a reduced intake). Two other pigs stopped eating, but did not vomit. Three pigs (57.4 ± 4.1 kg) were used as a comparison for the four pigs (50.5 ± 0.9 kg) infused with the ^{13}C-palmitate–methyl-β-cyclodextrin complex. Each pig given the ^{13}C-palmitate–methyl-β-cyclodextrin complex was infused under both fed and fasted

conditions, on two separate, non-consecutive days. The pigs receiving the ^{13}C-palmitate–albumin complex were infused only under fed conditions. Under fed conditions, pigs received one-tenth of their daily ration, hourly, beginning 2 h before basal samples were collected and continuing throughout the collection period, so that the entire ration was consumed over a 9-h period. Under fasted conditions, collections began following an 18-h fast. Feed refusals were recorded. Rectal body temperatures were recorded during two of the infusions for each infusate.

2.2. Infusate preparation

[1-^{13}C]-Palmitate (potassium salt; 99AP) was obtained from Mass Trace Inc. (Woburn, MA). In the present investigation, [1-^{13}C]-palmitate was bound to one of three potential carriers: (i) essentially fatty acid-free albumin (Sigma Chemical Co., St. Louis, MO: bovine, A9205; porcine, A-9422; and porcine essentially globulin-free, A1173; see Table 1 for details); (ii) methyl-β-cyclodextrin (Aldrich Chemical Co. Inc., Milwaukee, WI; 33,261-5); and (iii) 2-hydroxypropyl-β-cyclodextrin (Aldrich Chemical Co. Inc.; 33,260-7). The [1-^{13}C]-palmitate (potassium salt) was dissolved in pyrogen-free deionized H_2O and then added to a 5% w/v albumin solution (5 g albumin per 100 ml sterile physiological saline) as described by Wolfe (1992) using 3 g of [1-^{13}C]-palmitate per 100 g essentially fatty acid-free albumin. The solution was prepared at 60°C, filtered through a 0.2-μm sterile filter into a sterile bottle and stored at room temperature until used the following day. The [1-^{13}C]-palmitate (potassium salt)–cyclodextrin complexes were prepared as follows. Methyl-β-cyclodextrin or 2-hydroxypropyl-β-cyclodextrin was dissolved in sterile phosphate-buffered saline (PBS, GIBCO-BRL, Life Technologies, Grand Island, NY; 14190-151) at 50–60°C at 20 g of β-cyclodextrin per 100 ml PBS (20% w/v). The [1-^{13}C]-palmitate (potassium salt) was directly dissolved in one of the 20% cyclodextrin solutions at 3 g of palmitate per 100 g β-cyclodextrin. The mixtures were prepared at 50–60°C and filtered through a 0.2-μm sterile filter into a sterile bottle and stored at 4°C until infused. The [1-^{13}C]-palmitate–methyl-β-cyclodextrin mixture was reheated to approximately 30°C, if necessary, to clarify the solution before infusion. The hydroxypropyl-β-cyclodextrin mixture was not used in infusion studies for reasons described below. The final infusate enrichments ($99.22 \pm 0.06\%$) and palmitate concentrations were determined by gas chromatography-mass spectrometry (Metabolic Solutions Inc.; Merrimack, NH). The final palmitate concentration after filtering of both the 5% albumin and the methyl-β-cyclodextrin infusate preparations was $75.2 \pm 1.8\%$ of the calculated values. During the preparation of the 7% albumin infusate, a greater proportion of the palmitate was lost, and the final palmitate concentration was 66.7% of the calculated amount. Values used in the palmitate oxidation calculations were those determined by mass spectrometry analysis.

