High Omega-3 Fat Intake Improves Insulin Sensitivity and Reduces CRP and IL6, but does not Affect Other Endocrine Axes in Healthy Older Adults

Authors
P. D. Tsitouras[1], F. Gucciardo[1], A. D. Salbe[2], C. Heward[3], S. M. Harman[1]

Affiliations
[1] Kronos Longevity Research Institute, Phoenix, AZ, USA
[2] Obesity and Diabetes Clinical Research Section, NIDDK/NIH, Phoenix, AZ, USA
[3] Kronos Science Laboratories, Phoenix, AZ, USA

Key words
- omega-3
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Abstract

Aging diminishes hormone secretion and target cell responsiveness, possibly due to loss of cell membrane fluidity or alteration of membrane phospholipids affecting signal transduction. We investigated whether a high ω-3 polyunsaturated fatty acid diet would improve endocrine function in 6 men and 6 women aged over 60 years. Subjects first ate an isocaloric control diet for 6 weeks, followed by an 8-week experimental diet, which included 720 g of fatty fish weekly plus 15 ml of sardine oil daily. In the last week, we measured RBC membrane fatty acids on each diet, performed pituitary, adrenal, hepatic, and Leydig cell endocrine provocative testing, and assayed selected cytokines. We also assessed insulin sensitivity utilizing octreotide insulin suppression testing and assessed free fatty acid (FFA) responses to isoproteronol. Insulin sensitivity increased significantly after 8 weeks on the ω-3 diet and FFA responses trended lower. Serum C-reactive protein was significantly reduced and a trend towards lower IL-6 was noted. No differences were found in other metabolic parameters, adiponectin levels, or hormone responses. We conclude that, in older people, high ω-3 consumption increases insulin sensitivity, may reduce FFA mobilization by catecholamines, and reduces inflammatory markers, but did not alter endocrine responsiveness after 8 weeks.

Introduction

Changes in hormone levels and responsiveness of hormone target cells are well-documented concomitants of normal aging in humans and laboratory animals. For example, serum testosterone concentrations decrease with age in men [1–3], as does the testosterone secretory response to gonadotropins [4, 5]. Growth hormone (GH) secretion and blood levels of IGF-I diminish with age [6–10] along with pituitary somatotropic responses to growth hormone releasing hormone [11, 12]. Altered metabolic responsiveness of liver, muscle, and adipose tissue has been found in older humans [13–16]. The mechanisms of action of many hormones require signal transduction across target cell membranes by receptor proteins. However, investigations of aged cells have generally failed to reveal alterations in numbers or chemical integrity of receptors sufficient to account for the magnitude of the effects of aging [17–20]. In addition, post-receptor cascades appear to be intact when stimulated by alternative pathways not requiring transmembrane signaling [21]. Thus, the attenuation of endocrine signaling could be related to aging changes in the membrane itself. It has been shown that aging is associated with reductions in polyunsaturated fatty acids (PUFA) content of phospholipids and accumulation of peroxidized and cross-linked fatty acids, trans-fatty acids, and cholesterol esters in biological membranes. Moreover, it has long been known that metabolic function is affected by fatty acid composition of cell membranes [22]. In 1985 Panek et al. [23] found that a diet high in saturated fatty acids impaired norepinephrine storage and release in sympathetic neurons, leading them to hypothesize that, "…dietary manipulations alter the lipid environment of receptor proteins, which may result in the perturbation of specific membrane-associated processes." Consistent with this hypothesis, in rats, fish oil feeding improves LDL receptor activity in liver, lowers blood sugar levels, increases muscle cell membrane Glut-4 glucose transporter number, and increases insulin mediated glucose transport into muscle. A diet high in ω-3 PUFA may also improve glucose tolerance in

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Correspondence
P. D. Tsitouras, MD
Kronos Longevity Research Institute
2390 E. Camelback Road
– Suite 440
Phoenix
85016 AZ
USA
Tel.: +1/602/778 74 93
Fax: +1/602/778 74 90
tsitouras@kronosinstitute.org

nondiabetic adult [24] and elderly humans [25]. Membrane transduction of adrenergic signaling in parotid gland cells from old rats improved when membrane fluidity was increased using *S*-adenosylmethionine [26]. Such results suggest that manipulation of membrane chemistry might reverse age-related changes in transmembrane signaling. Significant alterations in fatty acid composition of cell membranes can be achieved fairly rapidly (4–8 weeks), in epithelial cells and erythrocytes of rats and humans by altering dietary fat intake [27–29]. The effects of a diet high in ω-3 PUFA on endocrine regulation in aging humans have not been extensively studied. We, therefore, investigated whether a diet enriched in ω-3 PUFA might increase transmembrane signaling mediated responses of hormone secreting and endocrine target organs in men and women over 60 years of age.

