

# Estimation of the Absorption Coefficient of Stearic Acid in SALATRIM Fats

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SALATRIM is the name given to a family of triacylglycerols that contain saturated long-chain fatty acids (LCFA) and short-chain fatty acids (SCFA). The major LCFA component is stearic acid, and the SCFA components are acetic, propionic, and butyric acids. The component acids are positioned statistically with respect to the glycerol backbone. SALATRIM compositions contain up to five triacylglycerol structure types (i.e., LSS, SLS, LLS, LSL, and LLL, where S and L refer to the relative positions of SCFA and LCFA substituents on glycerol). It has been shown that SALATRIM fats deliver only about half the calories of common fats and oils. A predictable portion of this caloric reduction is due to the SCFA components. The balance of the caloric reduction is due to the incomplete absorption of stearic acid that is liberated by the action of digestive lipases. We have used data from a caloric availability study in rodents involving SALATRIM compositions which differed in their total molar S/L ratio to show that the coefficient of absorption for stearic acid is a function of the relative proportions of the various triester structure types. A mathematical expression was developed to correlate  $Abs_{St}$  with the molar S/L ratio. The values of  $Abs_{St}$  obtained fall predictably in line with those reported for model stearate containing LCFA triacylglycerols. A value for  $Abs_{St}$  predicted by this methodology was also found to be consistent with results obtained with human subjects, providing a bridge between the rodent and human models. The apparent smooth variation of the absorption coefficient with triacylglycerol structure is not unlike that reported for fully caloric fats and oils.

## INTRODUCTION

It has been suggested that a healthier diet for the average American would include a reduction in dietary calories derived from fat from a current average of 36% to a level of 30% (U.S. Department of Health and Human Services, 1988). The SALATRIM family of triacylglycerols offers a range of functionally diverse triacylglycerol-based fats that can provide a substantial reduction in calories compared to corn oil (Finley et al., 1994b). The source of the caloric reduction in SALATRIM is due to the presence of short-chain fatty acids (SCFA) and saturated long-chain fatty acids (LCFA), principally stearic acid. The SCFA components are inherently lower in caloric value relative to their LCFA counterparts (*CRC Handbook of Chemistry and Physics*, 1989), while the reduced caloric value of stearic acid (Hoagland and Snider, 1942; Carroll and Richards, 1957; Nolen, 1981; Mitchell et al., 1989; Chen et al., 1989; Apgar et al., 1987; Awad, 1989) can be attributed to its relatively poor absorption from the gastrointestinal tract (Carroll, 1957; Clarke et al., 1977).

The fraction of stearic acid absorbed from an LCFA triacylglycerol composition during digestion is dependant on the particular triacylglycerol structure. Mattson (1959) correlated a low coefficient of absorption for stearic acid in the case of tristearin. Later research using specifically synthesized triacylglycerol structures containing oleic (O) and stearic (St) acids revealed that the coefficient for absorption ( $Abs_{St}$ ) for stearic acid was a function of its position and concentration in the molecule (Mattson et al., 1979). Specifically, in the presence of adequate dietary calcium and magnesium,  $Abs_{St}$  was 0.98 for OStO, 0.55 for StOO, 0.37 for StOSt, and 0.59 for StStO. Such apparent diversity for this parameter prompted our decision to derive an estimate for  $Abs_{St}$  in SALATRIM fats by drawing from experimental data that have recently been reported on these materials. The data sources used have been from caloric bioavailability assays in rats (Finley et al., 1994b) and a human clinical trial (Finley et al., 1994b,c).

## EXPERIMENTAL METHODS

The 11 SALATRIM compositions employed were prepared by the interesterification of different starting molar ratios of tributyrin and hydrogenated canola oil (Klemann et al., 1994). After removal of excess tributyrin, each product was examined for its molar ratio of short-to-long-chain acids (molar S/L ratio) by proton NMR using a Varian VXR-300 spectrometer. The S/L ratios of these compositions varied between 0.51 and 1.99. Caloric availability assays were carried out at Nutrition International Laboratories located in East Brunswick, NJ. The method for determining the caloric bioavailability is described by Finley et al. (1994a,b). The rodent studies on SALATRIM materials involved addition of 21% of each composition to a basal diet. Control groups received the same basal diet to which corn oil had been added in increments between 0.0 and 21.0%. The assay involved monitoring the weight gains for all animals over a 14-day period during which growth was rapid. Total dietary calories were designed to ensure that animals consumed all food presented. The average weight gain for each test and control group was used to compute the caloric availability for each SALATRIM composition. The details of this growth assay and the associated calculations have been reported (Finley et al., 1994a,b).

