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Individual Differences in the Impact of Acute Psychological Stress on Plasma Fatty Acids

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Résumé

Le stress psychologique altèrerait les niveaux d'acides gras (AG), mais nous en savons peu sur les AG les plus affectés et sur l'influence des différences individuelles. *Objectif:* Cette étude vise à évaluer l'impact immédiat d'un protocole de stress psychologique sur le profil d'AG plasmatiques d'adultes en santé et à déterminer si des différences existent en fonction de l'âge, du sexe et du niveau de base en Omega-3 des participants. *Méthodes:* 182 hommes et femmes en santé (41.43±11.47 ans) ont été exposé à un protocole de stress standardisé en laboratoire composé de quatre stresseurs interpersonnels. Le profil d'AG des participants a été extrait du plasma de deux prises de sang, l'une lors d'une période de repos et l'autre immédiatement après le protocole de stress. Des régressions linéaires hiérarchiques ont été effectuées sur les scores de changement (différence entre les valeurs post-stress et pré-stress) et incluaient des interactions, introduites «pas-à-pas», en contrôlant pour les covariables potentielles. *Résultats:* Le protocole de stress a mené à des changements significatifs au niveau du profil d'AG. L'acide oléique a augmenté post-stress dans l'ensemble de l'échantillon (p<.05). Les changements observés pour les autres AG étaient influencées par des caractéristiques individuelles des participants. Les niveaux basal en Omega-3 étaient modérateur des changements en Omega-3 (β=-.387), AG saturés (β=.276), AG polyinsaturés (β=-.243), and le ratio Omega-6/Omega-3 (β=.384). L'âge était modérateur pour les changements en Omega-3 (β=.202), AG saturés (β=-.210) et pour le ratio Omega-6/Omega-3 (β=-.191) (tous à *ps*<.05). Une interaction Sexe par Age était significative pour les variables Omega-6 (β= -0.160) et AG polyinsaturés (β=-0.146), indiquant de grandes augmentations engendrées par le stress chez les hommes plus âgés et les femmes plus jeunes. *Conclusion:* Le stress psychologique a mené à des changements significatifs sur le profil d'AG, mais ceux-ci étaient influencés par les caractéristiques individuelles des participants. De manière général, le profil d'AG suggère davantage de risque cardiovasculaire chez les individus âgés, plus particulièrement chez les hommes et chez ceux qui consomment moins d'Omega-3.

Mots-clés : stress, acides gras, Omega-3, âge, sexe, D.psy

Abstract

Psychological stress may compromise fatty acids (FAs) levels. Which FAs are most targeted by stress and whether individual differences exist has received little attention. *Objective:* The current study sought to evaluate the immediate impact of exposure to psychological stress on the plasma FAs profile of healthy adults, and to determine whether these responses differ according to age, sex, and participant's basal Omega-3 values. *Methods:* 182 healthy men and women (41.43±11.47 yrs) were exposed to a standardized laboratory stress protocol involving four interpersonal stressors. The FAs profile of participants was obtained from plasma in blood drawn at rest and immediately post-stress. Hierarchical linear regressions on change scores (post-stress values minus baseline) included interaction terms entered stepwise and controlled for potential confounders. *Results:* The stress protocol led to significant changes in the FAs profile. Oleic acid increased post-stress in the overall sample (p<.05). Changes in other FAs were influenced by individual characteristics. Basal Omega-3 moderated change in Omega-3 (β=-.387), saturated FAs (β=.276), polyunsaturated FAs (β=-.243) and the Omega-6/Omega-3 ratio (β=.384). Age moderated change in Omega-3 (β=.202), saturated FAs (β=-.210) and Omega-6/Omega-3 (β=-.191)(all ps<.05). Significant Sex by Age interactions for Omega-6 (β= -0.160) and polyunsaturated FAs (β=-0.146), revealed particularly large stress-related increases in these parameters among older men and, to a lesser degree, younger women. *Conclusion:* Psychological stress led to significant changes in FAs profile, though the impact was influenced by individual characteristics. Overall, the FAs profiles were suggestive of greater cardiovascular risk among older individuals, especially men, and among those who consume less Omega-3.

Keywords : stress, fatty acids, Omega-3, age, sex, D.psy

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AA: Arachidonic acid

ALA: Alpha-linolenic acid

BP : Blood pressure

CAD : Coronary artery disease

CRP : C-reactive protein

DHA : Decosahexaenoic acid

ECG : Electrocardiogram

EPA : Eicosapentaenoic acid

FA : Fatty acid

GC-FID : Gas chromatograph-Flame ionisation detector

HF : High frequency

HR : Heart rate

HRV : Heart rate variability

IL : Interleukin

LF : Low-frequency

LOG: Logarithm

SQRT : Square root

TNF-α : Tumor necrosis factor-alpha

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Individual Differences in the Impact of Acute Psychological Stress on Plasma Fatty Acids

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Introduction

Chronic psychological stress has been associated with coronary artery disease (CAD) development and progression independently of traditional risk factors (Backe, Seidler, Latza, Rossnagel, & Schumann, 2012; Richardson et al., 2012; Steptoe & Kivimaki, 2012, 2013). Acute stress (including man-made or natural disasters) also increases risk for coronary events in vulnerable individuals (Aoki et al., 2012; Chida & Steptoe, 2010; Holman et al., 2008; Stalnikowicz & Tsafrir, 2002; Steptoe & Kivimaki, 2012). While the mechanisms underlying the relation between stress and CAD remain unclear, physiological and behavioural correlates of stress are likely involved. This paper will focus specifically on the potential role of stress on fatty acid (FAs) profile, which has been linked to CAD risk.