Materials and Methods

Subjects

Twelve healthy men and women 60–75 years of age (mean 66.1 ± 4.5 years) participated in this study. All subjects completed the study. They were recruited via mass mailing of study brochures, and advertisements in local media. They were nonobese (BMI 22–30), nonsmokers, and free of chronic illness or debilitating conditions such as diabetes, cancer (except noninvasive skin cancer), lupus or severe rheumatoid arthritis, symptomatic coronary heart disease, liver or renal failure, or endocrine disease requiring hormone replacement (except menopause or adequately treated hypothyroidism) or cognitive impairment as assessed by a score ≥ 26 on the Mini Mental Status Exam (MMSE). If hypertensive, the blood pressure was well controlled without use of a calcium channel or α- or β-adrenergic blocking agent. History of food allergies, especially allergy to fish, was a reason for exclusion. Subjects consuming more than one serving per week of fatty fish or taking fish oil supplements were not eligible for study, nor were those engaged in high level competition sports (e.g., master athletes). The Institutional Review Board of the Arizona State University approved the study protocol and all research subjects signed informed consent documents.

Procedures

Qualification for study

At first contact, prospective subjects were advised that the study would entail a 6-week control diet during which they would be asked to eliminate all fatty fish while consuming four 6-ounce portions of nonfatty fish per week, to be followed by an 8-week experimental period during which they would consume four 6-ounce portions of fatty-fish per week and 1 tablespoon of fish oil per day. Prospective subjects were further advised that foods containing trans-fatty acids (examples of which were given) were to be minimized during the entire study. Upon verbal assent to the above guidelines, prospective subjects were asked to attend a screening interview. At the screening interview, prospective subjects were asked to complete the Block Food Frequency Questionnaire to assess usual intake [30, 31]. A diet history was completed for meal frequency and composition. At this time, they were also asked to taste and test the fish oil. Individuals who confirmed that they found the fish oil as well as the meal guidelines acceptable were deemed suitable for study. The protocol was fully reviewed with the subjects and written informed consent was obtained. Subjects provided a medical history and underwent physical examination, as well as cognitive evaluation (MMSE), EKG, and laboratory screening (SMA-24, CBC with platelets, thyroid function tests, lipid profile). Height and weight were measured and recorded. Blood was taken after a 12-hour fast.

Diets

During the first meeting with enrolled subjects, participants were asked to select four nonfatty fish items (orange roughy, halibut, haddock, trout, swordfish, and flounder) that would be provided to them each week for the first 6 weeks of study. Dietary guidelines for the study were reviewed, including provision of lists of fatty fish and foods containing trans-fatty acids to avoid during this period. Subjects were asked to use only olive oil for food preparation and salad dressing. Prior to week 3, instructions on completing a 3-day diet record were provided to the subjects. Except for servings of fish, and vitamin and oil supplements, diets were not supplied to research subjects by the investigators. Rather, volunteers purchased and consumed food based on detailed, individualized dietary plans provided and explained to them by a research nutritionist (ADS). Compliance was monitored by phone interviews with a dietician at 2-week intervals, during which 24-hour dietary recalls were recorded and during the 3rd week of each diet by a 3 day dietary diary that included a weekend day and 2 nonconsecutive weekdays.