The calculations used to relate the observed caloric availability to the coefficient of absorption for stearic acid were performed on a spreadsheet using Microsoft Excel. The model designed for this purpose followed that reported by Peters et al. (1991). Graphical curve fitting was done using the utilities found in Lotus Freelance software.

## STRUCTURE OF TRIACYLGLYCEROLS CONTAINING SHORT- AND LONG-CHAIN FATTY ACIDS

The SALATRIM family of triacylglycerols is produced by the base-catalyzed interesterification of triacylglycerols containing short-chain fatty acids (SCFA) and long-chain fatty acids (LCFA). Excess volatile SCFA triacylglycerols are virtually completely removed from the crude product by vacuum steam deodorization, leaving essentially only five glycerol triesters. Two of these have one LCFA and two SCFA moieties (LSS + SLS), two more have two LCFA

**Table 1. Experimental Caloric Availability Values for SALATRIM 4CA Compositions<sup>a</sup>**

molar S/L ratio	kcal/g obsd <sup>b</sup>	molar S/L ratio	kcal/g obsd <sup>b</sup>
0.51	2.56	1.39	4.46
0.79	3.54	1.52	5.07
1.15	4.63	1.76	5.32
1.26	4.59	1.80	5.76
1.31	4.32	1.99	6.39
1.36	5.56		

<sup>a</sup> Compositions prepared by interesterifying different molar ratios of tributyrin and hydrogenated canola oil and removing excess tributyrin. <sup>b</sup> All test materials fed at 21% of diet, studies T-216, T-306, and T-343.

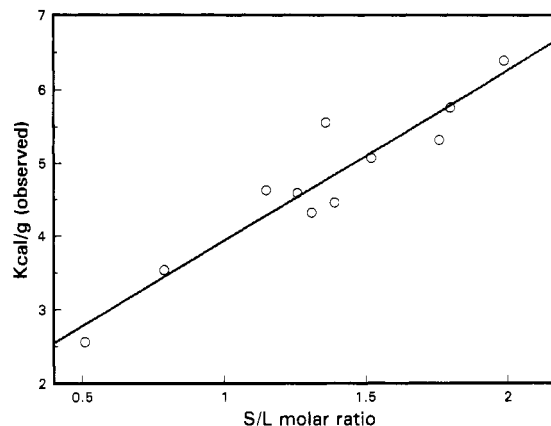
moieties and one SCFA moiety (LLS + LSL), and the fifth has only LCFA groups esterified to glycerol (LLL). The products are both qualitatively and quantitatively consistent with compositions predicted by random reaction statistics (Klemann et al., 1994).

As predicted by reaction statistics, the relative concentrations of the unsymmetrical isomers (i.e., LSS and LLS) in each isomeric pair should dominate by a factor of 2 to 1. This prediction has been experimentally verified by high-resolution NMR (Henderson et al., 1994). The compositional description of SCFA + LCFA triacylglycerol mixtures is simplified considerably by this finding. Since the relative amount of each isomer in a pair is a constant, only the mole fractions of the three triester classes (i.e., LSS + SLS, LLS + LSL, and LLL) need be considered to fully describe any composition. These mole fractions can themselves be determined by using statistically derived equations and the relative mole fractions of starting SCFA and LCFA triglycerides. The mole fractions of the three triester classes can be converted to weight fractions by applying the appropriate molecular weights. Alternatively, or to confirm this calculation, any one of several analytical tools can be applied to measure either the weight fraction, mole fraction, or SCFA/LCFA ratio in any product (Klemann et al., 1994).

#### DERIVATION OF ABS<sub>ST</sub> FROM CALORIC BIOAVAILABILITY STUDIES IN RATS

The interesterification of different starting molar ratios of tributyrin and hydrogenated canola oil was used to produce 11 SALATRIM compositions that contained different total molar ratios of short-to-long-chain acids. The molar S/L ratios of these compositions varied between 0.51 and 1.99. These compositions were employed in a caloric bioavailability assay in rodents (Finley et al., 1994b).

