

Evaluation of the relationship of Dairy and Meat consumption  
(as CLA content) with resting metabolic rate

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## **Abstract**

The primary goal of this research was to extract the dietary milk and meat consumption from three day food records of a previous study entitled "Food Habits and Behaviors, Metabolic Rate and Body Composition in College Females ages 18-26" to estimate the ingested conjugated linoleic acid (CLA). (1) In addition to the three day food records, the original data included using The Nutrition Electronic Tool (NET) with 38 college females to include self-reported markers of food habits, nutrition attitudes and dieting behaviors. Body fat was estimated using the BodPod® two compartment model, and resting metabolic rate was estimated using the MedGem® indirect calorimeter. The three day food records had been previously evaluated using Food Processor SQL. This current study used the quantities of foods recorded on the three day food records to estimate CLA content of the diet. The main focus was to examine the data for predictive value of estimated CLA consumption (from lean meat and low fat milk) for resting metabolic rate.

Previous evaluation of this data indicated that consumption of lean meats and low fat dairy products improved body fatness and resting metabolic rate. These findings begged the question if CLA found in these food substances was a potential bioactive predictor of increased RMR. Once meat and milk products were recorded from the three day food records, total CLA content was manually estimated for each record using published values (2). CLA exists in various isomers with the c9t11 and t10c12 isomers as the most commonly studied. Published values of CLA content in foods allowed for estimation of the two more popular isomers for separate evaluation in this study.

Evaluation for influence of CLA and the primary isomers in the diet followed a manual stepwise regression for the dependent variable Resting Metabolic Rate. Independent variables of interest considered in the resting metabolic rate modeling process included: waist, age, markers of nutrition attitudes, dieting behaviors, total food habits marker, body image, projected energy balance, BMI and weight alongside the CLA and CLA isomer variables.

Results indicated that the best models of regression did not include CLA or the c9t11 isomer in the models. The best model for resting metabolic rate included the variables waist, height, age, meat preference, and Food Habit Questionnaire total. This study remains consistent with the previous findings. The data does not support an influential role for the CLA as estimated from three-day dietary records, but it does support a strong role for dietary habits and attitudes. Surprisingly, the insignificant beta values obtained for CLA suggests an inverse relationship of what was originally hypothesized.

This study was more specific to the CLA dietary components than the original study. Electronic estimation of dietary CLA was not possible using current Food Processor SQL so this estimation was carried out manually. For future studies to be more accurate, database software that contains CLA content would be highly desirable and would likely produce more accurate values to include all foods.

## **Introduction**

Because there has been a growing problem with obesity in our society, it is important to learn all that we can about health and diet to try to reverse the current population's trends towards obesity and to teach proper physical activity, nutrition, and eating habits. It is important to keep finding new ways to examine our diet and to learn more about the influence of certain foods on the body. Studies to identify methods to improve metabolic rate or decrease body fatness work towards overcoming obesity.

In a previous study by Tonya Sue Orchard entitled "Food Habits and Behaviors, Metabolic Rate and Body Composition in College Females ages 18-26", metabolic rate was best predicted by subject's attitudes and self reported food habits (questionnaire) concerning the use of lean dairy and meat products when evaluated using linear regression modeling. (1) This current study aims to re-evaluate the data using predicted conjugated linoleic acid (CLA), a known bioactive component of the meat and milk consumed. It is hypothesized from the previous literature that diets that include ruminant meats and dairy products will have more CLA thus may elevate the resting metabolic rate.

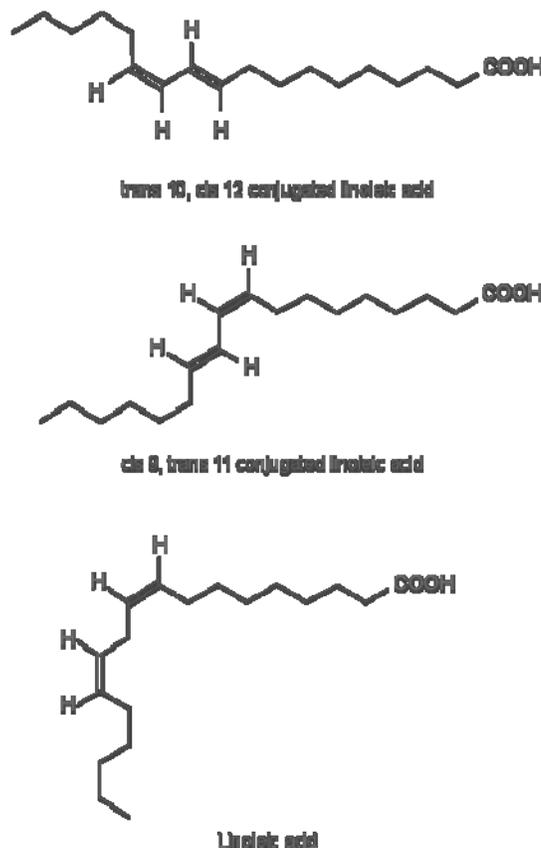
### Specific Aims:

