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Adiponectin levels are reduced, independent of polymorphisms in the adiponectin gene, after supplementation with α-linolenic acid among healthy adults

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Abstract

Our first aim was to determine whether an isocaloric intervention using α-linolenic acid (ALA) in the form of flaxseed oil would alter adiponectin levels among overweight, otherwise healthy, males and females, and our second aim was to test for any potential modification of this intervention by 2 single nucleotide polymorphisms (276 and 45) in the adiponectin gene. Subjects included healthy adult males and females (~81% female; average age, 38 years) with increased waist circumference (mean, 99 cm) and body mass index (mean, 30 kg/m^2) who were free of chronic disease, not taking medications, and sedentary. Subjects met weekly with a registered dietician for 8 weeks. The control subjects (n = 27) were instructed not to alter their habitual diet and the ALA group (n = 30) was instructed to follow an enriched ALA diet by using flaxseed oil capsules (increasing ALA to 5% of total energy intake) and to lower their dietary fat consumption by a commensurate amount. Diets were analyzed using the Food Intake and Analysis System (v. 3.0, University of Texas School of Public Health, 1998). Fasting blood samples were obtained before and after the 8-week intervention. We found significant decreases (P = .02) in adiponectin (10.12 μg/mL pre, 9.23 μg/mL post) in the ALA group as compared with the control group (7.93 μg/mL pre, 8.10 μg/mL post) after the intervention. We also saw a decline in adiponectin in all genotype groups with the greatest decline among those carrying the rare T allele of single nucleotide polymorphism 276. There were no significant changes in fasting insulin, glucose, or quantitative insulin sensitivity check index values as a result of this intervention. In conclusion, this study suggests that supplementing with ALA for 8 weeks may lower adiponectin levels among healthy individuals, and this effect appears to be independent of polymorphisms in the adiponectin gene. Although the change in adiponectin in response to the ω-3 fatty acids was not accompanied by any change in glucose, insulin, or quantitative insulin sensitivity check index, long-term implications of such a decrease should be considered in future studies.

1. Introduction

Adiponectin is an adipocyte-derived peptide that is inversely associated with plasma C-reactive protein and tumor necrosis factor α as well as obesity, insulin resistance, and heart disease [1-3]. It is considered antiatherogenic because it has been shown to inhibit the expression of tumor necrosis factor α in macrophages as well as expression of adhesion molecules in the endothelium [2]; furthermore, it was recently found to be inversely associated with carotid intima media thickness in men [4]. Variation in the gene that produces adiponectin has also been associated with obesity, insulin resistance, and adiponectin levels. Specifically, the single nucleotide polymorphisms (SNPs) G276T (intron 2) and T45G (exon 2) have been associated with varying levels of adiponectin, risk of type 2 diabetes mellitus as well as body mass index (BMI), fasting insulin, and glucose [5,6].

Dietary ω-3 fatty acids (n-3 and n-6 fatty acids) including α-linolenic acid (ALA, 18:3 n-3) and the long-chain polyunsaturated fatty acid eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) have been associated with lowering proinflammatory markers including C-reactive protein [7-10]. Furthermore, ω-3 fats may be associated with increasing insulin sensitivity [11,12]. Little work however has considered the effects of ω-3 fats on anti-inflammatory factors including adiponectin [13,14], and few studies have considered the potential genetic moderation of dietary interventions. Therefore, the purpose of this study was to determine if an isoenergetic diet supplemented with 5% of

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energy from ω-3 fats, specifically ALA, alters adiponectin levels or estimates of insulin sensitivity. Furthermore, we sought to determine if variation in SNP 276 or 45 of the adiponectin gene modified any of these potential associations.

2. Methods

2.1. Participants

Participants were recruited from Colorado State University (Fort Collins) students, faculty, and staff from the Fort Collins community through mass emails, flyers, and advertisements in local papers and publications. Participants included abdominally overweight/obese (waist circumference [WC] >81 cm for females, WC >94 cm for males) males and females aged 20 to 68 years who were free of diabetes, known heart disease, or other inflammatory condition including asthma, arthritis, or other major medical problem as determined by a health history questionnaire. Other exclusion criteria include regular physical activity (>2 d/wk); women who were pregnant or using hormone replacement therapy or oral contraceptives; use of medication known to alter inflammatory factors; tobacco users; or individuals with habitual diets high in polyunsaturated fatty acid (PUFA) (PUFA/saturated fat ratio, >0.7, n-6/n-3 ratio <7:1) as determined by baseline diet analysis. In addition, subjects had stable weight for a minimum of 3 months before the study.