FAs are important constituents of lipids, and consist of carbon chains of variable length, differing in the extent to which they have one (monounsaturated), two or more (polyunsaturated) or no double bonds (saturated) between two carbon atoms (see table 1). The configuration of the double bond (*cis* or *trans*) may further differ (Tvrzicka, Kremmyda, Stankova, & Zak, 2011). *Trans* FAs have been reported to increase CAD risk via adverse effects on serum lipids (Mensink, Zock, Kester, & Katan, 2003), endothelial function (Lopez-Garcia et al., 2005) and inflammation (Dariush Mozaffarian, Katan, Ascherio, Stampfer, & Willet, 2006). In contrast, monounsaturated (e.g. oleic acid) and especially polyunsaturated FAs (e.g. Omega-3 and Omega-6) have been shown to reduce CAD risk (Deckelbaum & Calder, 2010; Delgado-Lista, Perez-Martinez, Lopez-Miranda, & Perez-Jimenez, 2012; Farvid et al., 2014; Schwingshackl & Hoffmann, 2012). For example, a meta-analysis of 8 randomized controlled trials that examined the impact of increased consumption of polyunsaturated FAs found that for every 5% increase in energy consumed from polyunsaturated FAs, there was a corresponding 10% reduction in risk for CAD events (Mozaffarian, Micha, & Wallace, 2010). The Omega-6/Omega-3 ratio has also been proposed as an important factor to consider in primary and secondary CAD prevention, with higher Omega-6/Omega-3 ratios associated with greater pro-inflammatory eicosanoids production (Nicholson, Khademi, & Moghadasian, 2013).

Table 1.

FAs classification and nomenclature (Tvrzicka et al., 2011)

Name	Type	Nomenclature
Palmitic acid	Saturated	16:0
Stearic acid	Saturated	18:0
Oleic acid	Monounsaturated	18:1 n-9
Omega-3		
Alpha-linolenic acid (ALA)*	Polyunsaturated ω-3	18:3 n-3
Eicosapentaenoic acid (EPA)	Polyunsaturated ω-3	20:5 n-3
Docosahexaeonic acid (DHA)	Polyunsaturated ω-3	22:6 n-3
Omega-6		
Linoleic acid (LA)*	Polyunsaturated ω-6	18:2 n-6
Gamma-linolenic acid	Polyunsaturated ω-6	18:3 n-6
Dihomo-gamma-linolenic acid	Polyunsaturated ω-6	20:3 n-6
Arachidonic acid (AA)	Polyunsaturated ω-6	20:4 n-6

Note.*Essential fatty acid precursor for each category.

The association between **acute** stress and **total** plasma free FAs content has been the subject of numerous studies in humans. Overall, these investigations have noted an increase in total free FAs content ranging from 5 to 150% (*mean increase of 54% ± 44),* during or after exposure to acute stress (type, number, and duration of stressor was variable across studies) compared to baseline (for systematic reviews, see (Dimsdale & Herd, 1982; Niaura, Stoney, & Herbert, 1992).

However, the impact of acute stress on individual FAs categories remains to be examined. This appears a major lacuna given that different FAs have different implications for health, and because several studies in humans and in animals suggest that FAs are released from adipose tissues in a differential manner (Lafontan & Langin, 2009; Leclerf, 2009). FAs may be released according to their molecular structure; FAs with longer carbon chains appear to be mobilized (i.e. released into circulation from fat storage) to a lesser extent than those with shorter chains, and polyunsaturated FAs (particularly C20:5 n-3 and C20:4 n-6) are mobilized to a greater extent than monounsaturated and saturated FAs. Further, in an *in vivo* study (Connor, Lin, & Colvis, 1996) performed in rabbits, it was observed that polyunsaturated FAs are mobilized to a greater extent than other FAs by ACTH, a hormone involved in the neuroendocrine stress response. It has thus been suggested that FAs may be

differentially released during an acute stress response and that FAs generally associated with a better cardiovascular health may be released in priority. Hibbeln and Salem (1995) hypothesized that recurrent or chronic stress could thus lead to degradation of reserves of these protective polyunsaturated FAs.