- 1) Estimate the CLA content of the subject's diets using published values.
- 2) Evaluate the estimated CLA consumption with metabolic rate using regression modeling in relation to information from the previous study.

## Review of Literature

Conjugated Linoleic Acid (CLA) was discovered in the 1980's by Michael Pariza and his colleagues. (3) CLA's formation in food is thought to happen in at least two ways: 1) free-radical-type oxidation of linoleic acid effected by fat exposure to air, aging, heat treatment, and possibly protein quality, and 2) microbial enzyme isomerization of linoleic acids in the rumen of cattle and sheep. (4) From the discovery of this new family of compounds, two main isomers, figure 1, were isolated and studied, these being 9-*cis*, 11-*trans* and 10-*trans*, 12-*cis*, each of which have two conjugated double bonds.

Figure 1:



Other forms of the CLA compound exist but have not been the focus of much research. Of the two isomers c9t11 is more predominant than the t10c12 isomer; however both of these are used in most of the CLA research to date because they have shown the most bioactivity.

CLA is found naturally in many types of foods. Some examples include dairy products like cheese, milk, and ice cream,

lean meats like ground beef, veal, and lamb, even poultry, seafood and processed foods like beef franks, as well as some commercially produced oils like sunflower, safflower, and canola. CLA content tends to be lower in products like the oils and higher in animal products, especially ruminant products. (5) Ruminants are unique because they digest food in two steps, first by eating the raw material, fermenting and regurgitating a semi-digested form known as cud from the fore-stomach. The cud is further masticated and swallowed for continued fermentation. The fermentation process is thought to produce the CLA for incorporation into the animal's meat and milk. (5) The actual CLA content in these products is not constant and may depend on a number of factors: diet, season, breeding type, number of lactations, and current stage of lactation. (4) CLA content tends to be highest in grazing over grain-fed animals. (4) Not only does actual CLA content vary, but likely so does the ratio of the isomers. As for the oils, CLA is not naturally present, but is created in relatively small amounts as a result of industrial processing. (3)

Because this study will evaluate the estimated CLA in foods, it is important to explore if food preparation methods alter CLA content. The 1994 study by Shantha *et al* suggested homemade food preparation had no effect on CLA content. The study included testing CLA content for foods prepared through baking, frying, baking, broiling, and microwaving. (6) Storage like freezing and refrigeration also did not have an effect on the CLA content. However, the fermentation cultures, processing temperatures, and ripening periods could affect final overall content of CLA in the product. Two forms of bacteria were found to convert linoleic acid to the c9t11 isomer of CLA, those being

*Propionibacterium* spp. and *Lactobacillus* spp. Each of these strains could be used to enrich yogurts and cheeses in CLA during the fermentation process. (7)

Other studies suggested CLA was possibly responsible for modulating effects on energy and protein metabolism in animals like mice, rats, hamsters, and pigs as well as a few human studies. CLA has been shown to decrease body fat content and increase the body protein content as well as body water content in a rodent model. (8) Interestingly females showed a greater decrease in body fat than the males. (8)

The isomers (c9t11 and t10c12) likely produce different health effects. The t10c12 isomer is thought to change body composition through potential reduced body fatness, enhanced body water, enhanced body protein, and effects on the immune system. (9) One proposed mechanism to reduce body fatness is that it inhibits adipocyte lipoprotein lipase activity, which reduces the lipid uptake into adipocyte tissue. (10) The c9t11 isomer is thought to be more beneficial to inhibit the initiation and promotion of cancer and the t10c12 isomer is proposed as being responsible for the most weight (fat) loss. (9) The varied literature suggests that different isomers may produce different effects within the body, and it may be interesting to determine the blend of isomers to consume for maximal and desired benefits.