The results of the caloric bioavailability assays are provided together with the molar S/L ratio for each composition in Table 1. The data are also presented graphically in Figure 1 to demonstrate the linear correlation ( $r = 0.9438$ ) observed between the caloric availability and the respective molar S/L ratios. To probe the relationship between the stearic acid absorption coefficient and the caloric availability of these materials, an acid profile was constructed for each composition by utilizing an analytical fatty acid profile for hydrogenated canola oil, together with the experimentally determined molar S/L ratio. These data were arrayed after the method of Peters et al. (1991) in a matrix format to determine and assemble the individual caloric contributions of the acid and glycerol components. An example of one such matrix is provided in Table 2. All of the information necessary to predict the caloric availability of each test material either is available in the literature or can be estimated with reasonable certainty, except for the coefficient for absorption for stearic acid. The constants in each horizontal

**Figure 1.** Plot of caloric availability vs molar S/L ratio of SALATRIM fats prepared by interesterification of different ratios of tributyrin and hydrogenated canola oil.**Table 2. Data and Matrix Used To Estimate Abs<sub>ST</sub> for SALATRIM 4CA<sup>a</sup>**

component	wt fraction <sup>b</sup>	gross energy (kcal/g)	absorption coefficient	contribution to caloric value (kcal/g)
C4:0	0.129	5.91	1.0 <sup>c</sup>	0.762
C16:0	0.034	9.32	0.85 <sup>c</sup>	0.269
C18:0	0.745	9.5	Abs <sub>ST</sub>	7.078 Abs <sub>ST</sub>
C18:1	0.007	9.5	0.99 <sup>d</sup>	0.066
C20:0	0.019	9.65	0.42 <sup>e</sup>	0.077
C22:0	0.007	9.82	0.29 <sup>e</sup>	0.020
C24:0	0.004	9.95	0.15 <sup>e</sup>	0.006
glycerine	0.133	4.31	1.0	0.573
H <sub>2</sub> O	(0.078)	3.21 <sup>f</sup>		(0.250)
totals	1.000			C <sup>g</sup>

<sup>a</sup> SALATRIM is a reduced-calorie fat, in this case prepared from tributyrin and fully hydrogenated canola oil. <sup>b</sup> Composition derived for a material having a final molar S/L ratio of 0.51. <sup>c</sup> Estimated. <sup>d</sup> Carroll and Richards (1957). <sup>e</sup> Peters et al. (1991). <sup>f</sup> Heat of formation of water. <sup>g</sup>  $C = 7.078 \text{ Abs}_{ST} + 1.523$ ;  $C = 2.56 \text{ kcal/g}$  from experiment [cf. Table 1 and Finley et al. (1994b)].

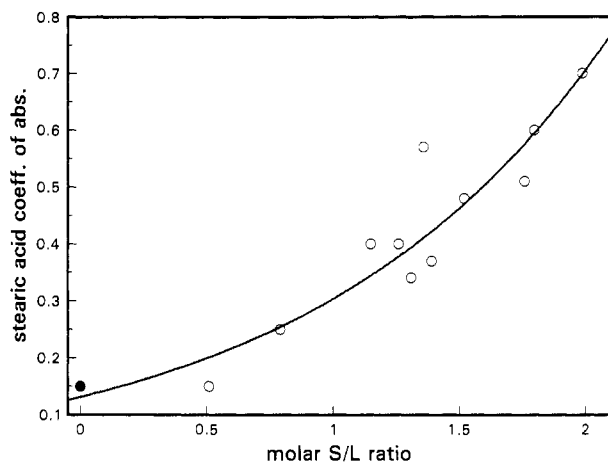
**Table 3. Estimated Values for the Absorption Coefficient of Stearic Acid in SALATRIM Compositions<sup>a</sup>**

molar S/L ratio	Abs <sub>ST</sub>	molar S/L ratio	Abs <sub>ST</sub>
0.51	0.15	1.39	0.37
0.79	0.25	1.52	0.48
1.15	0.40	1.76	0.51
1.26	0.40	1.80	0.60
1.31	0.34	1.99	0.70
1.36	0.57		