2.3. Stability of palmitate–β-cyclodextrin complexes

As with the palmitate–albumin mixture, the palmitate–methyl-β-cyclodextrin mixture remained in solution when it was cooled to room temperature. Unlike the palmitate-albumin mixture, it also remained in solution when stored at 4°C for more than 2 weeks. When the palmitate–methyl-β-cyclodextrin mixture was stored for periods of over 1 month, the palmitate began to precipitate, but it quickly resolubilized when heated to approximately 30°C. Upon cooling, the palmitate–methyl-β-cyclodextrin remained in solution. The palmitate solubilized in 2-hydroxypropyl-β-cyclodextrin precipitated out of solution when cooled to 25–30°C. It could be resolubilized with heating, but would only remain in solution if kept warm. Therefore, we determined that the 2-hydroxypropyl-β-cyclodextrin complex was not suitable for the in vivo infusion studies.

2.4. Infusion protocol

A 90-min primed-continuous (0.6 or 1.2 mmol kg^{-1} h^{-1}, fed or fasted conditions, respectively) infusion of $NaH^{13}CO_3$ (Mass Trace Inc.) was used to prime the CO_2/bicarbonate pool (as described by Wolfe, 1992) and to determine basal CO_2 production. Immediately following the $NaH^{13}CO_3$ infusion, [1-^{13}C]-palmitate solutions were infused at a constant rate (0.068 μmol ^{13}C-palmitate min^{-1} kg^{-1}) for 4 h. The [1-^{13}C]-palmitate-albumin mixture was administered at a rate of 0.89 ml h^{-1} kg^{-1} to achieve the target tracer infusion rate. Because the [1-^{13}C]-palmi-

tate-methyl-β-cyclodextrin mixture was four-fold more concentrated than the albumin complex, the solution was infused at one-fourth the volume h^{-1} kg^{-1}. Breath and blood samples were collected during a basal, pre-infusion period, and when isotopic enrichment plateau was achieved (60–90 min for bicarbonate infusion and 120–180 min for palmitate infusion). Additional samples were collected to ensure that a plateau had been achieved (bicarbonate: *t* 30, 45, 60, 70, 80 and 90 min; palmitate: *t* 30, 60, 90, 120, 135, 150, 165 and 180 min), but these were not included in the calculations. One animal was infused with the palmitate–methyl-β-cyclodextrin mixture for 360 min with samples taken every 30 min for further verification that the plateau was achieved before the 120–180-min window. Samples (breath and/or heparinized blood) were injected into evacuated vials for measurement of $^{13}CO_2$ enrichments. Blood samples were collected into heparinized syringes and plasma was harvested after centrifugation for measurement of plasma enrichments of [1-^{13}C]-palmitate. Breath and plasma enrichments were determined by mass spectrometry (Metabolic Solutions Inc., Merrimack, NH).

2.5. Mass spectrometry analysis

[1-^{13}C]-Palmitate enrichment in plasma and infusate was determined by gas chromatography-mass spectrometry (GCMS) on a Hewlett Packard 5989A system (Palo Alto, CA) using a Restek Rtx-1 capillary column. Extracted fatty acids were derivatized with addition of MTBSTFA/acetonitrile (1:1 v/v) to produce a *t*-BDMS derivative. Quantification of the natural and [1-^{13}C]-palmitate fatty acid derivatives was carried out by GCMS in the electron impact mode. $^{13}CO_2$ enrichment in breath and heparinized whole blood samples was determined by gas isotope ratio mass spectrometry (IRMS) on a Europa Scientific (Crewe, UK) 20/20 stable isotope analyzer. The breath samples were loaded directly on the IRMS via a Europa Scientific ABCA sample purification system. Blood samples were first acidified with a saturated citric acid solution to liberate the carbon dioxide and the released gas loaded on the IRMS in the same manner as the breath samples.