The influence of the isomers has extended to other animal models as well. A porcine model study suggested a reduction of subcutaneous fat and an increase in lean mass in both males and females (11), but this finding has not been replicated. A few fish studies have been performed using carp, tilapia, and rockfish. Groups of fish were given various levels of CLA in their daily diet for 8 weeks and it was concluded that fish grew better given a 1% CLA diet rather than larger amounts (2.5%, 5%, and 10%). This

finding in fish is unique in that the lower intake of CLA was most beneficial in comparison to the rodent studies where higher intakes provide better results. (4) However, in order to get a high enough concentration of CLA into the fish tissue for likely human health benefit, the 5% CLA diet was most appropriate, but it did hinder fish growth compared with the 1% treatment. (4)

Recent literature demonstrates interest in using this supplement in humans. A meta-analysis by Whigham *et al* summarizes 18 human studies looking at CLA and its correlation to fat mass and weight loss. (12) According to Whigham, seven studies showed a decrease in body fat, ten studies reported no statistical differences in body fat, and one study had no placebo group so those results were not taken into consideration. The studies that did show a decrease in body fat were longer duration (> 12 weeks) when compared with the studies that did not have any statistical differences. Whigham hypothesizes that had some of the null studies spanned a longer time period, the CLA affect on the body would have been more positive. One twelve week study demonstrated that 3.2 g of CLA per day does cause a modest, but significant reduction in fat loss of about 0.09 kg/wk. (12) This study consisted of 48 participants, 13 men and 35 women. This may seem like a small weight loss but, on average, Americans tend to gain 0.4 kg of weight each year (12), so CLA use may be valuable in maintaining current weight status.

While it would be beneficial to have longer studies to obtain more accurate data, some shorter studies also seem to be effective when mixed products of CLA are used in the form of over the counter supplements to aid in human weight loss. In a study by Zambell (2000), CLA supplements were given to overweight women for 64 days (5-6 weeks) and there was no effect on body composition. However, data reported in a study

by Thom (2001) stated that when CLA was given as TONALIN® mixture there was a body fat decrease after 3 months (12 weeks) of the supplementation. TONALIN® can be purchased over the counter and is a product based on the fatty acid CLA and provides the compound in ample amounts through a pill form. (13) According to the manufacturer, TONALIN® is purported to reduce body fat and support the maintenance of lean muscle mass, and reduce the amount and size of fat cells throughout the body. The product insert states that fats may be more readily broken down and fats are said to be transported and burned for energy more efficiently. (13) These are structure function claims that have yet to be substantiated in the human model according the literature reviewed for this paper.

Health claims of CLA have also been made by a study from Gnadig *et al* that suggested CLA may be capable of more than just weight loss effects. (3) A Major claim is that it could inhibit many chemical-induced cancers of the mammary system, stomach, colon, and skin. According to Gnadig, animal studies have shown that CLA can inhibit carcinogenesis at each of the major stages of initiation, promotion, progression, and metastasis. Gnadig also contends that the influence of CLA in atherosclerosis is controversial. While some studies have shown that it lowers LDL cholesterol and raises HDL cholesterol other studies have shown increases in each. Other CLA health benefit claims are that it can reduce hypertension, reduce symptoms of diabetes in some cases, reduce body fat gain, reduce catabolic effects of immune stimulation, reduce inflammation, and enhance immune function. It is clear from the lack of support literature that more studies need to be completed to confirm these claims.

Though CLA is thought to have a lot of health benefits, it is also thought to have some adverse effects when taken in large doses. The main concerns of large dose CLA consumption are the possible induction of fatty liver, insulin resistance, and/or unfavorable changes in serum lipids. (10) A weight loss supplementation study by Riserus and Ulf suggested that the *t10c12* isomer may have induced insulin resistance in abdominally obese men. (14) The same study also demonstrated other possible negative CLA effects such as oxidative stress and inflammation, which in theory could lead to cardiovascular disease. (14) A study with breast feeding women demonstrated an overall lower milk fat when taking CLA supplements leaving less energy available for the nursing infant. (15)

Many studies have been reported on CLA and its various isomers. These studies have included both animal and human models, each of which had unique results. Some of the data suggests positive effects while other data indicate CLA is not effective as a dietary supplement. In the future, long term studies need to be designed in a prospective double blind placebo fashion to be more helpful in determining the physiological influences of CLA and the main isomers. A well designed study might include measures of IR and glucose disposal, liver enzymes, and fatty infiltration evaluation and blood lipid panels in addition to changes in body weight and composition. The research to date on the efficacy of CLA as a dietary component or supplement is inconclusive.