Qualifying subjects were first placed on an isocaloric western (control) diet for 6 weeks. This diet contained 30% of calories as triglycerides of which 30% were polyunsaturated (corn or safflower oil, high in ω-6 PUFA), 40% monounsaturated (olive oil) and 30% saturated (butter, animal fats). Subjects were encouraged to eliminate trans fats from their diets. Carbohydrates supplied 50% of calories, of which two thirds were complex starches high in fiber (pasta, bread, whole wheat, etc.). The remaining 20% of calories came from protein, including fish, beef, pork, chicken, eggs, and cheese. As a control, subjects consumed six 180 g servings per week of nonfatty fish (cod, haddock, etc.). They were encouraged to consume 2 servings of fruit and 3 servings of vegetables or salad per day. In addition, they took 15 ml of a 50/50 mixture of olive and corn oils (placebo supplement) and 400 IU of vitamin E daily. They were also given 400 mg/day of vitamin C, and a multivitamin preparation containing minimum adult requirements of thiamine, niacin, folate, and vitamin B12. The total caloric intake was adjusted at 2-week intervals by the dietician to maintain stable body weight.

Prior to week 7, participants were asked to select four fatty-fish items (including fresh, smoked, and canned salmon, herring, mackerel, anchovies, and sardines) that would be provided to them each week for the next 8 weeks of study. Dietary guidelines for the study were reviewed, including provision of lists of nonfatty fish and foods containing trans-fatty acids to avoid during this period. Subjects were asked to use only olive oil for preparing foods and salad dressings and to consume 1 tablespoon of fish oil per day. From week 7 to 14 (8 weeks), subjects were switched to the experimental diet, which also contained 30% of calories as triglycerides, of which 30% were polyunsaturated (walnut, flax, or canola oil high in ω-3 PUFA), 40% monounsaturated (olive oil), and 30% saturated (butter, animal fats). Carbohydrate and protein content were the same as in the standard diet, with the difference that subjects consumed fatty (salmon, tuna, sardines, etc.), rather than nonfatty fish. In addition, subjects took 15 ml per day of deodorized, stabilized, sardine oil (donated by Cardinova International, Uppsala, Sweden) equiva-

lent to 4–5 g of the essential ω-3 PUFA's, EPA and DHA (analysis supplied by the manufacturer) and the same supplemental vitamins as in the standard diet.

Random 24-hour recalls were conducted weekly throughout the study. Prior to week 10, instructions on completing a 3-day diet record were again given.

Testing procedures

During the 6th week (control diet), and the 14th week (experimental diet), we performed endocrine and other laboratory tests. These included the collection of fasting blood samples for measurement of serum glucose, hormones and cytokines, lipid profiles, plasma free fatty acids (FFA), and red cell membrane fatty acid composition. Standard clinical laboratory profile (SMA-24, CBC), and TSH measurements were also obtained. Endocrine provocative and special testing was carried out over a three day period, and consisted of: ACTH stimulation test [32] with measurement of both cortisol and dehydroepiandrosterone (DHEA) responses; glucagon stimulation test [33–35] for glucose response; a human chorionic gonadotropin (hCG) stimulation test [36–38]; combined gonadotropin releasing hormone (GnRH) and growth hormone releasing hormone (GHRH) pituitary stimulation test [4, 11], as well as low dose graded incremental isoproterenol infusion test [39] with measurements of free fatty acid response. The hCG (Organon, Roseland, NJ) was given as a subcutaneous injection of 5000 units and serum testosterone (T) responses were evaluated at 24 and 48 hours. GnRH (Abbott Laboratories, Chicago, IL) and GHRH (Eli Lilly & Co., Indianapolis, IN) clinical reagents were administered as single bolus intravenous doses of, respectively, 1 μg and 0.5 μg/kg, with blood sampled for luteinizing hormone (LH), follicle stimulating hormone (FSH) and growth hormone (GH) levels at 0, 15, 30, 60, and 120 minutes. We also assessed insulin sensitivity/glucose utilization using the octreotide insulin suppression test [40]. In order to estimate the time interval for cell membrane ω-3 PUFA's to return to baseline after the experimental diet was discontinued, red blood cell (RBC) membrane fatty acid profiles were also measured at weeks 16, 18, and 20.

