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ERYTHROCYTE LINOLEIC ACID, BUT NOT OLEIC ACID, IS ASSOCIATED WITH IMPROVEMENTS IN BODY COMPOSITION IN MEN AND WOMEN

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Abstract

Scope—Supplementation with linoleic acid (LA; 18:2Ω6)-rich oils increases lean mass and decreases trunk adipose mass in people. Erythrocyte fatty acids reflect the dietary pattern of fatty acid intake and endogenous metabolism of fatty acids. The aim of this study is to determine the relationship of erythrocyte LA, with aspects of body composition, insulin resistance and inflammation. Additionally, we tested for relationships of oleic acid (OA) and the sum of long chain omega-three fatty acids (LC-Ω3-SUM), on the same outcomes.

Methods and Results—Men and women (N=139) were evaluated for body composition, insulin resistance, and serum inflammatory markers, interleukin-6 (IL-6) and c-reactive protein (CRP) and erythrocyte fatty acid composition after an overnight fast. LA was positively related to appendicular lean mass/body mass index (ALM/BMI) and inversely related to trunk adipose mass. Additionally, LA was inversely related to insulin resistance and IL-6. While there was an inverse relationship between OA or LC-Ω3-SUM with markers of inflammation, there were no relationships between OA or LC-Ω3-SUM with body composition or HOMA-IR.

Conclusions—Higher erythrocyte LA was associated with improved body composition, insulin resistance and inflammation. Erythrocyte OA or LC-Ω3-SUM was unrelated to body composition and insulin resistance.

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AUTHOR CONTRIBUTIONS

MAB, RRA and JKK-G developed the rationale and experimental design for this secondary analysis. RMC and J-Y K conducted laboratory analyses. RRA and BEB developed the statistical models for the analyses of data. All authors wrote and edited this work.

CONFLICTS OF INTEREST

There are no conflicts of interest by any authors for this work.

Key terms

Linoleic acid; unsaturated fatty acids; body composition; erythrocyte fatty acid composition

INTRODUCTION

Linoleic acid (LA; 18:2Ω6) is the predominant polyunsaturated fatty acid in the Western diet[1]. Accumulating evidence indicates that LA is associated with improvements in insulin sensitivity[2], reductions in cardiovascular disease risk[3], and changes in body composition[4–6]. In a double masked, randomized, controlled, crossover trial, 16 weeks of high-LA oil (6.4g LA/day) supplementation in obese women with type 2 diabetes reduced trunk adipose mass, fasting glucose and HOMA-IR values and increased lean mass[6].

Additional randomized controlled trials have demonstrated dietary LA to decrease liver fat, abdominal adipose mass and inflammatory markers. Abdominally obese subjects supplemented with LA had decreased liver fat and inflammatory markers compared with subjects who were supplemented with palmitic acid-rich foods [5]. In a separate study, when healthy, normal weight subjects were instructed to consume an excess of 750 kcal/day from muffins rich in LA or palmitic acid, those who consumed the high-LA muffins had a nearly 3-fold greater increase in lean mass compared to those who consumed the high-palmitic acid muffins, even though both groups gained similar amounts of weight over the seven week intervention[4].The change of lean mass was positively related with changes in plasma LA. In contrast, those that consumed the high-palmitic acid muffins had a nearly 2-fold greater increase in visceral adipose mass and accumulated more lipids in the liver than the high-LA muffin group.

Both plasma and erythrocyte fatty acids have been correlated with dietary fat consumption [7] but the fatty acid composition of erythrocytes has been suggested to more strongly reflect long term dietary intake of polyunsaturated fat,[7, 8] as well as changes in the metabolism of fatty acids [9, 10]. These reports [4–6] raise the compelling question of whether the status of LA measured in erythrocytes is associated with differences in body composition. We hypothesized that increased LA is associated with higher lean mass and decreased trunk adipose mass, insulin resistance and inflammatory markers in men and women. Our primary goal was to determine the relationship of LA status in non-supplemented individuals with aspects of body composition, insulin resistance, and inflammation. Additionally, we tested for possible relationships of two other fatty acid groups, oleic acid (OA) and the sum of long chain omega three fatty acids (LC-Ω3-SUM), also purported to contribute to cardiometabolic health[11, 12], on body composition, insulin resistance and inflammatory markers.