## Methods

This research was built upon data previously collected in the parent study entitled “Food Habits and Behaviors, Metabolic Rate and Body Composition in College Females ages 18-26”. (1) In the parent study, 38 women between the ages of 18-26 were measured for height, weight, waist circumference, body fatness (BodPod®), resting energy expenditure (REE per MedGem® method), three day diet record, and eating habits and attitudes by on-line questionnaires. The descriptive results for these subjects are included in Table 1.

<b>Characteristic</b>	<b>Mean</b>	<b>Range</b>	<b>Std. Dev</b>
Age	21.29	19-25	1.58
Ht (cm)	163.8	152-176.5	6.50
Wt (kg)	62.94	38.2-115.7	12.45
Waist (cm)	72.8	56.5-110	9.22
BMI	23.4	15.2-41	3.94
Fat Mass(kg)	17.6	5.9-53.3	8.17
Lean Mass(kg)	45.4	32.4-62.5	6.21
%Body Fat	27	14.1-46	7.31
RMR	1454	990-1830	194.7

\*BMI = Body Mass Index

\*\*RMR = Resting Metabolic Rate

Table 1. Subject characteristics (n=38)

The on-line (NET) questionnaire consisted of three sections of interest; The Food Habits Questionnaire (FHQ) (16), the Nutrition Attitudes Survey (NAS) (17), and the Tendency to Diet Scale (TDS) (17). The three day diet and activity records were analyzed using Food Processor SQL® software (18) to estimate detailed information for the nutrient content of each food consumed as well as the presumed energy balance of the diet. The statistical analysis of the parent study consisted of reliability and validity studies for the NET tool as well as linear regression analyses for the best predictors of RMR and body

fatness. The analyses were completed using SPSS 14.0®. (19) In addition to demonstrating good reliability and validity of the NET tool, the primary findings indicated resting metabolic rate was best modeled with protein intake, waist circumference and percent body fat as the independent variables. Exploration of modeling the significant predictors of body fat included waist circumference and meat consumption (as estimated from the questionnaire).

This current study explored the predictive value of meat and dairy consumption as estimated CLA from the 3-day diet records for the dependent variable resting metabolic rate. Dietary CLA and isomers were not estimated by Food Processor SQL and were estimated manually for each subject using average published values. (2) To accomplish this, meat and dairy products were extracted and recorded by hand from the database of 3-day diet records. Published average values of CLA and its isomer contents were used in an excel spreadsheet to estimate (calculate) CLA in the subject's diet. An example calculation sheet is included in Appendix 2. Because the published values did not include all of the dairy and meat products reported by the subjects, and because some food items on Food Processor SQL are not descriptive enough, some food assumptions needed to be made. Also, in addition to these assumptions, some foods consumed that were potentially high in CLA but not listed in the CLA reference were compared with similar foods using the USDA Food Composition Database website to determine how similar their fatty acid profiles were and to project how much CLA might be available.

#### List of Assumptions

Brie cheese assumed as cheddar

1 slice of pizza assumed as 0.5 oz mozzarella

Tuna assumed as shrimp

Halibut assumed as trout

White fish assumed as trout

Parmesan assumed as cheddar

Dinners with cheese (1 cup) assumed as one slice processed cheese

Cream cheese assumed as sour cream

Swiss cheese assumed as cheddar

Cheesecake assumed as sour cream

Provolone cheese assumed as mozzarella

Eggnog assumed as whole milk

Soy not taken into consideration

After the average CLA and isomer contents were estimated for the three days for each subject, the values were imported/merged into the parent study SPSS file. The entire dataset was then evaluated using linear regression analysis for insight into the relationships among variables including this potentially bioactive ingredient. As outlined in Appendix 1, the Variables of interest that were used in modeling included: waist, age, weight, height, body mass index, body density, and energy balance. Variables included from the questionnaires included: tendency to diet, the sum of the food habits questionnaire, NAS dietary helplessness, NAS food exploration, NAS meat preference, NAS health concern, and a variable for how you want to look compared to your perception of how currently look. The new variables for CLA and its isomers were also included. The variables of interest used in regression modeling for both studies are listed in Appendix 1 for comparison. SPSS 16.0® was used for this current analysis. (19)