Laboratory methods

Blood was drawn from fasting subjects, immediately processed, and stored at −80 °C until analyzed. All analyses were performed at Kronos Science Laboratories, Inc. (Phoenix, AZ), a CLIA certified specialty clinical reference laboratory. Complete Blood Counts (CBC) were performed on a GEN-S automated analyzer (Beckman Coulter, Miami, FL) and routine clinical laboratory measures (clinical chemistries, lipid profiles, and FFA) were carried out by standard methods using a SYNCHRON LX-20 automated analyzer (Beckman Coulter, Fullerton, CA). Hormones were analyzed by immunofluorescence methods, performed on an automated Immulite 2000 (Diagnostics Products Corporation, Los Angeles, CA). In order to reduce error due to day-to-day interassay variance, serum samples from individual subjects after control and experimental diet periods were assayed at the same time. Minimum detectible doses (MDD) and intra-assay coefficients of variance were as follows: serum TSH (MDD = 0.004 IU/ml, CV < 4.7 %), insulin (MDD = 2 IU/ml, CVs < 4.5 %), testosterone (MDD = 15 ng/dl, CVs < 6.4 %), estradiol (MDD = 15 pg/ml, CVs < 5.9 %), hGH (MDD = 0.01 ng/ml, CVs < 3.8 %), IGF-1 (MDD = 20 ng/ml, CVs < 4.5 %), DHEA-sulfate (MDD = 3 mg/dl, CVs < 5.3 %), cortisol (MDD = 0.2 μg/dl, CVs < 5.9 %), and hs-CRP (MDD = 0.01 mg/dl, CVs < 6.0 %). Serum estrone (E1) was measured by radioimmunoassay (Diagnostic Systems Laboratories, Inc., Webster, TX) with MDD = 1.2 pg/ml and CVs < 6.5 %. Interleukin IL-6 was assayed using an in-house ELISA method with MDD = 0.625 pg/ml and CVs > 4.2 %. Determinations of RBC membrane fatty acid profiles were by gas chromatography using a variant of the method of Ohta et al. [41]. FAME Standards for 34 fatty acids were obtained from Sigma-Aldrich, St. Louis, MO and the percent of total fatty acids determined for each of the 34. Coefficients of variance for the major fatty acids were as follows: stearic acid: C18:0 (CV = 14.2 %), C18:1ω9cis (CV = 9.2 %), linoleic acid: C18:2ω6cis (CV = 10.7 %), AA, C20:4ω6 (CV = 11.2 %), EPA, C20:5ω3 (CV = 38.3 %), and DHA, C22:6ω3 (CV = 13 %). Total plasma adiponectin concentrations were determined via radioimmunoassay (Linco Research, St. Charles, MO).

Analytical and Statistical methods

Data from the 24-hour recalls were analyzed with the Diet Analyzer Program (version 5.1). utilizing the ESHA database (ESHA Research, Salem, OR). Statistical testing was carried out using JMP software (SAS Institute, Cary, NC) on a Macintosh G4 computer. All analyses for significant differences were performed for the group as a whole and separately for men and women. Two-sided tests were employed and level of statistical significance was set at 0.05, with a Bonferroni adjustment for multiple comparisons. Comparisons of baseline/nonstimulated hormones, cytokines, and other metabolic parameters measured during control and experimental diets were performed utilizing paired *t*-tests. For stimulation tests, peak and change from baseline (delta) values were identified and areas under stimulation curves (AUC) were calculated by trapezoidal integration. We compared peak, maximum delta and AUC responses generated from each set of provocative test data during control and experimental diets using repeated measures ANOVA.

Results

Table 1 shows demographic, anthropometric, and lipid data at baseline (after control diet) and after the high ω-3 diet. There was no significant change in body weight at the end of each diet period. Men weighed more than women, both before and after the experimental diet, but BMI's were not significantly different. The ratio of ω-6 to ω-3 lipids in erythrocyte membranes was dramatically lowered after the 8-week high ω-3 diet period ($p < 0.0001$), indicating substantial incorporation of ω-3 fatty acids into the RBC membranes (**Table 1**, **Fig. 1**). Fasting morning serum FFA and total, LDL, and HDL cholesterol after the high ω-3 diet period were not significantly different compared to the control diet period, for all subjects considered together. VLDL and LDL/HDL ratios (data not shown) were also unchanged. There was a significant reduction of serum triglycerides for the group as a whole and for women, but in men this reduction was not statistically significant. There was a substantial reduction in resting FFA levels of men (but not women) after the high ω-3 diet.