To our knowledge, only one study has examined the association of (chronic) stress with individual FAs in humans. The circulating FAs profile of 19 medical students (16 in the second year) and 20 laboratory workers was measured over a 2-year period (Williams, Kiecolt-Glaser, Horrocks, Hillhouse, & Glaser, 1992). Because of their challenging school program, students were considered to experience greater psychological stress. Levels of some Omega-6's (especially C18:2 n-6) were significantly lower among students than controls across both years, while monounsaturated C16:1 was higher in students than in controls during the examination periods in Year 2. However, whether students were actually more stressed than lab workers was not measured, though they were more generally distressed. Lifestyle differences between the two groups were not controlled for in analyses, and whether participants were in fasting state for the blood draw was not specified, yet these may have a significant impact on FAs metabolism and levels (Hillbrand & Spitz, 1997). Moreover, FAs content in the two groups was measured at different times and the implication of this on results was not addressed.

Thus, while stress appears to have an impact on total plasma free FAs content, only one study in humans, performed more than 20 years ago, has measured the impact of chronic (but not acute) stress on individual FAs content. Whether individual differences may further influence the impact of stress on FAs content is currently unknown. However, there is reason to believe that this may be the case. Sex/gender differences have been reported in psychological and physiological responses to stress (Cohen & Janicki-Deverts, 2012; Kudielka & Kirschbaum, 2005), as well as in tonic levels of FAs (Lohner, Fekete, Marosvölgyi, & Desci, 2013). For instance, studies have found a higher contribution of polyunsaturated fats, especially Omega-3, to total lipids in women than in men (Decsi & Kennedy, 2011; Lohner et al., 2013). Age differences in stress exposure and reactivity have similarly been described (Stawski, Sliwinski, Almeida, & Smyth, 2008; Uchino, Birmingham, & Berg, 2010). Individual differences in basal levels of Omega-3 may also influence FAs response to stress. Indeed, two experimental studies showed that Omega-3 supplementation reduced

cardiovascular, autonomic, neuroendocrine, and free FAs responses to laboratory stress when compared to a control group who did not receive Omega-3 supplements or to their own reactions before supplementation (Carter, Schwartz, Yang, & Joyner, 2013; Delarue et al., 2003).

The objective of this study is thus to evaluate the immediate impact of exposure to a standardized laboratory stress protocol on the plasma FAs profile of healthy adults, and to determine whether these responses differ according to age, sex, and basal Omega-3 values, while controlling for potential confounders. It is hypothesized that acute psychological stress will lead to differential changes in levels of individual FAs categories. Based on the literature suggesting a differential release of FAs according to their molecular structure (Lafontan & Langin, 2009), we expect increases in polyunsaturated FAs levels in particular. Finally, we postulate that sex, age, and basal Omega-3 levels will moderate the relations between acute stress and change in FAs profile, but literature provides little information allowing to make specific assumptions regarding the nature of these influences.

Methods

This study was part of a broader prospective investigation on the psychophysiological correlates of intermediary CAD risk factors.

Participants

From 2005 to 2007, 199 healthy working adults (81 men, 118 women) aged between 20 and 64 years old (M=41, SD=11.45) were recruited using advertisements in community centers and newspapers within the greater Montreal area. Eligibility criteria were no (a) utilisation of mental health services within the past year, (b) current known health problems (e.g. hypertension, diabetes, asthma) or use of medication with the potential to alter cardiovascular, immune or neuroendocrine functions, (c) cognitive or learning impairment that can impair ability to complete the questionnaires or to understand the instructions, and (d) current use of hormone replacement therapy. To obtain a broad age distribution, participants were selected to provide three similar sized age groups (18-34 years; 35-44 years; and 45-65

years). Women were oversampled in order to include a pool of post-menopausal women (n=34), for a separate component of the overall study.

Procedure

Eligible participants were scheduled for a laboratory appointment at the Montreal Heart Institute at 8:00 a.m. during weekdays to control for circadian rhythms. They were asked to abstain from eating, drinking (other than water), smoking, and from strenuous exercise for 12h prior to testing. They were also requested not to use alcohol or recreational drugs during the 24h period before their appointment. Participants who did not respect these conditions or who showed physical symptoms of illness (e.g., cough or fever) were returned home and another appointment was scheduled. A same-sex research assistant who was trained to maintain a neutral expression and tone throughout the laboratory session was assigned to each participant.

After providing written consent, participants were equipped with physiological apparatus to monitor blood pressure and ECG for a separate component of the study (see Gentile, Dragomir, Solomon, Nigam, & D'Antono, 2015 for details). A 20-minute adaptation period during which they completed various questionnaires ensued. Participants then rested quietly during a 10-minute baseline period, after which they were exposed to 4 interpersonal stressors of 5 minutes each. Each stressor was preceded by a 5-minute autogenic relaxation procedure and a 2-minute preparation phase and was followed by a 5-minute recovery period. Blood samples were collected immediately following the baseline and the final recovery periods.

Participants received a 200 dollars compensation for time and travel. The Research and Ethics Boards of the Montreal Heart Institute approved this study.