2.6. Calculations and statistics

The palmitate entry rate [R_a(palmitate)], the CO_2 production rate [$R_a(CO_2)$] during the bicarbonate infusion, the palmitate oxidation rate, and the percent of palmitate uptake oxidized were calculated as follows (Wolfe, 1992):

$$R_a(\text{palmitate}) = \left((E_{ip}/E_{palm}) - 1\right) * F_{palm}$$

$$R_a(CO_2) = \left((E_{ib}/E_{bCO_2}) - 1\right) * F_{bicarb}$$

Palmitate oxidation

$$= \left(E_{pCO_2} * R_a(CO_2)\right)/E_{palm}$$

Palmitate uptake oxidized

$$(\%) = \left(\left(E_{pCO_2} * R_a(CO_2)\right)/F_{palm}\right) * 100$$

where E_{ip} is the isotope enrichment of the ^{13}C-palmitate infusate, E_{palm} is the plateau enrichment of plasma palmitate, F_{palm} is the infusion rate of the ^{13}C-palmitate infusate, E_{ib} is the isotope enrichment of the ^{13}C-bicarbonate infusate, E_{bCO_2} is the plateau enrichment of plasma or breath CO_2 during the ^{13}C-bicarbonate infusion, F_{bicarb} is the infusion rate of the ^{13}C-bicarbonate infusate, and E_{pCO_2} is the plateau enrichment of plasma or breath CO_2 during the ^{13}C-palmitate infusion.

The primary comparisons of interest were (i) between fed pigs receiving either the albumin or the methyl-β-cyclodextrin complex in order to evaluate whether the results were similar for the two methods and (ii) between fed and fasted states in pigs receiving the methyl-β-cyclodextrin complex to determine if this method could detect changes in palmitate oxidation. To compare data from fed pigs infused with the albumin vs. the methyl-β-cyclodextrin complex, ANOVA was used. For this comparison, the level of significance was set at $P < 0.1$ and the degrees of freedom within samples set at 4, to reduce likelihood of a type II error, concluding that there is no difference between infusion methods, when a difference does exist. For the fed and fasted states of pigs receiving the methyl-β-cyclodextrin complex infusion (each pig studied under both fed and fasted conditions), the average plateau values were compared using paired *t*-tests and the level of significance was set at $P < 0.05$. Data in tables are expressed as mean ± standard error of the mean (S.E.M.).

3. Results

3.1. Albumin infusion

In an initial infusion with a higher concentration of porcine albumin (7%), the pig vomited within 60 min of initiation of the palmitate–albumin complex infusion (Pig A, see Table 1); rectal temperature was unchanged at T_{30} min, elevated by +0.5°C at T_{60} min, +1.0°C at T_{90} min, and +1.5°C at $T_{120-180}$ min. Frequent temperatures were also taken in a second pig infused with a 5% albumin complex. It also had elevated rectal temperature within 60 min of the initiation of infusion of the complex. Pigs had normal rectal body temperatures the day following the infusion. Seven similar pigs received infusions of palmitate complexed with 5% albumin. One of these pigs was severely affected by the palmitate–albumin complex infusion and displayed symptoms of anaphylaxis. The pig was distressed, suffered from extremely labored breathing and exhibited diffuse erythema or flushing of the skin. The catheters of this pig also lost patency shortly after the initiation of the albumin infusion, and therefore no palmitate data were available from this animal. Albumin without palmitate bound to it was also infused into one pig and similar adverse responses were observed.

The palmitate oxidation data obtained from the pigs infused with palmitate–albumin complexes are presented in Table 1. Most of these pigs reacted negatively to infusion of albumin (i.e. vomiting, shaking, fever, and/or rapid breathing). Pigs infused with the palmitate–albumin complexes had reduced feed intakes (an average reduction of 28% for total daily intake). Most lost interest in feed within 1 h after initiation of palmitate–albumin complex infusion and, on average, consumed less than 20% of the feed offered during this infusion. The variation in feed consumed, as well as the other symptoms, probably contributed to the variation in the individual values for palmitate oxidation. The appetites of all pigs returned by the day following the infusion. Four pigs did not vomit and two pigs infused with the [1-^{13}C]-palmitate–albumin mixture continued to consume their diet throughout the infusion period; albeit one at a reduced level. Summarized oxidation data using the palmitate–albumin complex for three pigs is presented in Table 2 for a comparison with the pigs receiving the methyl-β-cyclodextrin complex.