Data analysis followed stepwise manual regression using the variables of interest to determine which variables had statistical significance in predicting resting metabolic rate as the dependent variable. The manual stepwise regression procedure was

accomplished using the SPSS scripting utility, first with univariate models and then exploring the best predictors in the univariate models ( $p \leq 0.05$ ) to proceed with bi-, tri-, and multivariate models to find the best overall significant predictors for RMR. Variables with a p-value greater than 0.1 were excluded from the next step (p Out) during the modeling procedure while variables demonstrating p-values less than 0.05 (p In) were considered for inclusion in the next step model. The adjusted R-squared value for each regression model estimated how much of the variation in the dependent variable was explained by the predictor variables in the model. Additionally, the  $R^2$  value of each model was compared to the previous step model  $R^2$  using an F-test to ensure the addition of each variable significantly contributed to the model. Finally, the underlying assumptions of normal distribution for the independent variables were examined plotting the predicted values against the residual values, and evaluating their distribution where the apriori probability of a type I error was set greater than  $p=0.20$ .

## Results

The most significant variables to estimate RMR in linear regression modeling included waist, height, age, NAS meat preference, and Food Habit Questionnaire (FHQ) total. The model entry sequence was waist, age, NAS meat preference, height, and FHQ total, with each variable significantly contributing to the next model. These variables combined are a strong predictor of resting metabolic weight with an  $R^2$  of 0.517 and an adjusted R square value of 0.441. This means that approximately 44% of the variability in RMR can be explained by these variables in this group of young women. Each variable entered into the model significantly contributed to the model according to the F-test on the difference in  $R^2$ . Finally, to check that the model was able to meet the assumption of normal distribution of variables, the residuals were plotted against the predicted values and no pattern could be identified. Additionally, the residuals followed a normal distribution ( $p= 0.930$ )

**Coefficients<sup>a</sup>**

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error	Beta		
(Constant)	-2246.313	776.418		-2.893	.007
Waist	7.107	2.693	.337	2.639	.013
Height	8.414	3.833	.281	2.195	.036
Age	49.792	16.671	.403	2.987	.005
NAS meat pref	22.440	6.754	.477	3.323	.002
FHQ total	3.183	1.554	.290	2.048	.049

a. Dependent Variable: RMR

Other models under consideration for the dependent variable were body fat (Brozek variable) and body density, however their overall significance and  $R^2$  values were not as strongly predicted as resting metabolic rate. Other independent variables in RMR models included body silhouette selection (lookdiff) and body mass index together. Another significant model that was a fairly strong predictor of RMR was BMI, Age, NAS meat preference, and height. This was similar to the final selected model only it did not include the FHQ total and the adjusted  $R^2$  accounted for 39.8% of the variability in RMR.

Estimated dietary CLA and its isomer were not included in the final model and the p-values for these variables in the complex models were typically in the  $p=0.15-0.30$  range and ironically demonstrated a negative beta value. With pre-determined model entry and removal criteria employed in this study, the CLA and isomer variables did not enter the model at any step.

## **Discussion**

An aspect of the previous experiment that seemed of significance to further analyze was that of the women's responses to the food survey questionnaires in relation to their answers for meat and dairy preference. Women who chose to consume dairy and/or meat were more likely to have a higher resting metabolic rate according to the regression analysis. It was hypothesized that the CLA content from these foods could be to the underlying reason why these women demonstrated a leaner body mass and higher RMR.

While CLA was analyzed in relation to the variables of choice in comparison to resting metabolic rate, it never displayed as a significant variable in the modeling process or in the final model. In addition to the CLA results yielding non-significant p-values, CLA demonstrated an inverse relationship with RMR in the modeling process and this is counter to the other results in the literature.

The final model in this experiment included the sum of the meat preference variable from the Nutrition Attitude Survey, demonstrating once again that a willingness and preference to eat meat predicts a higher RMR. We speculate that this may be a surrogate marker for unrestrained eating attitudes which may yield the higher RMR. The willingness of women to eat meat and dairy may be a factor in higher RMRs, but this study was unable to confirm a role for the CLA content of the diet. Further work on food contents of CLA likely needs to be researched in order to conclude whether or not CLA is a strong predictor of RMR. This data does not support an influential role for the CLA on RMR as estimated from three-day dietary records, but it does support a strong role for dietary habits and attitudes.

In a study with better CLA estimation, it would be interesting to further compare the genders in the human model similar to the study by Park *et al* where female rats lost more weight with CLA supplementation than males. (8) If a male population were estimated in the same way, would their results show up differently in reference to CLA consumption? Or would the results remain the same for the male population? Because women have more fatty tissue than men, and because CLA is stored in adipose, this seems unlikely, however it would be an interesting comparison.