As shown in **Table 2**, baseline glucose, insulin, LH, FSH, GH, and cortisol were not significantly different after the high ω-3 diet period as compared to the control diet period for all subjects considered together, nor were testosterone or DHEA in men or estradiol or estrone in women. However, in both sexes considered together, we observed a statistically significant ($p < 0.008$) reduction of serum C-reactive protein following 8 weeks of high

Table 1 Unstimulated (Baseline) demographic, anthropometric and lipid values before and after high ω-3 diet (mean ± SEM)

	Men		Women		All	
Variable	**Control**	**ω-3 diet**	**Control**	**ω-3 diet**	**Control**	**ω-3 diet**
age (years)	67.5 ± 5.5	–	64.7 ± 3.3	–	66.1 ± 4.5	–
weight (kg)	85.4 ± 10.5	85.9 ± 10.2	70.1 ± 7*	68.6 ± 6.5*	77.7 ± 11.6	77.3 ± 12.2
BMI (kg/m^2)	27.2 ± 1.7	27.4 ± 1.4	25.9 ± 2.1	25.4 ± 2.2	26.6 ± 2	26.4 ± 2
RBC ω-6/ω-3 ratio	5.7 ± 1.1	3.2 ± 0.4††	5.2 ± 1.4	2.7 ± 0.5††	5.5 ± 1.2	3 ± 0.5††
total cholesterol (mol/l)	4.36 ± 0.67	4.66 ± 0.8	5.15 ± 1.03	4.95 ± 0.8	4.76 ± 0.92	4.81 ± 0.77
LDL cholesterol (mol/l)	3.04 ± 0.81	3.16 ± 0.81	3.47 ± 0.93	3.23 ± 0.69	3.26 ± 0.86	3.19 ± 0.72
HDL cholesterol (mol/l)	0.92 ± 0.11	0.98 ± 0.05	1.40 ± 0.44*	1.48 ± 0.44*	1.16 ± 0.4	1.23 ± 0.39
triglycerides (mmol/l)	1.39 ± 0.68	1.2 ± 0.68	0.9 ± 0.45	0.61 ± 0.25†	1.14 ± 0.61	0.9 ± 0.58†
free fatty acids (mg/dl)	0.70 ± 0.19	0.48 ± 0.1†	0.63 ± 0.31	0.60 ± 0.31	0.67 ± 0.24	0.54 ± 0.22

*p < 0.05 for difference men vs. women

†p < 0.05 for difference before and after ω-3 diet

††p < 0.002 for difference before and after ω-3 diet

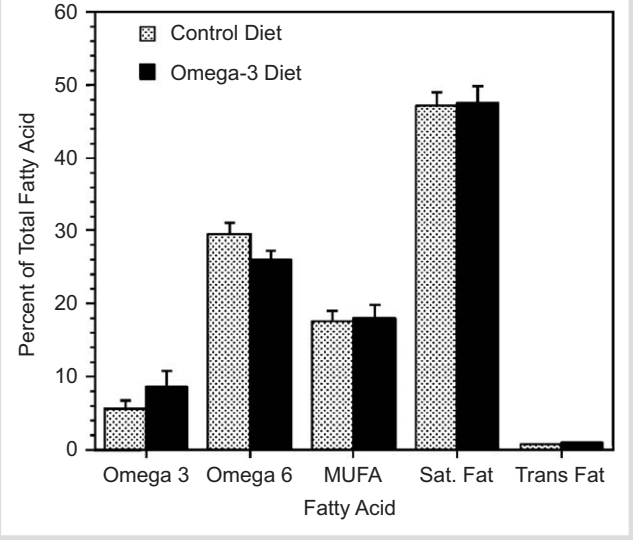

Fig. 1 Fatty acid composition of red blood cell membranes in men and women aged >60 years before and after high ω-3 diet. Mean (± SEM) values for percentages of total fatty acids represented by ω-3 and ω-6 polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids, and trans-fatty acids measured in extracts of isolated RBC membranes from 12 men and women over age 60 years after 6 weeks of a control diet (stippled bars) and 8 weeks of a diet with high ω-3 fatty acid content (black bars).

ω-3 intake (**Table 2**). We also observed a statistically nonsignificant, increase in adiponectin levels ($p < 0.18$), and a nearly significant trend towards a decrease of IL-6 ($p = 0.057$) after the high ω-3 diet.