MATERIALS AND METHODS

Study Design

Data from two randomized, controlled trials were combined for this secondary analysis. In brief, each study examined the relationships of past depression and impact of daily stressors [13] or marital quality [14] on triglyceride clearance and other aspects of metabolism in a

postprandial timeframe following the administration of a standardized test meal. The cohorts were fully described previously[13, 14]; Data, where appropriate for the aims of this study, were combined and subject characteristics are shown in Table 1. Typical dietary intake of energy was measured by averaging results from three 24-hour dietary recalls, physical activity by questionnaire and metabolic data were obtained using indirect calorimetry [13, 14]. Both studies were approved by The Ohio State University Biomedical Institutional Review Board and all participants completed informed consent and privacy notifications.

The design of both studies consisted of a double-blind randomized crossover study that tested two meal challenges during two separate study visits [13, 14]. Generally healthy adults were recruited from the greater Columbus, Ohio (US) area and participated in visits at the Ohio State University Wexner Medical Center at the Center for Clinical Research. Exclusion criteria for both studies included any notable chronic health problems, smoking, alcohol or drug abuse, diabetes, or any prescriptions other than birth control.

Men and women enrolled were from diverse socioeconomic and education backgrounds (Table 1). Blood samples obtained after a 12-hour fast were used for this secondary analysis; blood samples collected post-prandially after the test meal challenge were not used in these analyses.

Exclusion criteria for each study were described previously [13, 14]. From the original cohort, N=144, data from three subjects removed due to possible sickness at one of the visits that would have affected markers of inflammation and data from two subjects where biochemical analysis of HDL were extremely high N=139 for these analyses.

Fatty acid composition of erythrocytes

Fatty acid composition of erythrocyte samples was determined from blood collected at the first visit for each subject in both studies. Whole blood was collected in EDTA tubes on ice and centrifuged (4°C, 1700 × g, 10 minutes). Erythrocyte fatty acid methyl esters were prepared using boron trifluoride in methanol[15, 16]. Fatty acid methyl esters were analyzed by gas chromatography (Shimadzu, Columbia, MD) using a 30-m Omegawax 320 (Supelco-Sigma) capillary column. Conditions of gas chromatography include: helium flow rate was 30 ml/min, oven temperature started at 175°C held for 4 min then increased to 220°C at a rate of 3°C/min. Retention times were compared to authentic standards for fatty acid methyl esters (Supelco-Sigma, St. Louis, MO and Matreya, Inc., Pleasant Gap, PA) and fatty acids are reported as percent of total identified. The intra-assay coefficients of variation were 3.8% for OA, 4.2% for LA, 13.5% for EPA, 4.8% for DPA and 6.5% for DHA. The sum of the three long chain Ω3 fatty acids = EPA+DPA+DHA, e.g., LC-Ω3-SUM. Fatty acid composition of erythrocytes is shown in Table 2.

Body mass and composition

Body mass index (kilograms of body weight per meters height^2), was calculated from body weight (Healthometer standard beam scale; Sunbeam® Products, Inc, Boca Raton, FL) and height (using a wall mounted Harpenden stadiometer; Holtain Limited, Crymych, UK). Body composition was analyzed using dual x-ray absorptiometry (DXA) [13, 14](Lunar iDEXA, Lunar Corp, Madison, WI). In brief, a whole body DXA scan was used to measure

lean mass and trunk adipose. For lean mass, coefficients of variation within people with BMI>30 m/kg^2 were 0.37% CV for total lean mass, 1.09% CV for ALM and 1.8% CV for trunk adipose on this instrument. Data for each variable is expressed in absolute (kg) and relative terms (% body mass). Unadjusted measurements of adipose and lean mass as measured by DXA are reported in Supplemental Table 2. We report the relationship of LA to lean mass as adjusted by body mass (ALM/BMI) since many of our subjects were overweight or obese; the Foundation for National Institutes of Health recommends this measurement to include BMI since BMI of people in the US is, on average quite variable and may be very highki[17].