Estimation of CLA was likely inaccurate in this study because there was not a complete list of foods with CLA content and the CLA found in similar foods can be so variable. Some foods entered into the database were mixed foods such as a burrito with cheese where it was necessary to parse out an estimate of how much cheese would have been in the food. CLA values were estimated as closely as possible using this logic and the posted values, but this still leaves room for error above and beyond the usual error assumed in estimating dietary records. While a lot of CLA research has been done in the past, it is still difficult to make an accurate estimate of overall consumption because actual content within each item depends on the diet of the animal. The best way to get an accurate measurement would likely be to do a study where all food consumed was collected as duplicate meals and chemically analyzed to get the total CLA consumption measurement.

There is still a lot more research that needs to be done in regard to CLA and its use. While it may offer some health benefits, it could also potentially be harmful if consumed in large amounts from supplementation. This study did not show any CLA significance in relation to resting metabolic rate, but because the meat preference

variables and the food habits stayed in the model, it still seems that there is something about eating meat that increases one's metabolism. Further analysis would need to be done to examine these parameters. This study found no support for a positive influence of CLA on resting metabolic rate.

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## APPENDIX 1

Variables of Interest for RMR model

Parent Study:

Ht  
Wt  
Waist  
Age  
NASsum1 Helplessness  
NASsum2 Exploration  
NASsum3 Meat Preference  
NASsum4 Health Concern  
TDS Total  
Sum of FHQ Category Averages  
Fat Mass (kg)  
Lean Mass (kg)  
% Body Fat  
Grain Average  
Vegetable/Fruit Average  
Dairy Average  
Meat Average  
Fat Average  
Other Foods Average  
FHQ Average  
DIS Total 1-9  
Calories  
Protein  
Carbohydrate  
Total Fat

This Study:

Ht  
Wt  
Waist  
Age  
NASsum1 Helplessness  
NASsum2 Exploration  
NASsum3 Meat Preference  
NASsum4 Health Concern  
TDS Total  
Sum of FHQ Category Averages  
Density  
lookdiff  
Energy Balance  
BMI  
mgCLA  
mgCLA Isomer

## APPENDIX 2

Food	Reported serving	ESHA Grams fat	CLA per gram	calc CLA	c9t11%	c9t11 total
<b>DAIRY PRODUCTS</b>						
<b>Milk</b>						
	whole		0.0055	0	92.00%	0
	2%		0.0055	0		0
	skim		0.0055	0		0
Butter			0.0047	0	88.0%	0
Sour Cream			0.0046	0	90.0%	0
Plain Yogurt			0.0048	0	84.0%	0
Nonfat Yogurt			0.0017	0	83.0%	0
Ice Cream			0.0036	0	86.0%	0
Sharp Cheddar Cheese			0.0036	0	93.0%	0
Mozzarella Cheese			0.0049	0	95.0%	0
Colby Cheese			0.0061	0	92.0%	0
Cottage Cheese			0.0045	0	83.0%	0
American Processed Cheese			0.005	0	93.0%	0
<b>MEAT</b>						
				0		0
Fresh Ground Beef	1 serving	14	0.0043	0.0602	85.0%	0.05117
Beef Round			0.0029	0	79.0%	0
Veal			0.0027	0	84.0%	0
Lamb			0.0056	0	92.0%	0
Pork			0.0006	0	82.0%	0
<b>POULTRY</b>						
				0		0
Chicken	1.5 serving	25.35	0.0009	0.022815	84.0%	0.0191646
Fresh Ground Turkey			0.0025	0	76.0%	0
<b>SEAFOOD</b>						
				0		0
Salmon			0.0003	0	0.0%	0
Lake Trout			0.0005	0	0.0%	0
Shrimp			0.0006	0	0.0%	0
<b>PROCESSED FOODS</b>						
				0		0
Beef Frank			0.0033	0	83.0%	0
Turkey Frank			0.0016	0	70.0%	0
Peanut Frank			0.002	0	0.0%	0
<b>CANNED FOODS</b>						
				0		0
Spam			0.0013	0	71.0%	0
Baked Beans			0.0007	0	56.0%	0
Corned Beef			0.0066	0	85.0%	0
<b>VEGETABLE OILS</b>						
				0		0
Safflower			0.0007	0	44.0%	0
Sunflower			0.0004	0	38.0%	0
Canola			0.0005	0	44.0%	0
			total	0.083015	total	0.0703346