<sup>a</sup> Estimated from the observed caloric availability values (cf. text and Table 1).

line in this matrix are multiplied together to yield the extreme right-hand vertical column of individual contributions to the caloric availability. A sum taken of this column provides the total caloric availability as an algebraic expression, as is shown in the final footnote in Table 2. Since the caloric availability for each composition is also known (cf. Table 1), the coefficient for absorption for stearic acid is the only unknown in this algebraic expression. Therefore, the appropriate value for the caloric availability can be inserted and the expression can be solved for Abs<sub>ST</sub>. By reiterating this process for each SALATRIM composition, estimates for the absorption coefficient of stearic acid at each respective molar S/L ratio were obtained. The results of this exercise are summarized in Table 3 and are presented graphically in Figure 2.

The stearic acid coefficient of absorption value of 0.15 reported for tristearin (Mattson, 1959) is included at a



**Figure 2.** Plot of the absorption coefficient of stearic acid vs the molar S/L ratio of SALATRIM compositions and tristearin: (open circles) SALATRIM compositions; (solid circle) tristearin.

molar S/L ratio of zero in Figure 2. This point, together with the points determined experimentally, can be fit with the exponential function given in eq 1 which relates the

$$\text{Abs}_{\text{St}} = 0.13085 e^{(0.84109 \times \text{S/L})} \quad (1)$$

absorption coefficient for stearic acid to the molar S/L ratio for any member of the SALATRIM family of fats ( $r = 0.9466$ ). Application of this relationship, along with the total acid profile (i.e., the methodology outlined by way of example in Table 2) will enable prediction of the caloric availability for any composition.

#### ESTIMATION OF $\text{Abs}_{\text{ST}}$ FROM THE RESULTS OF A HUMAN CLINICAL TRIAL

Determination of the amount of stearic acid that is not absorbed (relative to the total amount ingested) provides a method for estimating  $\text{Abs}_{\text{St}}$  that can extend the work just described above in rodents to a human model. The necessary experimental work in this case was part of a human clinical trial carried out on SALATRIM fats, and the results of this study have been reported (Finley et al., 1994b,c). The results can be used to estimate the absorption coefficient for stearic acid by comparing the amount of fecal stearic acid excreted while a SALATRIM fat is in the diet relative to the amount of fecal stearic acid excreted on diet containing a control fat such as coconut oil.

Such a comparison was made for human subjects receiving either 60 or 45 g of added dietary fat per day. Since the SALATRIM fat employed in this study contained 57.0% of its total mass in the form of stearic acid (Finley et al., 1994b,c), the respective dosages in terms of stearic acid were 34.2 and 25.7 g per day. The results of this study can be interpreted to show  $0.22 \pm 0.05$  or  $0.16 \pm 0.06$  g of excess stearic acid excreted per day per gram of ingested dietary lipid, respectively. Since the absorption coefficient for stearic acid is the ratio of the stearic acid absorbed to the total stearic acid ingested, eq 2 can be used to relate the available data. Referring to eq 2,  $W_{\text{tmc}}$  is the weight of test material consumed per day,  $St_e$  is the excess stearic acid excreted per gram of lipid consumed per day, and the constant is the weight fraction of stearic acid in the ingested test material. By inserting the appropriate respective values into eq 2,  $\text{Abs}_{\text{St}}$  can be determined for both dietary fat levels tested.

$$(W_{\text{tmc}} \times 0.57) - (W_{\text{tmc}} \times St_e) / (W_{\text{tmc}} \times 0.57) = \text{Abs}_{\text{St}} \quad (2)$$

The results of these calculations provide values for  $\text{Abs}_{\text{St}}$  of  $0.61 \pm 0.15$  and  $0.72 \pm 0.28$ , respectively, for the subjects who received test material at either 60 or 45 g per day. Using eq 1 and the molar S/L ratio of 1.78 for the SALATRIM fat employed in the clinical trial, an absorption coefficient of 0.58 for stearic acid can be predicted.