2.2. Experimental design

The study design was approved by the institutional review board at Colorado State University and the subjects gave their informed consent. Upon inclusion, subjects underwent baseline testing including the determination of percentage of body fat by dual-energy x-ray absorptiometry (DXA; Lunar DPX-IQ, v.4.5c, Lunar, Madison, WI), a fasting blood sample, and anthropometric measurements. Each subject recorded 7 days of detailed food records for assessment of baseline dietary intake. The 7-day diet record was entered into the Food Intake and Analysis System (FIAS v. 3.0, University of Texas School of Public Health, 1998) to determine total energy and baseline PUFA and n-6/n-3 ratios.

Participants were then matched on BMI and WC and randomly assigned to either the isocaloric control or ALA condition. The control group was instructed to maintain their normal diet throughout the 8 weeks of the study. Based on the calculation of total energy from baseline 7-day food records, subjects in the ALA group increased ALA intake to 5% of total energy via flaxseed oil capsules (Veg-Omega 3 Organic Flax Oil capsules from Spectrum Naturals, Petaluma, CA, and Barlean's Fresh ExPressed Flax Oil capsules from Barlean's Organic Oils, Ferndale, WA). The average ALA content based on gas chromatography in our laboratory was 57%; other fatty acids included ~18% linoleic acid, ~16% oleic acid, and ~9% saturated fatty acid. The ALA group, was provided instruction on how to reduce their dietary fat intake by a similar amount as that provided by the capsules to maintain the isoenergetic nature of the study. Subjects were advised to decrease their intake of high-fat foods, whole and 2% milk products, and added fats. Subjects were also provided lists of lower-fat alternatives to these foods. Diet records of both groups were assessed at weekly visits together with the weekly weigh-ins to ensure compliance to the diet prescriptions and weight stability. The ALA group received supplements at this time in unlabeled containers, and they were unaware of the type of supplement they were receiving. There were no adverse affects reported during or after using the ALA supplement. Compliance was determined by a capsule count at each visit after varying amounts of capsules were distributed the week prior. Assessment of erythrocyte membrane fatty acid composition, using gas chromatography, also provided a biomarker of dietary compliance.

2.3. Anthropometrics

Waist circumference was measured at the level of the umbilicus using a Gulick spring-loaded measuring tape. Height and weight were obtained from subjects dressed in lightweight clothes with their shoes removed. Body mass index was calculated as the weight in kilograms divided by the square of height in meters. To assess the percentage of body fat, we obtained DXA scans using a Lunar DPX-L total body scanner (Lunar DPX-IQ, v.4.5c).

2.4. Assays

Fasting blood samples were obtained from the anticubital vein after an overnight fast. All subjects' pre- and posttest samples were analyzed on the same plates to decrease any potential between-assay variation in assessing pre- and posttest blood samples. Adiponectin was assessed using Quantikine enzyme-linked immunosorbent kit (R&D Systems, Minneapolis, MN). Coefficient for intra-assay variation was less than 5% and that for interassay variation was less than 7%. Glucose levels were determined using the hexokinase/glucose 6-phosphate dehydrogenase method and were analyzed with the ELx808 automated plate reader (Bio-Tek Instruments, Winooski, VT). Coefficient for intra-assay variation was less than 4% and that for interassay variation was less than 5%. Plasma insulin levels were measured using the DSL-10-1600 ACTIVE Insulin enzyme-linked immunosorbent kit (Diagnostic Systems Laboratories, Webster, TX). Coefficient for intra-assay variation was less than 3% and that for interassay variation was less than 6.5%. Erythrocyte membrane fatty acid composition was determined by gas chromatography using standard procedures [15,16].