Laboratory tasks

Tasks were chosen because they were shown to lead to significant affective and physiological reactions (al'Absi, Bongard, & Lovallo, 2000; D'Antono, Moskowitz, Miners, & Archambault, 2005). To add an additional challenge, participants were informed that they would be filmed and evaluated during each stress task.

Neutral reading task: In this first task, participants read an affectively neutral text on Antartica's geography in front of a same-sex research assistant.

Role-plays: In the second and third tasks, participants were asked to play the role of a supervisor giving feedback to an employee whose performance has been mediocre. This task had two conditions. In the first one, the script provided agreeable and constructive feedback (ex. I can see you tried hard) while the other script provided a similar number of quarrelsome comments (ex. I am not impressed by your performance). Participants were asked to enact the script as accurately as possible with the help of a confederate who played the part of the supervisee. The order in which the two scenarios were administered was counterbalanced across participants.

Debate: This final task involved engaging in a non-scripted debate about abortion. Participants were asked to adopt a partisan position and alternate between speaking and listening for 1-minute periods with the research assistant debating the opposite position. Participants began the debate, allowing them to debate actively for 3 minutes and listening for 2 minutes. A sheet containing various arguments was provided to help the participant prepare for the debate and was allowed during the debate.

In the current sample, the stress protocol led to significant changes in emotional valence and autonomic and cardiovascular activity (Lévesque, Moskowitz, Tardif, Dupuis, & D'Antono, 2010). The stress protocol lasted a total of 68 minutes. Studies have shown that a change in total FAs level can occur within 5 to 15 minutes of a stress onset (Joborn et al., 1990; Villani & Singer, 1991). The duration of the stress protocol was thus sufficient to observe a change in FAs levels.

Measures

Sociodemographic and anthropomorphic : data on sex, age, ethnicity, weight, height, marital status, income, and years of schooling were collected.

Medical variables and health behaviors: information regarding personal and family medical history, daily consumption of tobacco, caffeine and alcohol, and hours of physical activity were reported by participants.

During testing, blood was drawn using a butterfly needle inserted into the median cubital vein of the non-dominant arm. Blood was collected and placed on ice (i) in tubes

containing EDTA as anticoagulant, and (ii) in one tube containing heparin. After 30 minutes, the tubes were centrifuged for 30 minutes at 3000 X *g* at 4°C. Plasma was frozen at -80°C until assayed.

Fatty acids: Plasma were processed for quantitative profiling of fatty acids using a previously described sample preparation method, without the separation of lipid classes (Gelinas et al., 2011). Briefly, a solution of chloroform: methanol (2:1) containing 100uL of plasma was sonicated and filtered. Then, 50 µl of nonadecanoic acid (C19:0) as external standard was added. FAs were transmethylated with acetyl chloride and the hexane phase was collected and transferred in a vial (Lepage & Roy, 1986). Samples were injected on a gas chromatograph coupled to a flame ionisation detector (GC-FID) equipped with an Agilent DB-WAX polar capillary column (60m; 0.25mm ID; o.25 µm thickness). High purity hydrogen was used as a carrier gas at a constant flow of 1.9ml/minute (40°C for 7 minutes, increased by 10°C /minute until 120°C and then 1°C /minute until 180°C and 0.5°C /minute until 218°C). FAs were identified according to their retention time, and concentrations were calculated using standard curves and the external standard.

Lipids: Plasma samples were analyzed for lipid determination at the Montreal Heart Institute. These determinations were made using respective reagent Flex on the multianalyzer Dimension RxL Max (Dade Behring Diagnostics, Marburg, Germany) with heparinized plasma as simultaneously as possible following blood draw.

Data reduction: C18:2 n-3, C20:5 n-3 and C22:6 n-3 were combined to create the Omega-3 variable. For the Omega-6 variable, C18:2 n-6, C18:3 n-6, C20:3 n-6 and C20:4 n-6 were combined. The polyunsaturated FAs variable included C18:2 n-3, C20:5 n-3, C22:6 n-3, C18:2 n-6, C18:3 n-6, C20:3 n-6 and C20:4 n-6. The saturated FAs variable was created by the addition of C16:0 and C18:0. Omega-6 was divided by Omega-3 to create the Omega-6/Omega-3 ratio.

Statistical Analysis

Analyses were based on 182 participants (76 men and 106 women) for whom complete FA data were available. To normalize their distributions, a logarithm transformation (log) was applied to saturated FAs while a square root (sqrt) transformation was applied to oleic acid.

Pearson bivariate correlations were employed to examine the associations between FAs and potential covariates, including sociodemographic, behavioural (smoking, physical activity, caffeine, alcohol consumption) and physiological variables (total cholesterol). Potential covariates were chosen based on existing literature regarding stress and FAs. Variables were included as covariates in analyses if they correlated with FAs at $p<0.20$.

To assess if the laboratory stress protocol induced changes in individual FAs content, repeated measure ANOVAs were performed comparing baseline and post-stress values for the whole sample and by sex.