#### DISCUSSION

Since a review of the literature on the subject of the absorption coefficient of stearic acid provided no unambiguous choice for  $\text{Abs}_{\text{St}}$  in triacylglycerols that contained short- and long-chain saturated acids, it was decided to investigate the effect of structure on  $\text{Abs}_{\text{St}}$  in a caloric bioavailability study of SALATRIM fats. The element of structure unique to the SALATRIM family is the simultaneous presence of short- and long-chain acids esterified to glycerol. The molar S/L ratio for SALATRIM compositions provides a simple way of assessing the impact of the three classes of triester structure types present (i.e., LSS + SLS, LLS + LSL, and LLL triesters, which contain one, two, or three LCFA moieties, respectively).

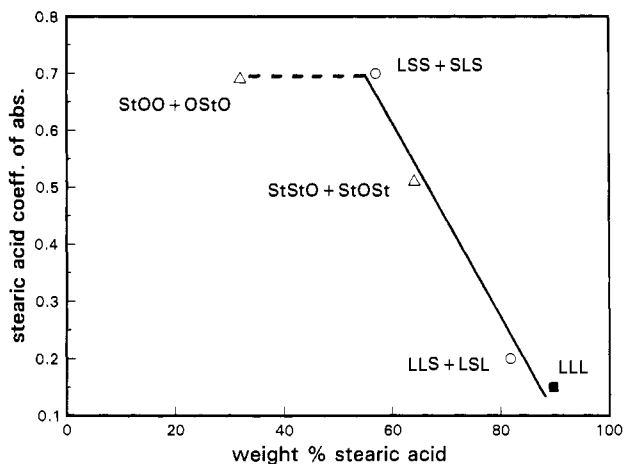
The results reported in Table 1 and Figure 1 reveal an apparent linear relationship between the observed caloric availability and the molar S/L ratio ( $r = 0.9438$ ). Since the apparent caloric availability increases with decreasing stearic acid content, the observed correlation cannot be explained in terms of test material compositional differences. If, for example, the coefficient of absorption for stearic acid were a constant, then the effect of composition would predict the exact opposite result (i.e., caloric availability would be expected to decrease with decreasing stearic acid content or increasing molar S/L ratio).

To estimate  $\text{Abs}_{\text{St}}$  for each caloric availability result, a modification of the methodology used by Peters et al. (1991) was employed. As shown by way of example in Table 2, each distinct molecular fragment that makes up an aggregate triacylglycerol composition can be arrayed in a matrix with its appropriate weight fraction, gross energy (from bomb calorimetry), and absorption coefficient to yield a series of contributions to the total available calories per gram. When all of the necessary constants are known, this matrix approach permits an estimate of the caloric availability to be made (Peters et al., 1991). In the present investigation, this approach to the determination of the caloric availability was used in reverse. That is, all matrix elements (including an experimentally derived value for the total caloric contribution) were supplied except for  $\text{Abs}_{\text{St}}$ . Each composition (which varied only in its molar S/L ratio) was first formulated by adjusting the ratio of short-to-long-chain acids until the proper molar S/L ratio was achieved. Once composition was fixed, the product for each line in the matrix was determined. By inserting the appropriate experimentally derived value for the total caloric contribution, this matrix can be solved for the remaining unknown,  $\text{Abs}_{\text{St}}$ . The results of these calculations produced a value of  $\text{Abs}_{\text{St}}$  for each respective SALATRIM composition, as shown in Table 3. A graphical representation of the data is provided in Figure 2. The literature value for tristearin is included in the plot (Mattson, 1959). A linear fit to the data was rejected in favor of a nonlinear exponential fit since the latter ultimately afforded a higher correlation coefficient ( $r = 0.9466$ ). The mathematical expression for the line drawn through the data in Figure 2 is given by eq 1. Application of this relationship, along with the total acid profile (i.e., the methodology outlined by way of example in Table 2),

**Table 4. Stearic Acid Coefficients of Absorption for Model Triacylglycerols**

entry	abbreviations <sup>a</sup>	acids modeled	wt % St	Abs <sub>St</sub>
1	StOO + OStO	stearic, oleic	32.0	0.69 <sup>b</sup>
2	StStO + StOSt	stearic, oleic	64.0	0.51 <sup>b</sup>
3	LSS + SLS	stearic, butyric	57.0	0.70 <sup>c</sup>
4	LLS + LSL	stearic, butyric	81.8	0.20 <sup>c</sup>
5	LLL	stearic	89.7	0.15 <sup>d</sup>

<sup>a</sup> 2:1 mole ratio of unsymmetrical and symmetrical isomers of respective triacylglycerols: St, stearic acid; O, oleic acid; L, LCFA; S, SCFA. <sup>b</sup> Mattson et al. (1979). <sup>c</sup> This work, eq 1. <sup>d</sup> Mattson (1959).