HOMA-IR, interleukin-6 and C-reactive protein

Plasma insulin was analyzed by chemiluminescent-linked immunoassay (Immuliate 2000, Siemens Medical Solutions) and glucose by ELISA (YSI 2300 Stat Plus Glucose, Yellow Springs Instruments). Serum interleukin-6 (IL-6) was measured using an electrochemilluminescence method and quantified using the Meso QuickPlex SQ 120 (Meso Scale Discovery, Rockville, MD.) V-Plex kits were purchased from Meso Scale Discovery. The lower limit of detection for IL-6 is 0.06 pg/mL. Intra-assay coefficient of variation is 4% and the inter-assay (run-to-run) coefficient of variation is 6.4%. Serum C-reactive protein (CRP) was measured in human serum using a chemilluminescence method and analyzed using the Immulite 1000 (Siemens Healthcare Diagnostics, Inc., Deerfield, IL.) The analytical sensitivity for CRP is .01mg/L but functional sensitivity is 0.3 mg/L. The intra-assay coefficient of variation was 3.1% and inter-assay coefficient variation is 7.3%.

Statistical methods

Our primary interest was to test for the associations between LA, OA or LC-Ω3-SUM, and five outcomes: lean mass (BMI-adjusted ALM), trunk adipose mass, insulin resistance (HOMA-IR) and two inflammatory markers (IL-6 and CRP). In our secondary analysis, we tested for the possible mediating effect of trunk adipose on these relationships. Because one of the two studies enrolled married couples and partners' outcomes tend to be correlated, linear mixed effects models were used to account for within-couple correlations. Specifically, models included random couple-specific intercepts with random intercepts forced to be zero for individuals in the other study (not part of a couple in the study), thus capturing the positive correlation between responses from a spousal pair. Estimates of the within-couple correlation for each outcome, referred to as the intraclass correlation (ICC), are reported in Table 3. The Kenward-Roger degrees of freedom adjustment was used to control type I error in these models [18]. The within-couple correlation was estimated to be zero for the models with trunk adipose and IL-6 as outcomes, thus for these outcomes, models were equivalent to linear regression models.

Measurements of fasting HOMA-IR, IL-6, and CRP were obtained at both visits for each study; therefore, values for these outcomes were averaged for each subject. The remaining measures were only obtained at a single visit in each study. HOMA-IR, IL-6, and CRP data were right-skewed, thus analyses used natural log transformed (ln) values to better approximate normality of residuals. Therefore, results from models that used a log-transformed outcome are presented as changes in geometric means. Since data from two

studies were combined for this individual patient data meta-analysis, a fixed effect for study was included in all models [19]. The fixed effect of cancer history was also included in all models to account for the design of the parent studies, as one study included both female cancer survivors and benign control subjects[13]. Of note, women with a history of cancer were on average 10 years older than women without a history of cancer, but all other characteristics listed in Table 1 did not significantly differ between these two groups of women. To guard against potential confounding, all models further controlled for sex, age, and activity level (caloric expenditure per week). All analyses were conducted in SAS version 9.4 (Cary, North Carolina).

RESULTS

The combination of cohorts from two studies allowed for ample power to detect differences of the relationship of erythrocyte linoleic acid and body composition, metabolism and inflammation. In Table 1, general subject characteristics revealed that 53% of our cohort was obese and 29%, had metabolic syndrome.

Erythrocyte LA was significantly associated with BMI-adjusted ALM. Each 1 g/100g increase in LA correlated with a 0.019 unit increase in ALM/BMI, adjusting for sex, age, activity level, cancer history, and study ($p = 0.02$) (Table 3). On the contrary, erythrocyte LA was significantly *inversely* associated with trunk adipose and HOMA-IR: With a 1 g/100g increase of LA there was a 1.83 kg decrease in trunk adipose ($p = 0.0001$) and a 17% decrease in the geometric mean of HOMA-IR ($p = 0.006$) adjusting for sex, age, activity level, cancer history, and study. Similarly, after adjusting for sex, age, activity level, cancer history, and study, LA was inversely associated with inflammatory markers IL-6 and CRP, where a 1 g/100g increase in LA was significantly associated with a 15% decrease in the geometric mean of IL-6 ($p = 0.0003$) and a 26% decrease in the geometric mean of CRP ($p = 0.0005$). Because of the extremely strong inverse relationship of LA with trunk adipose, we examined whether trunk adipose mediated the effect of LA on lean mass, HOMA-IR, and the inflammatory markers. When trunk adipose was included as a covariate, the effect of LA on ALM/BMI and HOMA-IR diminished to near zero and was non-significant. However, including trunk adipose as a covariate reduced but did not completely eliminate the relationship between LA and IL-6 and CRP. After adjusting for trunk adipose, a 1 g/100g increase in LA was associated with an 8% decrease in the geometric mean of IL-6 ($p = 0.05$) and a 14% decrease in the geometric mean of CRP ($p = 0.07$).