The main analyses involved hierarchical lineal regressions on change scores (post-stress values minus baseline). Block 1 included covariates that were determined as relevant for the different FAs, baseline level of the FA under investigation, age, baseline Omega-3, and sex. In block 2, the interactions terms sex*age, baseline Omega-3*age, sex*baseline Omega-3, sex*age*baseline Omega-3 were entered stepwise. Interaction terms were created from centered variables. When interaction terms were significant, main effects were not interpreted as per recommendations. Interactions were evaluated visually using median splits for age or basal Omega-3 level.

Analyses were performed separately for Omega-3, Omega-6, oleic acid, saturated, polyunsaturated, Omega-6/Omega3. Additional analyses, not presented here due to space considerations, were performed on C18: 2 n-3, C20:5 n-3, C22:6 n-3, C18:2 n-6, C18:3 n-6, C20:3 n-6, C20:4 n-6, C16:0 and C18:0 and different ratios (*C20:5 n-3/C22:6 n-3, C18:2 n-6/C20:4 n-6, C18:3 n-3/C22:6 n-3,* saturated/polyunsaturated*)*. These are presented in Supplemental Table 8. No significant collinearity was found.

Since preliminary analyses showed that total FAs levels did not change significantly with stress, it was deemed more parsimonious and appropriate to examine absolute rather than relative (to total) values for each parameter.

Statistical significance was set at $p<0.05$.

Results

Participant characteristics are presented in Table 2. Women were, on average, slightly older than men as we oversampled for menopausal women. Men drank more caffeine and alcohol compared to women, but also exercised more.

Table 2

Sociodemographic and Behavioral Profile of Participants By Sex

	Men (*n*=76)	Women (*n*=106)
Age (years + *SD*)**	38.71 (11.14)	43.37 (11.40)
Body mass index (kg/m^2 + *SD*)	24.78 (4.11)	25.38 (5.62)
Years of schooling (years + *SD*)	15.88 (3.39)	16.15 (3.51)
Marital status *n (%*)		
Single	38 (50)	45 (42.45)
Married/living with someone	31 (40.79)	40 (37.74)
Separated/Divroced/Witdowed	7 (9.21)	21 (19.81)
Annual family income *n* (%)		
≤ $29,999	26 (34.21)	38 (35.85)
$ 30,000-59,999	24 (31.58)	41 (38.68)
≥ 60,000	26 (34.21)	27 (25.47)
Smoker *n* (%)	12 (15.79)	26 (24.07)
Cups of coffee or tea/week *n* (*SD*)*	2.89 (6.88)	1.09 (2.54)
Glasses of alcohol/week *n* (*SD*)***	5.29 (6.17)	2.74 (4.18)
Hours of exercise/week *n* (*SD*)***	4.66 (5.33)	2.57 (3.28)
Total cholesterol (mmol/l + SD)	4.65 (0.94)	4.85 (0.92)

Notes. p<0.05*, p<0.01**, p<0.001***

Individual differences in baseline FAs levels

Men showed higher baseline concentrations of oleic acid (C18:1 n-9). Individuals with higher baseline Omega-3 values showed significantly higher concentrations across most of the other FAs as well. Age was also associated with significant elevations across total, polyunsaturated, and saturated FAs. See Table 3 for correlations between age, baseline Omega-3 and FAs.

Table 3

Correlations between basal FAs levels, age and basal Omega-3.

Basal FAs	Age	Basal Omega-3
Omega-3	r=0.328***	r=1
Omega-6	r=0.390***	r=0.370***
Oleic Acid (C18:1 n-9)	r=0.288***	r=0.306***
Polyunsaturated	r=0.420***	r=0.539***
Saturated	r=0.320***	r=0.483***
Omega-6/Omega-3	r=-0.033	r=-0.693***
Total FAs	r=0.346***	r=0.445***

Notes. p<0.05*, p<0.01**, p<0.001***

Stress-related changes in FAs levels (univariate analyses)

In the overall sample, exposure to the stress protocol led to a significant increase in oleic acid. In subgroup analyses, the Omega-6/Omega-3 ratio increased significantly in men but not women. See Table 4 for details.

Table 4

Stress-related changes in fatty acids levels for the whole sample and by sex

	Whole sample		Men		Women	
FA	Baseline (mean±SD)	Final (mean±SD)	Baseline (mean±SD)	Final (mean±SD)	Baseline (mean±SD)	Final (mean±SD)
Omega-3	690.64 ± 169.46	686.01 ± 165.90	692.23 ± 171.58	671.46 ± 163.77	689.50 ± 168.73	696.44 ± 167.41
Omega-6	4161.75 ± 751.44	4192.85 ± 792.25	4154.09 ± 855.99	4210.73 ± 912.61	4167.24 ± 670.76	4180.03 ± 697.61
Oleic Acid (C18 :1 n-9)	3015.01 ± 1279.01	3063.63 ± 1301.90 *	3291.89 ± 1497.43	3332.71 ± 1523.08	2816.50 ±1059.72	2870.70 ± 1083.87 †
Polyunsaturated	4852.39 ± 829.16	4878.86 ± 871.63	4846.31 ± 928.69	4882.19 ± 981.32	4856.75 ± 754.33	4876.47 ± 788.54
Saturated	3478.52 ± 813.32	3495.95 ± 827.03	3611.57 ± 903.66	3618.27 ± 929.34	3383.13 ± 731.43	3408.24 ± 737.26
Omega-6/Omega-3	6.29 ± 1.54	6.36 ± 1.57	6.25 ± 1.59	6.53 ± 1.73 *	6.31 ± 1.51	6.25 ± 1.44
Total FA	11345.92 ± 2678.27	11438.4 ± 2767.52	11749.80 ± 3124.89	11833.17 ± 3228.15	11056.38 ± 2277.61	11155.41 ± 2359.18