As shown in **Table 3** and **Fig. 2**, mean serum glucose levels during the last 30 min of the octreotide test were significantly lower ($p = 0.03$) after the high ω-3 diet. Luteinizing hormone and FSH responses to GnRH, cortisol and DHEA responses to ACTH, hGH response to GnRH, testosterone response to hCG, and glucose response to glucagon were not significantly altered after 8 weeks on a the high ω-3 diet (**Table 3**). The serum FFA levels in response to graded isoproterenol doses appeared to be lower after high ω-3 diet at all isoproterenol doses and time points, but this difference did not reach statistical significance (**Table 3, Fig. 3**).

Discussion

Changes in hormone levels and responsiveness of hormone target cells are well-documented concomitants of normal aging in laboratory animals and humans. Serum testosterone concentrations decrease with age in men, as does the testosterone secretory response to gonadotropins [4]. Integrated 24-hour growth hormone (GH) secretion and blood levels of IGF-I also diminish with age in males and females [6–9] along with the pituitary GH

Table 2 Baseline hormone, adiponectin, and cytokine values after control and experimental diets

	Control diet		Omega-3 diet		
Measurement (Basal)	**Mean**	**SD**	**Mean**	**SD**	**p**
glucose mmol/l	5.10	0.51	5.27	0.58	ns
insulin pmol/l	70.8	53.5	72.9	34.0	ns
LH IU/l (men)	2.4	1.4	3.2	1.4	ns
FSH IU/l (men)	9.3	5.4	10.8	6.9	ns
LH IU/l (women)	15.9	3.8	16.6	8.6	ns
FSH IU/l (women)	66.1	7.8	64.8	21.7	ns
GH IU/l	0.22	0.19	0.28	0.48	ns
cortisol nmol/l	300.7	110.4	314.5	102.1	ns
DHEA nmol/l (men)	1.34	0.56	1.40	0.55	ns
DHEA nmol/l (women)	0.80	0.42	0.77	0.27	ns
testosterone nmol/l (men only)	14.7	4.55	14.23	4.6	ns
estradiol pmol/l (women only)	78.9	13.6	73.42	1.84	ns
estrone pmol/l (women only)	59.9	27	62.9	41.8	ns
CRP (mg/dl)	0.203	0.092	0.073	0.073	0.008
IL-6 (pg/ml)	1.375	0.942	0.833	0.227	0.057
adiponectin (units)	11.9	7.27	12.6	7.24	0.18

Table 3 Responses to endocrine provocative testing in men and women before and after high ω-3 diet

Test response	Control	ω-3 Diet	p
LH response to GnRH (AUC, [IU/l] * min)	7017 ± 4795	6881 ± 4755	ns
FSH response to GnRH (AUC, [IU/l] * min)	8929 ± 6282	8684 ± 6068	ns
cortisol response to ACTH (Δ, nmol/l)	672 ± 163	676 ± 174	ns
DHEA response to ACTH (Δ, nmol/l)	8.3 ± 4.2	6.9 ± 5.6	ns
GH response to GHRH (AUC, [IU/l] * min)	513 ± 297	596 ± 339	ns
glucose response to Glucagon (max, mmol/l)	8.21 ± 1.39	8.21 ± 1.22	ns
testosterone response to hCG (max, nmol/l)	29.9 ± 6.7	31.6 ± 3.1	ns
FFA response to isoproterenol (max, mg/dl)	0.87 ± 0.20	0.78 ± 0.30	0.16
steady state glucose-OIS test (mmol/l)	7.59 ± 3.54	6.19 ± 3.51	0.03