3.2. Methyl-β-cyclodextrin infusion

Pigs infused with the [1-^{13}C]-palmitate–methyl-β-cyclodextrin mixture showed none of the adverse side effects observed with albumin infusion. Rectal body temperature was unchanged during palmitate–methyl-β-cyclodextrin complex infusion. All pigs infused with this complex continued to consume meals fed hourly during the infusion and no loss of appetite was observed following the infusion. The effects of fasting as measured in pigs infused with the [1-^{13}C]-palmitate–methyl-β-cyclodextrin mixture are shown in Table 2. In the animals infused with the [1-^{13}C]-palmitate–methyl-β-cyclodextrin complex, fasting increased palmitate entry rate 1.8-fold ($P < 0.05$) and the

Table 2
Comparison of albumin and methyl-β-cyclodextrin as carrier for palmitate for the measurement of palmitate entry rate [R_a(palmitate)] and oxidation[1]

	Albumin[2] ($n = 3$)		Methyl-β-cyclodextrin ($n = 4$)			
	Fed		Fed		Fasted	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
R_a(palmitate), μmol min^{-1} kg^{-1}	4.09^{a}	1.03	1.35^{bA}	0.27	2.46^{B}	0.61
$R_a(CO_2)$, μmol min^{-1} kg^{-1}	803^{a}	30	1008^{aA}	87	627^{B}	22
Palmitate oxidation, μmol min^{-1} kg^{-1}	0.30^{a}	0.13	0.17^{aA}	0.02	1.44^{B}	0.36
% Palmitate uptake oxidized	7.3^{a}	2.1	13.2^{aA}	2.0	58.4^{B}	1.9

[1]Values with different superscripts within the same row are different (for fed albumin vs. fed methyl-β-cyclodextrin, lower case letters are used: $P < 0.1$; for methyl-β-cyclodextrin fed vs. fasted, upper case letters are used: $P < 0.05$).
[2]Means of pigs D, E, and G (see Table 1) are presented.

percentage of palmitate uptake oxidized by greater than eight-fold ($P < 0.001$). Fasting also increased palmitate oxidation ($P < 0.05$).

3.3. Comparison of palmitate oxidation

A comparison of palmitate oxidation measurements made in pigs infused with the [1-^{13}C]-palmitate–methyl-β-cyclodextrin mixture and pigs infused with the [1-^{13}C]-palmitate–albumin mixture is shown in Table 2. Palmitate oxidation values obtained under fed conditions when [1-^{13}C]-palmitate–methyl-β-cyclodextrin complex was infused were similar to those obtained using albumin as a carrier; however, the coefficient of variation was 1.7–3-fold lower with the methyl-β-cyclodextrin mixture. The similarity of plasma ^{13}C-palmitate and breath $^{13}CO_2$ enrichments (above baseline) for representative pigs using either albumin or methyl-β-cyclodextrin as carriers are shown in Fig. 1.