Note: Means and standard deviations (SD) are for non-transformed data for ease of interpretation. Results are expressed in umol/L.
SFA/PUFA: Saturated FAs/polyunsaturated FAs. $p<0.05$*, $p<0.01$**, $p<0.001$***, $p<0.10$ †

Moderation of stress-induced FAs changes by age, sex and baseline Omega-3 values

Table 5 contains the details of the various hierarchical regressions.

Omega-3. Significant main effects of age and baseline Omega-3 emerged. More specifically, older participants presented with an increase in plasma Omega-3 post-stress while their younger counterparts showed a decrease. Individuals with lower baseline Omega-3 plasma concentrations showed an increase in Omega-3 post-stress, whereas the opposite was observed in those with higher baseline Omega-3 levels.

Omega-6: While no significant main effects emerged, a significant Sex by Age interaction was found. Older men, and to a lesser degree younger women, showed particularly large increases in Omega-6 values post-stress. Changes were less remarkable among the other groups (see figure 1).

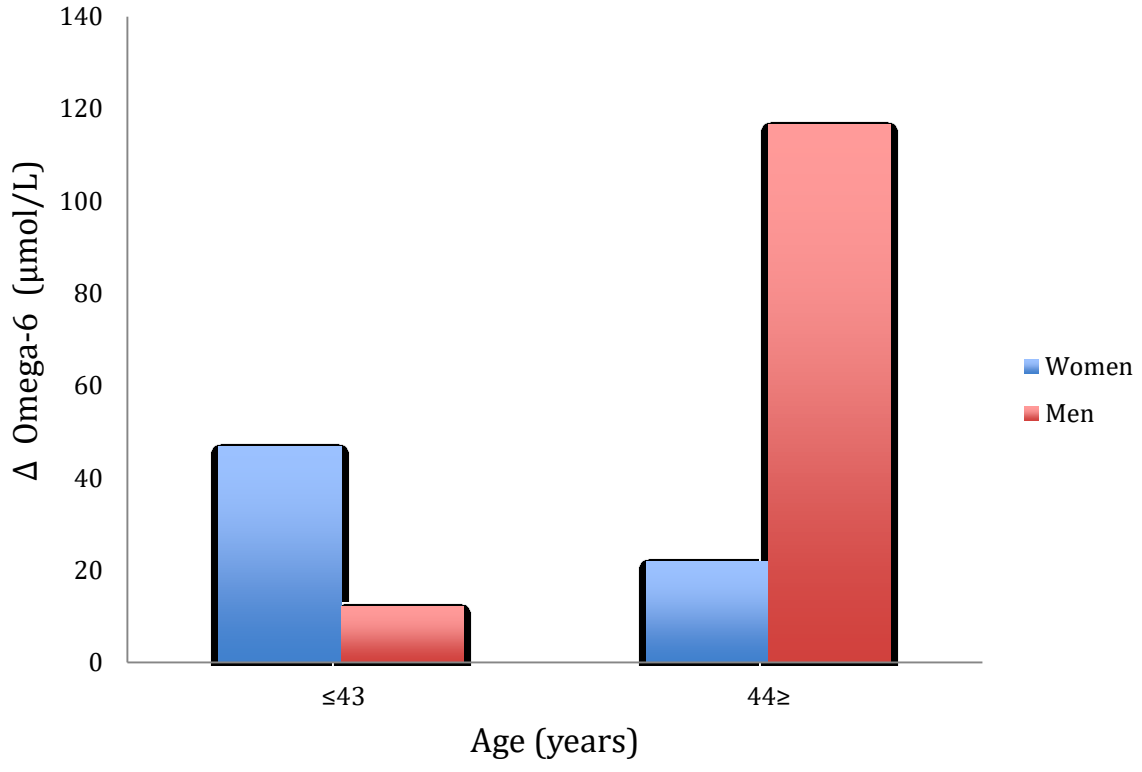

Figure 1. Mean Change in Omega-6 Following Stress is Sex and Age Dependent

Oleic Acid: No significant main or interaction effects were found.

Saturated FAs: Significant main effects of age and baseline Omega-3 emerged. Younger participants showed a slight decrease in saturated FAs post-stress, whereas older individuals showed a notable increase. Participants with higher basal Omega-3 presented with a decrease in saturated FAs post-stress, while those with lower basal Omega-3 levels showed an increase. No significant interaction effect was found.