Diet and physical activity may alter body composition, metabolism and inflammation. Therefore, we added these as covariates in our models to determine whether these, rather than LA status, were influencing our findings. In fact, these variables had little to no relationship to body composition, metabolism or inflammation in this cohort of healthy adults (results not shown).

In contrast to relationships between erythrocyte LA and body composition, HOMA-IR, and markers of inflammation, erythrocyte OA was not significantly associated with trunk adipose mass, nor was it significantly associated with HOMA-IR or ALM/BMI, with or without adjustment for trunk adipose. However, OA was significantly associated with

markers of inflammation after adjusting for trunk adipose, where a 1 g/100g increase in OA was associated with an 8% decrease in the geometric mean of IL-6 ($p = 0.04$), and a 16% decrease in the geometric mean of CRP ($p = 0.03$). When trunk adipose was excluded from these models, results were similar (Table 3).

Similar to OA, the LC-Ω3-SUM was not significantly associated with trunk adipose ($p = 0.92$), nor was it significantly associated with HOMA-IR, ALM/BMI, with or without adjustment for trunk adipose (Table 3). The LC-Ω3-SUM was not significantly associated with IL-6, but it was significantly associated with CRP after adjusting for trunk adipose, with a 1 g/100g increase in the LC-Ω3-SUM associated with a 13% decrease in CRP ($p = 0.047$). The magnitude of the association between LC-Ω3-SUM and CRP remained the same when trunk adipose was excluded from this model (Table 3).

DISCUSSION

Higher erythrocyte LA is associated with increased lean mass and decreased trunk adipose in middle aged, relatively healthy men and women while erythrocyte OA and LC-Ω3-SUM were not. To our knowledge, this is the first demonstration of such a robust relationship between a biomarker of LA status, e.g., erythrocyte LA, and favorable changes in body composition in adults who were not supplemented with high-LA oils.

A diet rich in LA may improve insulin sensitivity [20]. Our findings suggest that the insulin sensitizing effects may be linked with beneficial effects on lean mass and/or trunk adipose mass; a finding that may be corroborated by our previous finding that supplementation with high-LA safflower oil resulted in increased plasma levels of LA and decreased insulin resistance as measured by QUICKI[21]. Alternatively, LA may improve insulin sensitivity by decreasing lipid accumulation in liver [5], muscle [22] and/or pancreas[4]. This association between LA with insulin sensitivity may predict for reduced risk for conversion to type 2 diabetes over a 5-year follow-up period [23].

All three groups of unsaturated fatty acids were associated with decreased markers of inflammation in this cohort of relatively healthy adults. Evidence continues to accumulate showing that LA is linked with reduced inflammation[24]. A strong inverse relationship between IL-6 with lean mass and muscle strength was reported in a slightly older cohort [25]. Whether there was a relationship between LA and IL-6 or lean mass in this prior study was not reported. We[26, 27] and others [28–31]have reported that LC-Ω3 fatty acids are associated with decreased markers of inflammation. Results from studies seeking to determine a relationship between OA and inflammation have been mixed: Plasma OA was associated with elevated CRP [32].

During the years of data collection for these studies (December 2010 – September 2013) [13, 14], the consumption of LA was estimated to be 10 g – 20g / day for adults in the US [1]. Dietary sources for LA were traditionally from plant seed oils including safflower, sunflower, corn, and soybean. Within the last 5 years, modification of vegetable oils, e.g., safflower, sunflower, and soybean, has introduced a shift in fatty acid composition: OA was less than 15% in these oils but is now as high as 80% of fatty acids in the US market. The

shift of fatty acid composition has come at the expense of LA, which is now below 20% of the fatty acids in these commonly purchased vegetable oils. Based on our findings, the reduction of LA in vegetable oils available in the US market could significantly influence body composition and insulin resistance in people already at risk for cardiometabolic diseases. The impact of these modified vegetable oils for the health of consumers at-large is yet to be determined.