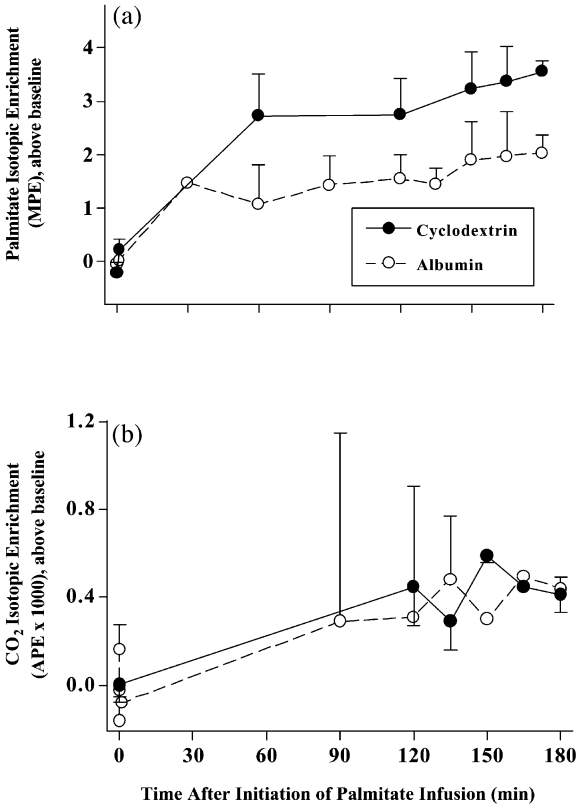

Fig. 1. Comparison of (a) plasma isotopic enrichment of [1-^{13}C]-palmitate (expressed as mol.% excess, MPE, above baseline) and (b) breath isotopic enrichment of $^{13}CO_2$ (expressed as at.% excess, APE, above baseline) from fed pigs during infusions of either the [1-^{13}C]-palmitate-methyl-β-cyclodextrin mixture (solid circles; $n = 4$) or the [1-^{13}C]-palmitate-albumin mixture (open circles; $n = 3$). Error bars represent S.E.M.

4. Discussion

In the present study, we developed and validated a new technique using methyl-β-cyclodextrin as a carrier (rather than fatty acid-free albumin) for palmitate in vivo infusion studies in growing pigs. In this study, the fed and fasted states were used as metabolic tools to elicit known states of low and high fatty acid oxidation to determine if the technique could measure these relatively extreme differences. With fasting, fatty acid oxidation should increase in the pig (Cunningham, 1967).

Fatty acid-free albumin has been the standard fatty acid-carrier used in most metabolic studies (Wolfe, 1992). However, in this study, the pigs infused with palmitate–albumin complexes (prepared using several sources of porcine and bovine fatty acid-free albumin) displayed a variety of adverse reactions. In addition, the experimental variation in the palmitate oxidation values was higher in the animals infused with albumin-bound palmitate. The palmitate oxidation values for the pigs receiving the palmitate–albumin mixture, while high for a fed animal, were lower than those observed in the fasted animals receiving the palmitate–methyl-β-cyclodextrin mixture. The pigs infused with the palmitate–albumin complex consumed two-thirds of their total diet before the initiation of the palmitate–albumin complex infusion, and although some regurgitated an unmeasured portion of the previously consumed diet, they were not ‘fasted’. Metabolically, some of the pigs infused with the palmitate–albumin complex were probably somewhere between the fed and fasted states. The elevated R_a(palmitate) value may have been due to the reduced feed intake in combination with the elimination of digesta from the stomach via the albumin-induced vomiting. The percent of the R_a(palmitate) which was oxidized in the pigs infused with the palmitate–albumin mixture was similar to the values observed in fed pigs infused with the palmitate–methyl-β-cyclodextrin mixture.

The palmitate oxidation rates and the percent of palmitate uptake oxidized in fed pigs obtained when methyl-β-cyclodextrin was used as a carrier for palmitate were not different to when albumin

was used as a carrier. The R_a(palmitate) values for fasted pigs were higher than the fed pigs infused with the palmitate–methyl-β-cyclodextrin mixture, demonstrating that the methyl-β-cyclodextrin method can detect expected differences in fatty acid oxidation. This was further supported by comparison with fasted and fed values in the literature. In pigs where methyl-β-cyclodextrin was used as a carrier, the palmitate oxidation increased 5–12-fold when the pigs were fasted as compared to oxidation rates observed in the fed-state. This was a similar percentage increase to that which was observed by Cunningham (1967) in fed and fasted pigs (65 kg) using radioisotopic techniques with infusion of ^{14}C-palmitate bound to ox albumin.