Polyunsaturated FAs: Significant main effects of age and basal Omega-3 were found, as was a significant Sex by Age interaction. Lower basal Omega-3 was associated with large increases in polyunsaturated FAs post-stress, whereas the opposite was observed in those with higher basal Omega-3 level. Evaluation of the Sex by Age interaction showed a particularly large increase in polyunsaturated FAs post-stress among older men, and to a lesser extent younger women (see figure 2).

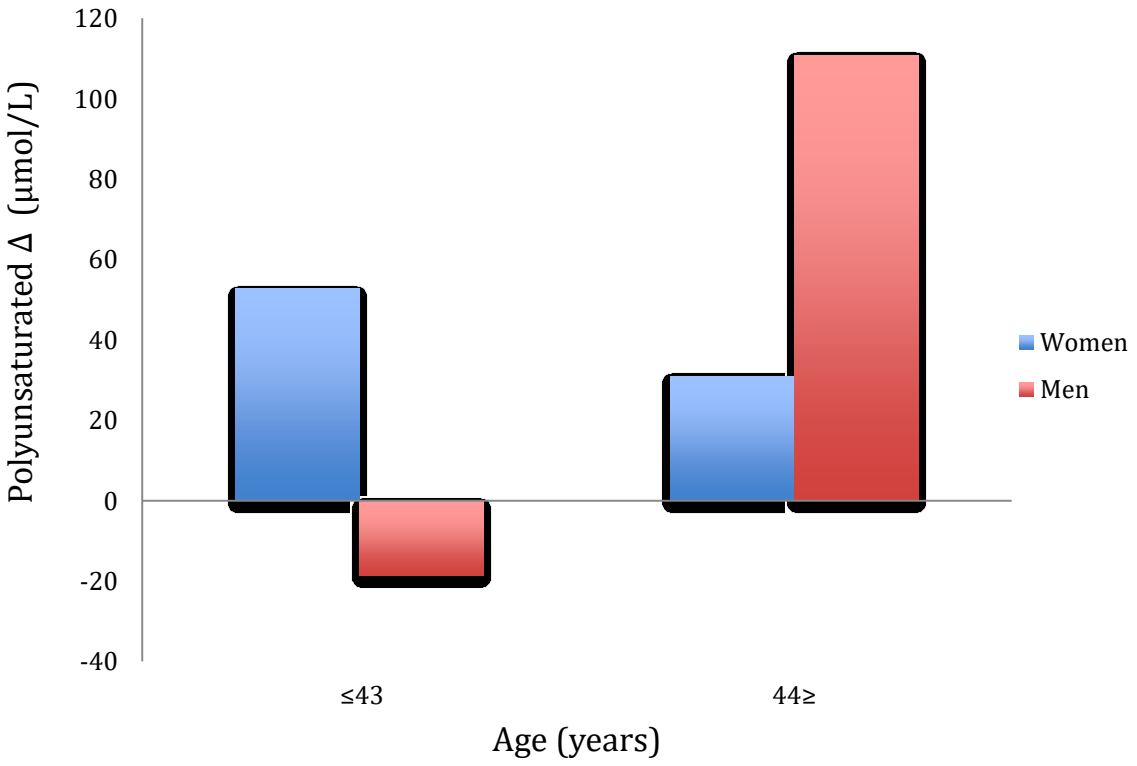

Figure 2. Mean Change in Polyunsaturated FAs Following Stress is Sex and Age Dependent

Omega-6/Omega-3: Main effects of age and basal Omega-3 were found. Younger participants showed a greater increase in the Omega-6/Omega-3 ratio than their older counterparts. Individuals with lower basal Omega-3 levels showed a decrease in the Omega-6/Omega-3 ratio while an increase occurred in those with higher basal Omega-3 levels.

Table 5

Summary of results of the hierarchical regressions

		F	df	R2	Sex	Age	Omega-3 (basal)	Significant interaction
Omega-3[2,3]	M1	7.024***	5,176	0.166	β=0.051 T= 0.715	β=0.202 T=2.702**	β=-0.387 T=-5.298***	
Omega-6[1,2,6,7]	M1	5.216***	8,173	0.194	β=-0.090 T=-1.278	β=0.036 T=0.417	β=-0.117 T=-1.534	
	M2	5.382***	9,172	0.220	β=-0.090 T=-1.278	β=0.035 T=0.417	β=-0.117 T=-1.534	**Sex*age** β=-0.160 T=-2.366*
Oleic Acid (C18 :1 n-9)[6,7]	M1	6.488***	7,174	0.207	β=-0.050 T=-0.698	β=-0.002 T=-0.019	β=-0.124 T=-1.653	
Saturated[1,7]	M1	4.169***	6,175	0.125	β=-0.022 T=-0.295	β=0.210 T=2.648**	β=-0.276 T=-3.347***	
Polyunsaturated[1,2,6,7]	M1	5.890***	8, 173	0.214	β=-0.049 T=-0.706	β=0.093 T=1.085	β=-0.243 T=-2.934**	
	M2	5.872***	9,172	0.235	β=-0.060 T=-0.874	β=0.092 T=1.082	β=-0.230 T=-2.798**	**Sex*age** β=-0.146 T=-2.172*