2.5. Genetic analysis

The buffy coat for DNA extraction was isolated from EDTA anticoagulated whole blood samples from each participant. DNA was extracted using the Pure Gene kit (Gentra Systems, Minneapolis, MN). Single nucleotide polymorphism 45 (T/G) in exon 2 (single nucleotide polymorphism database [dbSNP]

Table 1
Descriptive statistics at baseline for the ALA and control groups

	ALA group (n = 30)	Control group (n = 27)	*P*
	Mean ± SD	Mean ± SD	
Age	38.80 ± 11.97	38.15 ± 10.77	.83
Sex (% female)	80%	81%	.89
BMI (kg/m^2)	30.19 ± 5.50	30.46 ± 4.56	.84
WC (cm)	100.76 ± 11.36	99.60 ± 11.96	.71
Percentage of fat (%)	40.05 ± 8.29	41.16 ± 6.77	.58
Adiponectin (μg/mL)	10.12 ± 6.25	7.93 ± 4.25	.13
Insulin (μU/mL)	12.82 ± 10.08	14.61 ± 8.98	.031
Glucose (mg/dL)	98.40 ± 16.97	96.37 ± 13.61	.64
QUICKI	0.34 ± .05	0.33 ± .04	.64
SNP 45	TT = 78%	TT = 61%	.21
	GT = 22%	GT = 39%	
	GG = 0%	GG = 0%	
SNP 276	GG = 57%	GG = 52%	.77
	GT = 43%	GT = 44%	
	TT = 0%	TT = 4%	

no. rs2241766) of the adiponectin gene was detected through an engineered *Sma*I restriction enzyme site (New England Biolabs, Ipswich, MA). The primers used for amplification were 5′-GCAGCTCCTAGAAGTAGACTCTG-3′ and 5′-CTGTGATGAAAGAGGCCAGAAAC-3′. Digestion products were resolved on 2% agarose gels. Fragment sizes were assigned by comparison to known size markers. Single nucleotide polymorphism 276 (G/T) in intron 2 (dbSNP no. rs1501299) of the adiponectin gene was detected through *Bsm*I restriction enzyme site (New England Biolabs) and the primers used for amplification were 5′-GGCCTCTTTCATCACAGACC-3′ and 5′-AGATGCAGCAAAGCCAAAGT-3′. Digestion products were resolved on 2.5% agarose gels. Fragment sizes were assigned by comparison to known size markers. Genotypes were checked for deviations from Hardy-Weinberg equilibrium proportions.

2.6. Dietary data

Food records were analyzed using the Food Intake and Analysis System (v. 3.0, University of Texas School of Public Health, 1998), which uses the US Department of Agriculture database of food composition information (including individual fatty acids) for more than 7300 foods.

2.7. Statistical analysis

Descriptive statistics (means and SDs) as well as an independent-samples *t* test were performed to compare anthropometrics, percentage of body fat, and baseline adiponectin levels between controls and the ALA intervention group. A Mann-Whitney test was used to compare sex and genotype frequencies between groups. Because of their skewed distributions, adiponectin, insulin, glucose, and the quantitative insulin sensitivity check index (QUICKI) were log transformed before all analyses.

Because the dietary data and erythrocyte membrane fatty acid data were not normally distributed even after log transformations, a Wilcoxon singed rank test was used to compare the mean grams for the dietary variables and the mean membrane fatty acid change from baseline to posttesting among the ALA group and the control group. A 2-way repeated-measures analysis of variance (ANOVA) was used to determine time × group interactions for adiponectcin levels, fasting glucose, insulin, and QUICKI between the baseline and posttest ALA and control groups as well as when these variables were stratified by genotype. Age and BMI were controlled for in the analysis of adiponectin, glucose, insulin, and QUICKI. The n-6/n-3 ratio was calculated using the sum of arachidonic acid and linoleic acid divided by the sum of ALA, EPA, and DHA. The formula for the QUICKI is as follows: QUICKI = 1/[log (glucose, mg/dL) + log (insulin, μU/mL)]. Statistical significance was accepted at $P < .05$. All analyses were run using SPSS 13.0 (Statistical Package for Social Science, SPSS, Chicago, IL) for Windows.