We did not determine the association/dissociation properties of palmitate–methyl-β-cyclodextrin. However, in in vitro studies, β-cyclodextrin behaved similarly to defatted serum albumin (Ramadoss et al., 1976), and methylated β-cyclodextrin did not inhibit palmitate oxidation or impede palmitate bioavailability in vitro for *Mycobacterium leprae* (Ishaque, 1992). Our data suggested that this is true for in vivo studies as well.

Other studies using the pig model (Cunningham, 1967; Boyd et al., 1982; Dunshea et al., 1992) have utilized bovine or porcine albumin or autologous serum as a carrier for ^{14}C-fatty acids without negative side effects. However, relatively small amounts of radioactive tracers (as used in those ^{14}C studies) need to be infused to measure metabolite fluxes as compared to stable isotopic tracers, and therefore studies using radioactive fatty acid tracers require less total albumin to be infused. For example in the present study, we infused albumin at 39 mg kg^{-1} h^{-1}, and in a study using pigs of a similar weight, approximately 6 mg kg^{-1} h^{-1} of albumin was infused when a radioisotope was used (Dunshea et al., 1992), a difference greater than six-fold.

We did not determine the exact cause of the adverse reactions to albumin infusion, but the symptoms observed were consistent with anaphylaxis. Previous experience has shown that pigs are very sensitive to pyrogens and endotoxins in intravenous solutions and feed contaminants that do not appear to adversely affect some other species (Wray-Cahen, unpublished data). Use of essentially γ-globulin-free and low endotoxin preparations of bovine albumin reduced, but did not eliminate, the adverse reactions, nor did their use prevent the suppression of food intake. We were unable to obtain the fatty acid-free albumin routinely used in human studies to provide a direct comparison, but it is possible that pigs would experience heterologous anaphylaxis with albumin of human origin as well, irrespective of the purity. A porcine albumin comparable to that used in human studies was not commercially available. While severe adverse reactions to human serum albumin infusion in man are relatively rare, intolerance to infusion of human albumin, including pyrogenic and anaphylactic reactions (Ring et al., 1979; Stafford et al., 1988; Leach, 1991; Pool and McLeod, 1995), and possibly an increased risk of mortality (Cochrane Injuries Group, 1998), have been reported.

We did not observe any adverse side effects while using methyl-β-cyclodextrin as a non-blood-derived alternative to serum albumin as a carrier for [1-^{13}C]-palmitate. This could be due, in part, to the lower volume of methyl-β-cyclodextrin compared to albumin infused. However, this was not likely, because: (i) we infused one animal (data not shown) at a higher infusion rate (using 10% methyl-β-cyclodextrin) and observed no adverse reactions; and (ii) we have observed no adverse reactions in pigs receiving much higher infusion rates of other compounds (e.g. infusion of glucose during hyperinsulinemic–euglycemic clamps). Use of β-cyclodextrins may allow for a higher infusion rate of a fatty acid tracer than possible when albumin is used as a carrier. Cyclodextrins have some additional potential advantages over albumin. For example, methyl-β-cyclodextrin is more readily available than infusion-quality albumin and is more cost-effective. Cyclodextrins have potentially much less batch-to-batch variation and are free of contaminating biological substances, such as residual fatty acids and immunoglobulins. Refrigeration of the palmitate–albumin mixture can also lead to the formation of compounds that are pyrogenic in nature for humans (Wolfe 1992); this did not appear to be a problem with methyl-β-cyclodextrin.