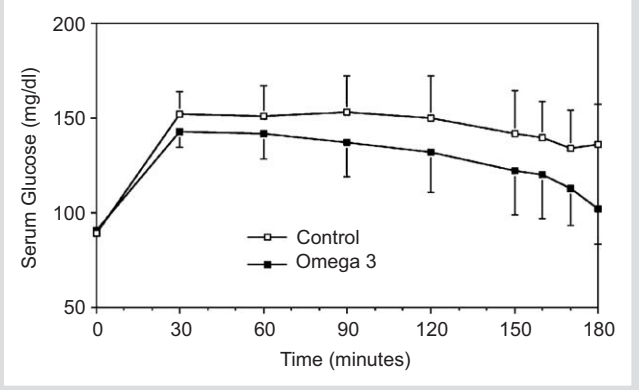

Fig. 2 Serum glucose during octreotide-insulin suppression tests in subjects aged >60 years before and after high ω-3 diet. Mean values (± SEM) are depicted for plasma glucose at time points from baseline to 180 minutes in 12 men and women over 60 years of age during simultaneous constant intravenous infusion of glucose (240 mg/m^2 BSA/min) and insulin (25 mU/m^2 BSA/min) with suppression of endogenous insulin production by a somatostatin analogue (Sandostatin®) 125 μg bolus followed by 0.5 μg/min infusion. Tests were conducted after 6 weeks of a control diet (unfilled squares) and 8 weeks of a diet with high ω-3 fatty acid content (filled squares).

response to various stimuli. Altered metabolic responses of liver, muscle, and adipose tissue to glucagon, insulin, and beta-adrenergic stimuli have all been described in older humans [13–16]. A possible common factor explaining decreased hormone secretion and action with aging is diminished transmembrane signal transduction. However, investigations of reduced hormonal responsiveness of aged cells have generally failed to reveal significant alterations in numbers or chemical integrity of membrane receptors sufficient to account for the magnitude of the effects of aging on function [17–20]. In addition, post-receptor cascades usually appear to be intact when stimulated by alternative pathways not requiring transmembrane signaling [21]. Another possibility is that the attenuation of signal transduction with aging could be related to changes in the membrane itself, which is to say the milieu in which membrane receptors must operate.

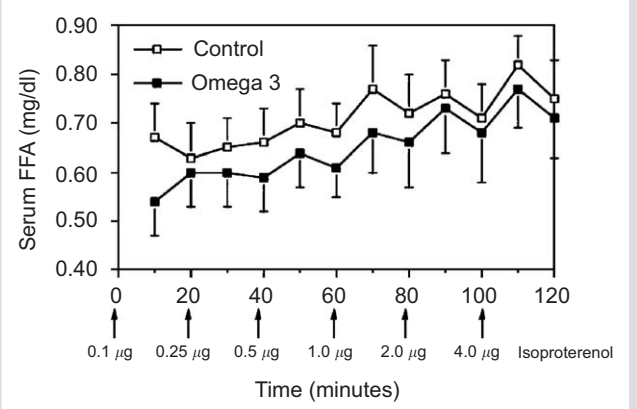

Fig. 3 Fatty acid responses to beta-adrenergic stimulation in men and women aged >60 years before and after a diet with high ω-3 fatty acid content. Mean values (± SEM) for total free fatty acids (FFA) are shown at baseline and 10 and 20 minutes after graded intravenous bolus injections of isoproterenol at six doses ranging from 0.1 μg to 4.0 μg. Tests were conducted after 6 weeks of a control diet (unfilled squares) and 8 weeks of a diet with high ω-3 fatty acid content (filled squares).

It has been shown that aging is associated with reductions in polyunsaturated fatty acid (PUFA) content of phospholipids [42] and accumulation of peroxidized and cross-linked fatty acids, trans-fatty acids, and cholesterol esters in biological membranes [43,44]. These alterations lead to a loss of membrane fluidity [43,45,46]. Cell membrane fluidity can be improved by increasing dietary PUFA [45,47], and changes in the fatty acid content of cell membranes can be achieved fairly rapidly (4–8 weeks) by altering dietary fat intake [27–29]. In old rats, substitution of corn oil, which is high in ω-6 (PUFA), for 12 weeks, resulted in near-normalization of the glycogenolytic response of liver cells to glucagon and of the lipolytic response of fat cells to beta-adrenergic stimulation [48]. Membrane transduction of adrenergic signaling in parotid gland cells from old rats improved when membrane fluidity was increased using *S*-adenosylmethionine [26]. These results suggest that manipulation of membrane chemistry might reverse age-related changes in receptor function. Our review of the literature revealed no prior studies utilizing controlled protocols to investigate the effects of a diet high in ω-3 PUFA on endocrine regulation in aging humans.