**Figure 3.** Plot of the absorption coefficient of stearic acid for model triacylglycerols: (circles) stearate + butyrate triacylglycerols; (triangles) stearate + oleate triacylglycerols; (square) tristearin; abbreviations are defined in Table 4.

provides a prediction of the caloric availability for any SALATRIM composition.

The nonlinear relationship between Abs<sub>St</sub> and molar S/L ratio is not surprising. If one takes the published values for Abs<sub>St</sub> developed for a series of triacylglycerol compositions made up of oleic (O) and stearic (St) acids (Mattson et al., 1979) and models an equilibrium mixture (as would be obtained by interesterification) containing the appropriate ratios of unsymmetrical to symmetrical triester structure types, values of Abs<sub>St</sub> of 0.69 and 0.51 can be calculated for these model StOO + OStO and StStO + StOSt compositions, respectively. If one then adds the 0.15 value of Abs<sub>St</sub> for tristearin to this array, clearly a plot of Abs<sub>St</sub> vs molar O/St ratio would be nonlinear and, in fact, would have positive curvature.

There is an important element of consistency between Abs<sub>St</sub> estimates for both SALATRIM and the model stearate-oleate triacylglycerols if one considers the weight fraction of stearic acid in each composition. If we select a stearate-butyrate model for our SALATRIM compositions, the data in Table 4 can be assembled. A plot of these data is provided in Figure 3. Inspection of Figure 3 reveals an excellent correlation ( $r = -0.9850$ ) between Abs<sub>St</sub> and the weight percent stearic acid at stearate levels greater than about 57%. At this point, Abs<sub>St</sub> appears to reach a saturation value of about 0.70 (although more data would clearly be needed to fully validate this observation). This comparison shows that SALATRIM composition has a similar predictable impact on the absorption of stearic acid as can be found in model stearate containing LCFA triacylglycerols.

It was desirable to compare the model for stearic acid absorption developed from caloric availability studies in rodents with some form of direct measurement of Abs<sub>St</sub> in humans. The availability of stearic acid excretion data from a human clinical trial involving SALATRIM per-

mitted the desired connection. The human clinical study (Finley et al., 1994b,c) employed a SALATRIM composition that had a molar S/L ratio of 1.78. Use of eq 1 provides an estimate for Abs<sub>St</sub> of 0.58. Observed values for Abs<sub>St</sub> could be obtained from test subjects who received either 45 or 60 g of added dietary fat per day. The values of Abs<sub>St</sub> derived from the data for these two groups were  $0.72 \pm 0.28$  and  $0.61 \pm 0.15$ , respectively. Since the experiment in question involved the measurement of nonabsorbed stearic acid in collected fecal samples, the relative error in such a measurement becomes larger as the amount of material to be assayed is decreased. This fact notwithstanding, the Abs<sub>St</sub> values determined experimentally in humans agree well the value that can be estimated from the caloric availability experiments in rodents.

## CONCLUSIONS

Data from a caloric availability study in rodents involving a family of SALATRIM compositions which differed only in their molar ratio of short-to-long-chain acids was used to develop a mathematical relationship between the absorption coefficient of stearic acid, Abs<sub>St</sub>, and the molar S/L ratio of the reduced calorie fat. If one focuses on the weight percent of stearic acid present, the model developed supplies estimates of Abs<sub>St</sub> that appear to be completely consistent with values available for model triacylglycerols that contain mixtures of oleic and stearic acids. The rodent-derived model for estimating Abs<sub>St</sub> also provides a consistent estimate for Abs<sub>St</sub> determined independently in a human clinical trial. This finding produces an important linkage between the rodent and human biological models with respect to the caloric reduction achievable with the SALATRIM family of fats.

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