Interest in cyclodextrins has been increasing, both for their use in drug delivery and formulation (Rajewski and Stella, 1997; Stella and Rajewski, 1997; Thompson, 1997) and for their potential as a lipid carrier in parenteral nutrition (Brewster et al., 1989; Ohtani et al., 1989; Irie et al., 1992; Thompson, 1997). Although infusion of unsubsti-

tuted β-cyclodextrin is unsafe due to its nephrotoxicity (Stella and Rajewski 1997), modified cyclodextrins vary with regard to this trait of their parent compound. Irie et al. (1992) infused hydroxypropyl β-cyclodextrin parenterally into humans without irritation. They also infused hydroxypropyl β-cyclodextrin parenterally into rabbits with no observed morphological changes in most organs (including liver). However, very high infusion rates of hydroxypropyl-β-cyclodextrin did result in some morphological changes in the kidneys, similar to those observed with large i.v. doses of glucose, sucrose, mannitol, or dextran (Irie et al., 1992). This may be associated with its route of clearance. Clearance of i.v.-administered hydroxypropyl-β-cyclodextrin, dimethyl-β-cyclodextrin, and β-cyclodextrin appears to be primarily via glomerular filtration, with approximately 90% of infused cyclodextrin appearing in the urine within less than 24 h (Frijlink et al., 1990; Pitha et al., 1994; Kubota et al., 1996; McCormack and Gregoriadis, 1996; Thompson, 1997). Grosse et al. (1999) reported the pharmacokinetics of methyl-β-cyclodextrin in rabbits. They reported a distribution time of approximately 30 min and a half-life of 7 h. Appearance in urine was not determined in that study, but methyl-β-cyclodextrin levels in the kidney were high within 4 h. It seems highly probable that methyl-β-cyclodextrin administered i.v. is also cleared by the kidneys.

The ability to tolerate oral or i.v. dosing of cyclodextrins appears to vary with species. Cats have demonstrated adverse reactions to high infusion rates of methyl-β-cyclodextrin (personal communication, Dr S.A. Center, Cornell University) and dogs appear to tolerate long-term dietary inclusion of β-cyclodextrin better than rats (Thompson, 1997). Methylated β-cyclodextrins with a high degree of substitution (di-methyl and tri-methyl β-cyclodextrin) have been found to have toxic effects when given parenterally, but both rabbits and mice have been infused with high doses of methyl-β-cyclodextrin without exhibiting any toxic effects (Grosse et al., 1999). The safety of i.v. administration of methyl-β-cyclodextrin has not been fully explored.

Although it was not measured in this study, there is the potential for methyl-β-cyclodextrin to bind or complex with other lipophilic compounds in the circulation. There is evidence that cyclodextrins have the capacity to bind to and remove cholesterol, vitamin A, bile salts, triglycerides and potentially other lipophilic compounds from the blood (Ferezou et al., 1997; Rajewski and Stella, 1997; Thompson, 1997). In pigs, 5 and 10% β-cyclodextrin diets reduced levels of cholesterol in blood and liver when a high cholesterol diet was fed (Ferezou et al., 1997). Addition of β-cyclodextrin to diets also reduced cholesterol levels in rats and dogs (Rajewski and Stella, 1997). It is possible that infused cyclodextrins may bind to other lipophilic compounds as well. The physiological result of β-cyclodextrin binding to lipophilic compounds may not be predictable — in vitro studies suggested that β-cyclodextrins may make some compounds more available to cells (Christian et al., 1999) and the cholesterol-lowering effect of oral β-cyclodextrin has not been consistent in different species (Thompson, 1997).

In conclusion, we have demonstrated the feasibility of using methyl-β-cyclodextrin as a substitute for albumin as a carrier for ^{13}C-palmitate in in vivo infusion studies to determine the rate of turnover and oxidation of plasma palmitate in pigs. The ^{13}C-palmitate–methyl-β-cyclodextrin complex was stable at room temperature and the palmitate tracer complexed with methyl-β-cyclodextrin has been demonstrated to be available in vivo. Methyl-β-cyclodextrin did not have the negative side effects that we have observed with albumin infusion into pigs. Therefore, it may be a promising non-blood-derived alternative to serum albumin for use as a fatty acid-tracer carrier in fatty acid oxidation studies in growing pigs.

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