There are a few limitations with this study. A first limitation of this study is that because it is a cross-sectional analysis, we cannot attribute any causal relationships between erythrocyte LA with body composition, insulin resistance, and inflammation. Based on the highly significant relationship of LA with trunk adipose, when trunk adipose was used as covariate in the models for lean mass, HOMA-IR, and inflammatory markers, the relationships between LA and these outcomes were blunted. One might interpret this to mean that the relationship of LA with lean mass and HOMA-IR may be attributed to an effect of LA on trunk adipose; however, we cannot attribute causality with these related outcomes. Mechanisms of action of LA on body composition and metabolism are still unclear but deserve further investigation.

A second limitation of this study is that we recruited generally healthy middle-aged men and women for the cohorts analyzed in this study: The findings may not be generalizable to the overall middle age adult population of the United States. For example, although the prevalence for the metabolic syndrome for adults ages 40–59 is 40.8% in the United States []; prevalence for the metabolic syndrome in our cohort was modestly lower at 29%. The prevalence of obesity in the United States is ~ 40% [34]; in our cohort, obesity prevalence was 53%. Finally, we assessed prior lifetime prevalence of major depressive disorder in each prior study [13, 14]: The prevalence of 29.3% [13] and 15.1% [14] spans the average estimate of lifetime depressive disorder of 20.1 [35]. As is true for many randomized controlled trials, our combined cohort was also slightly more educated and Caucasian than the population of the US on average. Larger, epidemiological studies with data of body composition using DXA, as well as erythrocyte fatty acid composition and measurements of metabolism and inflammation will help to address whether our findings are unique or are generalizable for adults of middle age.

In conclusion, the relationship of erythrocyte LA with improved body composition and markers of insulin resistance and inflammation in a relatively healthy adult cohort suggests that erythrocyte LA status may predict for better outcomes related to obesity, metabolic syndrome, diabetes and cardiovascular disease. Further work is needed to validate these findings in larger, more diverse groups of individuals. The utility of using diets rich in high-LA oils to improve body composition and insulin resistance should also be investigated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ALM	appendicular lean mass
CRP	c-reactive protein
DXA	dual x-ray absorptiometry
HOMA-IR	homeostatic model assessment for insulin resistance
IL-6	interleukin-6
LA	linoleic acid
LC-Ω3-SUM	sum of long chain omega 3 fatty acids
OA	oleic acid

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TABLE 1

Subject characteristics by sex

Variable	Women (N=99)	Men (N=40)	Overall (N=139)
Age, years*	46.2 (11.1)	39.7 (9.3)	44.3 (11.0)
BMI, kg/m^2	29.9 (6.0)	31.5 (5.0)	30.4 (5.7)
Waist, cm*	97.0 (12.8)	105.5 (14.2)	99.4 (13.8)
Trunk adipose, kg	17.6 (6.6)	18.7 (7.2)	17.9 (6.8)
Caloric expenditure, kcals/wk	2.5 (2.1)	3.5 (2.9)	2.8 (2.4)
Fasting triglyceride, mg/dl	121.1 (69.1)	124.2 (58.5)	122.0 (66.0)
ALM/BMI*	0.67 (0.09)	0.98 (0.15)	0.76 (0.18)
HOMA-IR*	1.19 (0.98)	1.45 (0.83)	1.26 (0.94)
Fasting IL-6*	1.72 (1.40)	1.15 (0.81)	1.55 (1.28)
Fasting CRP	4.35 (5.23)	2.52 (2.20)	3.82 (4.64)
Years married[1]	11.5 (6.7)	11.8 (6.8)	11.6 (6.7)
Obese[2*]	47 (47%)	27 (68%)	74 (53%)
Metabolic syndrome[3]	26 (26%)	14 (35%)	40 (29%)
Race			
White	79 (80%)	32 (80%)	111 (80%)
Black	19 (19%)	8 (20%)	27 (19%)
Other	1 (1%)	0 (0%)	1 (1%)
Education			
Graduate degree	31 (31%)	16 (40%)	47 (34%)
College degree	27 (27%)	11 (28%)	38 (27%)
Partial college	31 (31%)	6 (15%)	37 (27%)
High school graduate	10 (10%)	5 (13%)	15 (11%)
<11 years high school	0 (0%)	2 (5%)	2 (1%)

Data shown are mean (SD) or N (%)

[1] Data only collected in study with couples (N=43 women, N=40 men)

[2] Subjects classified as obese if BMI > 30

[3] Criteria from International Diabetes Foundation were used.