Despite the above reports, we observed no effects of 8 weeks of high ω-3 intake on resting hormone levels in men and women over 60 years of age and our provocative test results showed no changes in responses of endocrine target cells to ACTH, glucagon, GnRH, GHRH, or hCG. Although our data indicate that for erythrocytes, cells with a high turnover rate, 8 weeks of a high ω-3 diet was sufficient to dramatically reduce cell membraneω-6 to ω-3 ratios, 8 weeks may or may not be adequate to substantially alter the membrane composition of cells with lower turnover rates, such as endocrine secretory cells, hepatocytes, etc.

We observed one notable exception to the general lack of altered hormone responsiveness: the insulin sensitivity, as measured by the octreotide/insulin suppression test, improved significantly. The trend towards lower FFA mobilization in response to graded doses of isoproterenol points to one possible mechanism for this effect, since high FFA levels are associated with reduced insulin sensitivity [49,50]. We suggest that a decrease in ω-6 to ω-3

fatty acid ratios in cell membranes of adipocytes may modulate FFA mobilization response to -adrenergic stimuli. Another possible contributor is the reduction in inflammatory factors and/or increased adiponectin (see below). Studies in rats and mice have also found that ω-3 fatty acids improve insulin action in liver and muscle by reducing intracellular fat (long chain CoA and diacylglycerol) content, which occurs via a PPAR-dependent mechanism [51,52]. Such an alternative or complementary mechanism could have contributed to the observed improvement in insulin sensitivity.

Fish oil feeding of diabetic rats has been shown to produce lower blood sugar levels and higher myocyte membrane content of Glut-4 glucose transporters [53] as well as increased insulin induced glucose transport into muscle [54]. Population studies have also suggested that a diet high in ω-3 PUFA may improve glucose tolerance in nondiabetics [24], especially in elderly persons [25]. However, previous experimental studies in humans have not shown similar effects [55–59]. Differences in study design may account for the positive finding in our study. First, in prior studies the ω-3 fat was given as a supplement without attempts to control the diet, while in our study the total number of calories was maintained at isocaloric levels and diet implementation was reinforced by dietary diaries and by calling the subject on a bi-weekly basis to review compliance. The success of this approach is indicated by the maintenance of stable weight by all subjects. Second, diets were designed to provide low intake of saturated fatty acids, both during the control and the high ω-3 phases. Third, the bulk of fatty acids other than the supplement were given in the form of MUFA during both phases and during the ω-3 phase there was a substitution of ω-3 for ω-6 fats, with the total content of PUFA remaining stable. Finally, the amount of ω-3 fats (supplement plus fatty fish approximating 720 g per week) was much higher than the amounts used in previous studies. New studies examining ω-3 effects on adipocyte membrane composition, FFA mobilization, and insulin/glucose metabolism in a greater number of subjects, and, perhaps, with greater duration of high ω-3 intake, appear justified.

The serum lipid changes in our healthy elderly patients were similar to those observed in diabetics treated with high ω-3 diets [55,58]. It is of interest that the decline of FFA was more pronounced in men, whereas, the triglyceride reduction was more prominent in women.

We also observed changes in inflammatory markers after high ω-3 intake. There was a highly significant decrease in C-reactive protein and a borderline but nonsignificant reduction in IL-6. In addition, levels of adiponectin, a factor associated with reduced inflammation and improved insulin sensitivity, trended higher but did not reach statistical significance. These findings are consistent with clinical and epidemiological studies [60–62] reporting evidence of anti-inflammatory effects of high ω-3 diets and/or omega-3 supplements. The ω-3 fats are key components of the Japanese and Mediterranean diets, and are believed to contribute to the low heart disease rates in those regions in part by decreasing endothelial inflammation, as evidenced by decreasing C-reactive protein and, possibly, IL-6 [63–65].

In summary, our data indicate that diets sufficiently high in ω-3 fats cause substantial change in red cell membrane lipid composition and within 8 weeks observable reductions in certain inflammatory markers and an improvement in insulin sensitivity, but no other significant changes in hormonal or metabolic parameters known to be affected by aging. The sample size and duration of the study have not been large enough to detect small and/or highly variable changes in the parameters studied. The main purpose of this study was to identify physiological variables likely to justify more detailed investigation. Studies with larger number of subjects and of longer duration will be required to further investigate the effects of ω-3 intake on hormone and metabolic functions in the elderly.

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