3. Results

Table 1 shows descriptive statistics for these subjects. There were 30 subjects in the ALA group and 27 subjects in the control group. The mean age of the subjects was ~38

Table 2
Dietary intake presented as means and percentage of total calories at baseline and average intake for weeks 1 to 8 based on self-reported daily diet records

	ALA group (n = 30)			Control group (n = 27)		
	Baseline	Average intake (weeks 1-8)	*P*	Baseline	Average intake (weeks 1-8)	*P*
	Mean (%) ± SD	Mean (%) ± SD		Mean (%) ± SD	Mean (%) ± SD	
Total calories	1997.5 ± 485.5	1924.3 ± 516.5	.30	2171.1 ± 600.2	2062.9 ± 532.0	.16
Total fat (g, %)	81.0 (36.5%) ± 25.0	85.0 (38.6%) ± 20.1	.11	84.5 (34.3%) ± 32.8	89.0 (35.8%) ± 33.3	.11
PUFA (g, %)	16.3 (7.3%) ± 6.3	26.9 (12.1%) ± 7.2	.00	16.3 (6.7%) ± 5.6	17.2 (7.1%) ± 6.0	.22
MUFA (g, %)	30.8 (13.9%) ± 9.9	28.8 (13.1%) ± 7.1	.06	33.3 (13.4%) ± 14.6	34.2 (13.8%) ± 14.2	.26
SFA (g, %)	27.9 (12.6%) ± 10.6	23.2 (10.6%) ± 6.0	.00	28.6 (11.6%) ± 11.7	30.0 (12.2%) ± 11.8	.12
ALA (g, %)	1.4 (0.6%) ± 0.6	11.9 (5.3%) ± 3.9	.00	1.4 (0.6%) ± 0.5	1.5 (0.6%) ± 0.6	.25
n-6/n-3 ratio	10.6 ± 2.8	1.3 ± 0.3	.00	10.5 ± 2.5	10.2 ± 2.0	.72

MUFA indicates monounsaturated fatty; SFA, saturated fatty acid.

Table 3
Percentage of fatty acids present in erythrocyte cell membranes for the ALA group and control group before and after the intervention

	ALA group (n = 30)			Control group (n = 27)		
	Baseline	8 wk	*P*	Baseline	8 wk	*P*
	Mean % ± SD	Mean % ± SD		Mean % ± SD	Mean % ± SD	
SFA						
16:0 palmitic acid	20.22 ± 2.12	19.89 ± 1.01	.77	19.54 ± 1.05	19.91 ± 1.07	.10
18:0 stearic acid	17.88 ± 1.68	17.50 ± 0.71	.79	17.50 ± 1.45	17.73 ± 1.10	.40
MUFA						
18:1 oleic acid	15.80 ± 1.40	15.62 ± 1.10	.35	14.88 ± 1.36	15.20 ± 1.76	.48
PUFA						
18:2 linoleic acid	10.74 ± 1.39	11.31 ± 1.55	.01	10.87 ± 0.97	11.30 ± 0.91	.02
18:3 ALA	0.00 ± 0.00	0.19 ± 0.37	.03	0.00 ± 0.00	0.00 ± 0.00	1.00
20:4 arachidonic acid	15.97 ± 1.71	16.44 ± 1.15	.08	16.91 ± 2.41	17.30 ± 1.15	.41
20:5 EPA	0.05 ± 0.17	0.56 ± 0.62	.00	0.00 ± 0.00	0.00 ± 0.00	1.00
22:6 DHA	2.11 ± 0.33	2.98 ± 0.38	.00	2.21 ± 0.30	2.37 ± 0.24	.00
n-6/n-3 ratio	12.76 ± 2.44	7.96 ± 2.43	.00	12.84 ± 2.39	12.21 ± 1.66	.13

years with primarily females in both groups (~81%). The average WC, BMI, and percentage of fat were similar between groups as expected because the subjects were matched on WC and BMI. According to World Health Organization guidelines, this population was considered "high risk" for metabolic diseases based on their WC and obese based on their BMI [17]. The adiponectin levels, fasting insulin and glucose, and QUICKI were also similar between the groups at baseline. Table 2 shows the dietary intake at baseline and the average intake for weeks 1 to 8 in both the ALA group and the control group. As expected, there were significant increases from baseline in the ALA group for PUFA and ALA intake and decreases in the n-6/n-3 ratio when compared with the control group.