* Significant differences between men and women ($p < 0.05$)

TABLE 2

Fatty acid composition of erythrocytes.

Name	Structural form	Women		Men		Overall	
		g/100g	SD	g/100g	SD	g/100g	SD
Myristic acid	14:0	0.4	0.1	0.4	0.1	0.4	0.1
Palmitic acid	16:0	24.6	3.0	25.1	1.7	24.7	2.7
Palmitoleic acid *	16:1n7	0.6	0.5	0.5	0.4	0.5	0.5
Stearic acid	18:0	20.9	1.1	21.0	1.0	20.9	1.1
Oleic acid	18:1Ω9	11.9	1.2	11.7	1.0	11.8	1.2
Linoleic acid	18:2Ω6	11.1	1.2	11.5	1.2	11.2	1.2
α-Linoleic acid	18:3Ω3	0.2	0.1	0.1	0.1	0.1	0.1
Arachidonic acid	20:4Ω6	17.0	1.7	16.6	1.4	16.9	1.6
Eicosapentaenoic acid *	C20:5Ω3	0.5	0.3	0.4	0.2	0.5	0.2
Docosapentaenoic acid	C22:5Ω3	2.3	0.4	2.5	0.6	2.4	0.5
Docosahexaenoic acid *	C22:6Ω3	3.7	1.0	2.9	0.7	3.5	1.0
LC-Ω3-SUM[1] *		6.5	1.3	5.9	1.1	6.3	1.3

Data shown are average g/100g +/− SD

Sum of EPA+DPA+DHA

*Significant differences between men and women ($p < 0.05$)

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TABLE 3

Relationship between erythrocyte linoleic acid (LA) and body composition, HOMA-IR and inflammation in men and women

			Predictor											
			Linoleic acid				Oleic acid				LC-Ω3-SUM			
Outcome[1]	Adjusted for trunk adipose?	N	Coef.	SE	*p*	ICC	Coef.	SE	*p*	ICC	Coef.	SE	*p*	ICC
Trunk adipose		139	−1.83	0.46	0.0001	0	−0.28	0.51	0.59	0	−0.04	0.47	0.92	0
ALM/BMI	No	139	0.019	0.008	0.015	0.26	0.0033	0.008	0.70	0.23	0.0045	0.008	0.55	0.22
ALM/BMI	Yes	139	0.006	0.007	0.41	0.29	0.0028	0.008	0.71	0.29	0.0047	0.007	0.48	0.29
ln(HOMA-IR)	No	135	−0.19	0.07	0.006	0.05	−0.071	0.07	0.32	0.08	−0.059	0.06	0.35	0.13
ln(HOMA-IR)	Yes	135	−0.018	0.06	0.75	0.14	−0.051	0.06	0.37	0.14	−0.064	0.05	0.20	0.21
ln(IL-6)	No	133	−0.17	0.04	0.0003	0.34	−0.095	0.05	0.057	0.23	−0.05	0.05	0.27	0.25
ln(IL-6)	Yes	133	−0.082	0.04	0.053	0.43	−0.086	0.04	0.042	0.40	−0.05	0.04	0.22	0.39
ln(CRP)	No	131	−0.30	0.08	0.0004	0	−0.20	0.09	0.028	0	−0.14	0.08	0.083	0
ln(CRP)	Yes	131	−0.15	0.08	0.067	0	−0.17	0.08	0.028	0	−0.14	0.07	0.046	0

[1]Adjusted for age, sex, caloric expenditure per week, cancer history, and study

Coef. = estimated regression coefficient; SE = standard error; ICC = intraclass correlation