Importantly, we also assessed compliance to this intervention by considering changes in fatty acid content of the erythrocyte membranes from baseline to postintervention. Table 3 shows that most of the fatty acids did not significantly change; however, the 18:3 (ALA), 20:5 (EPA), and 22:6 (DHA) n-3 fatty acids all significantly increased in the ALA group. Furthermore, the n-6/n-3 ratio in the erythrocyte membrane significantly decreased in the ALA group. Interestingly, the percentage of DHA significantly increased in the control group; however, these changes were not of the same magnitude as those in the ALA group (30% increase in DHA in the ALA group vs 7% increase in the control group). The diet records (Table 2) corroborated these results showing ALA consumption increased from 1.4 to 11.9 g in the ALA group, whereas among the control group, ALA in the diet did not significantly change (1.4-1.5 g). Table 4 shows the results of 2-way repeated-measures ANOVA. As expected, BMI, WC, and percentage of fat did not change from baseline to postintervention because of the isoenergetic nature of the study. Adiponectin levels significantly decreased from baseline to postintervention in the ALA group when compared with the control group after controlling for age and BMI. There were no significant changes in fasting insulin, glucose, or QUICKI.

We also considered the above analyses after stratification by genotype using repeated-measures ANOVA (Table 5). We found that, similar to the results in Table 4, there were no changes in BMI, WC, and percentage of fat when stratified by genotype (data not shown). There were also no changes in

Table 4
Baseline and 8-week values for body composition, adiponectin, insulin, glucose, and QUICKI levels among the ALA and control groups

	ALA group (n = 30)		Control group (n = 27)		
	Baseline	8 wk	Baseline	8 wk	*P* [a]
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
BMI (kg/m^2) [b]	30.19 ± 5.50	30.18 ± 5.36	30.46 ± 4.56	30.48 ± 4.73	.91
WC (cm)	100.76 ± 11.36	101.08 ± 12.87	99.60 ± 11.96	98.59 ± 12.57	.17
Percentage of fat (%)	40.05 ± 8.29	40.33 ± 8.28	41.16 ± 6.77	41.19 ± 6.95	.47
Adiponectin (μg/mL)	10.12 ± 6.25	9.23 ± 5.54	7.93 ± 4.25	8.10 ± 4.20	.02
Insulin (μU/mL)	12.82 ± 10.08	13.77 ± 10.52	14.61 ± 8.98	15.84 ± 11.05	.78
Glucose (mg/dL)	98.40 ± 16.97	98.06 ± 19.88	96.37 ± 13.61	92.58 ± 15.11	.41
QUICKI	0.34 ± 0.05	0.34 ± 0.05	0.33 ± 0.04	0.33 ± 0.04	.67

[a] *P* value indicates results from 2-way repeated-measures ANOVA comparing changes in the ALA group with changes in the control group.
[b] Age and BMI were adjusted for in all analyses except for BMI.

Table 5
Baseline and 8-week values for adiponectin among the ALA and control groups stratified by SNP 276 and SNP 45 and their haplotypes

	ALA group			Control group			
	n	Baseline	8 wk	n	Baseline	8 wk	P [a]
		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Adiponectin							
276 GG	13	9.42 ± 4.15	8.94 ± 3.81	12	7.44 ± 3.33	7.49 ± 2.88	.45
276 GT or TT	10	11.78 ± 9.21	10.27 ± 7.92	11	9.02 ± 5.57	9.36 ± 5.71	.03
45 TT	18	9.47 ± 7.10	8.63 ± 5.97	14	9.11 ± 3.55	9.46 ± 3.99	.21
45 TG or GG	5	13.95 ± 4.08	12.70 ± 4.37	9	6.77 ± 5.61	6.70 ± 4.86	.19
276GG and 45TT	9	7.58 ± 2.36	7.41 ± 1.97	6	8.38 ± 1.61	8.41 ± 1.50	.94
276TX and 45TT	9	11.36 ± 9.67	9.86 ± 8.28	8	9.66 ± 4.56	10.26 ± 5.12	.06
276GG and 45GX	4	13.55 ± 4.59	12.39 ± 4.97	6	6.50 ± 4.44	6.57 ± 3.75	.27

[a] P value indicates results from 2-way repeated-measures ANOVA comparing changes in the ALA group with changes in the control group after controlling for age and BMI.

fasting insulin, glucose, or QUICKI when stratified by genotype (data not shown); however, adiponectin levels decreased in the ALA group as compared with the control group, after controlling for age and BMI, in all genotypic groups. Significant decreases in adiponectin were found in the ALA group compared with the control group in those carrying the T allele of SNP 276. Comparing the haplotype groups, adiponectin also declined in all groups with nearly significant decreases in the ALA group among those carrying the rare T allele of SNP 276 who were also homozygotes for the T allele of SNP 45.

4. Discussion

Overall, we found that adiponectin levels significantly decreased after supplementing abdominally obese but otherwise healthy males and females with 5% of calories (~11 g/d) from ALA. Furthermore, adiponectin decreased in all genotypic groups, with those carrying the rare T allele of SNP 276 showing the greatest decreases. The clinical significance of the small decrease in adiponectin (0.89 μg/mL) among the intervention group is not clear because we did not find any changes in insulin, glucose, or insulin sensitivity measured by the QUICKI after ALA supplementation. Importantly, however, this small decrease could have clinical relevance in relation to overall cardiovascular disease risk. For example, Hotta et al found among males and females, with an average age of 59 years, that adiponectin was significantly lower in diabetic patients with coronary artery disease (CAD) vs those without CAD and that these differences were quite small: 1.3 μg/mL in females (7.6 μg/mL without CAD vs 6.3 μg/mL with CAD) and 2.6 μg/mL in males (6.6 μg/mL without CAD vs 4.0 μg/mL with CAD) [18], suggesting that even small decreases in adiponectin can have clinical significance. Considering that our study was primarily among women (80%), with an average age of 38 years who were free of diabetes, known heart disease, or other inflammatory condition including asthma or arthritis, a decrease of 0.89 μg/mL may pose a significant risk for cardiovascular outcomes if these subjects continued with this ω-3 fatty acid supplement into their fifties. Future studies will be needed to further ascertain the long-term effects of ω-3 fatty acid supplementation on adiponectin levels.

There are 3 studies we are aware of that have considered the association of ω-3 fatty acids with adiponectin levels and only one of these studies considered an intervention among humans. Patel et al considered the effects of 1 g/d of Omacor (Reliant Pharmaceutical, Liberty Corner, NJ) (eg, EPA and DHA) given over 3 months among male, post–myocardial infarction patients. They did not find any significant changes in adiponectin levels compared to the usual-care control group, with the changes they did see being quite small (0.17 μg/mL decrease). They also did not find any major changes in metabolic variables related to glycemic control other than significant increases in insulin in the usual-care vs the Omacor group [14]. The second study was cross-sectional and found that, among healthy smokers and nonsmokers (average age, 38 years; mean BMI, 24 kg/m^2), those in the highest quintile of serum DHA had significantly increased adiponectin (18.02 vs 14.76 μg/mL). Furthermore, when they performed multivariate models, they found that ω-3 fatty acids contributed to adiponectin variance after controlling for age, BMI, waist-hip ratio, and the other fatty acids; this finding was only among nonsmokers [13]. The third study was conducted among mice, and although the results are difficult to extrapolate to humans, they found that replacing 15% of lipids with EPA and DHA (specifically, the replacement lipid consisted of 6% EPA and 51% DHA) improved insulin sensitivity as well as raised plasma adiponectin levels, independent of food intake or adiposity. They also found that the adiponectin gene expression was up-regulated in mature adipocytes after the intervention [12].

Although the study by Patel et al [14] is the most comparable to our study, there are still many differences. Their population was clinical (post–myocardial infarction) compared to our "healthy" young population, and thus, there

may be differences in underlying factors (eg, glucose regulation and inflammation) that may influence adiponectin levels. For example, 19% of the Omacor group and 26% of the usual-care group were diabetic as compared to our study where none were diabetic. Because adiponectin is significantly associated with insulin resistance [1-3], the response of adiponectin to an intervention may be quite different in a more insulin-resistant population vs an insulin-sensitive population. Flachs et al [12] hypothesized that there may be an inability to induce adiponectin in type 2 diabetic patients or in those with insulin resistance. They based this hypothesis on the review by Delarue et al [19] that shows that EPA and DHA prevent insulin resistance but do not necessarily reverse insulin resistance in humans. This may explain why Patel et al [14] did not see an increase in adiponectin or a change in insulin sensitivity among a group that were ~20% diabetic.

Comparing these results to our study, among an insulin-sensitive group, we found a decline in adiponectin with no change in insulin sensitivity. The lack of change in insulin sensitivity is not surprising based on the high insulin sensitivity our subjects exhibited at baseline. The decline in adiponectin may be attributed to a reduced demand for its anti-inflammatory actions in the face of high ω-3 fatty acids, as has recently been reported for interleukin 10, a related anti-inflammatory cytokine. A recent study found that treating RAW 264.7 cells (macrophage cell line) with ω-3 fats resulted in decreased interleukin 10 levels. The authors suggest that this decrease may be a secondary reaction to the anti-inflammatory effects of ω-3 fatty acids [20]. In the case of adiponectin, it may be that the insulin-sensitizing and potential anti-inflammatory effects of ALA compensate for adiponectin's effect and thus result in a decreased demand or requirement for adiponectin.

We must also consider the dosage and type of ω-3 fats given when comparing the above studies. From the comparison of the 2 EPA/DHA interventions, the Patel et al study gave 1 g/d of EPA and DHA; furthermore, Patel et al [14] provided the Omacor supplement that contained roughly equal percentages of EPA and DHA. Fernandez-Real et al [13] found only DHA, not EPA, was associated with adiponectin levels in healthy young adults. Comparing our results to those presented above, we did find that DHA in the erythrocyte membrane was positively correlated with adiponectin (r = 0.26, P = .05) among our total sample; however, after the 8-week intervention, the correlation no longer held (r = 0.12, P = .35) primarily because adiponectin levels declined in the ALA group. There were no associations between adiponectin and EPA and ALA erythrocyte membrane levels in our sample. Because our intervention included primarily ALA instead of EPA and DHA, it is difficult to compare to the above studies. We would however expect ALA to act in much the same way as EPA and DHA based on several studies that show that EPA and DHA up-regulate the adiponectin gene by acting as ligands for peroxisome proliferator–activated receptor γ, which is a transcription regulator of the adiponectin gene promoter, and that ALA has also been shown to be a principle ligand for peroxisome proliferator–activated receptor γ [21,22]. With little work done in this area, it highlights the need for more studies considering these associations.

Regarding the genetic modification of this dietary intervention, we found a decrease in adiponectin among those carrying the rare T allele of SNP 276 and the rare G allele of SNP 45, with the greatest changes among those carrying the T allele of SNP 276 (13% vs 9%). Most of the previous work has considered the adiponectin gene in relation to metabolic syndrome variables (eg, insulin, glucose, diabetes status, BMI, etc) without considering the response to a dietary intervention. For example, several studies have shown that the 276 common G allele is associated with lower adiponectin levels, greater insulin resistance, diabetes, and greater BMI compared with the rare T allele [23-26]. We did find lower baseline adiponectin levels among those with the 276 common G allele (8.47 ± 3.83 vs 10.34 ± 7.46 μg/mL, P = 0.31), but the differences were not significant. We also considered both SNP 276 and SNP 45 together (or the haplotype) as did other studies that have considered the adiponectin gene [5,24,26,27]. We found the greatest change in adiponectin in those who carried the rare 276 T allele and were homozygous for the common 45 (TT) allele.

There are several limitations that should be mentioned. This was not a "blinded" study; although the intervention group did not know what type of supplement they were receiving, they knew they were in the intervention group. However, knowledge of being in the intervention group was unlikely to influence any of the objectively measured outcomes in this study. The results of this study may not be generalizable to a "leaner" population. We recruited subjects with increased waist circumference and BMI, and although they were otherwise healthy, they may have had a greater response to this dietary intervention than a leaner group of subjects. Furthermore, because we only recruited participants from the Fort Collins community and the Colorado State University campus, our results may only be generalizable to this population. Because of the small sample size, the results of the genetic analyses should be interpreted with caution. Future studies should consider replicating these results with a larger sample size.

In conclusion, we found that adiponectin levels decreased after supplementing healthy males and females with 5% of their calories from ALA. This decline was seen in all genotype groups for SNP 276 and SNP 45 with the greatest decline occurring among those carrying the rare T allele of SNP 276. This is the only study we are aware of that has considered the effect of ω-3 fatty acids in the form of ALA on adiponectin levels among healthy adult males and females. Although the change in adiponectin in response to the ω-3 fatty acids was not accompanied by any change in glucose, insulin, or QUICKI, long-term

implications of such a decrease should be considered in future studies.

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