Omega-6/Omega-3[1,3,5]	M1	6.166***	6, 174	0.175	β=-0.100 T=-1.350	β=-0.191 T= 2.484*	β=0.384 T=-5.239***

Note. Covariates: [1]Body mass index, [2]Years of schooling, [3]Cups of coffee or tea/week, [4]Glasses of alcohol/week, [5]Hours of exercise/week, [6]Total cholesterol, [7]Cigarettes/week. M1=model 1, M2=model 2. $p<0.05$*, $p<0.01$**, $p<0.001$***, $p<0.10$ †.

Posthoc analyses

Previous literature suggests an important association between plasma FA activity and activity in other physiological systems involved in the stress response (Calder, 2009; Galli & Calder, 2009; Holm, 2003; Paolisso et al., 2000; Steptoe, Hamer, & Chida, 2007). Additional analyses were thus performed in order to evaluate whether stress-induced changes in FAs correlated with changes in other related physiological systems and whether the latter could be responsible for the pattern of results observed. We have previously reported on significant sex and/age differences in stress reactivity across cardiovascular (Gordon, Ditto, & D'Antono, 2012; Levesque et al., 2010), autonomic (D'Antono, Moskowitz, Miners, & Archambault, 2005), and inflammatory (Boisclair Demarble, Moskowitz, Tardif, & D'Antono, 2014; Girard, Tardif, Boisclair Demarble, & D'Antono, 2016) measures in this same sample. The inflammatory markers C-reactive protein (CRP), interleukin-6, and tumor necrosis factor-alpha (TNF-α) were examined as were changes in blood pressure (BP) heart-rate (HR) and autonomic (measured via high-frequency (HF) and low-frequency (LF) heart rate variability (HRV) activity. Please see Boisclair Demarble et al. (2014) and Levesque et al. (2010) for details on these various measures.

Pearson correlations between FAs and other stress responses revealed that stress-related changes in CRP were positively correlated with change scores in total FAs ($r=0.165$, $p<0.05$) and Omega-6 ($r=0.190$, $p<0.01$). HF-HRV change scores were negatively correlated with change scores in Omega-3 ($r=-0.152$, $p<0.05$) and polyunsaturated FAs ($r=-0.164$, $p<0.05$) and positively correlated with Omega-6/Omega-3 change ($r=0.155$, $p<0.05$) (see supplemental table 9 for full details).

However, while controlling for the relevant change scores in immune, cardiovascular and endocrine systems explained an additional percentage of vairiance, it did not change the significance of the results with the exception of polyunsaturated FAs for which the Sex*Age interaction was no longer significant ($\beta=-0.107$ $t=-1.543$, $p=0.125$) once the change score for HF-HRV ($\beta=-0.185$ $t=-2.653$, $p=0.09$) was added as covariate in Block 1. See supplemental table 10 for details.

Discussion

The aim of this study was to evaluate the impact of exposure to a standardized laboratory stress protocol on the plasma FAs profile of healthy adults, and to determine whether these responses differ as a function of age, sex, and basal Omega-3, while controlling for potential confounders. Results partially corroborated our hypotheses. While total FAs levels did not change significantly with stress, significant changes in oleic acid (monounsaturated FA) were observed post-stress. As expected, changes in Omega-3, Omega-6, saturated FAs, and associated ratios depended on the sex, age, and/or, basal Omega-3 levels of participants.

The fact that total FAs levels did not increase significantly post-stress is not consistent with previous literature (Dimsdale & Herd, 1982). However, this discrepancy may reflect the measure of FAs used in this versus other studies. Indeed, most studies assessed total plasma concentration of free FAs, which represent only a small fraction of FAs circulating in the blood stream. In the current investigation, we assessed total, but also individual plasma FAs, which included both free FAs and those bound to phospholipids and triglycerides (Richieri & Kleinfeld, 1995).

In contrast to previous literature on sex differences in plasma FAs, women did not show significantly greater baseline polyunsaturated FAs levels compared to men (Childs, Romeu-Nadal, Burdge, & Calder, 2008; Decsi & Kennedy, 2011), However, sex did influence the stress responses of Omega-6 and that of the combined polyunsaturated FAs variables, though this further depended on the age of our participants. More specifically, older men (and to a lesser extent younger women) showed the most notable increases in Omega 6, and polyunsaturated FAs more generally. Stress-induced changes in Omega-3, saturated FAs and Omega-6/Omega-3 ratio similarly depended on the participant's age but not on sex. Older participants showed large increases in Omega-3 and saturated FAs and a small increase in Omega-6/Omega-3 ratio following exposure to stress. Their younger counterparts, on the other hand, showed decreases in Omega-3 and saturated FAs, in addition to a larger increase in the Omega-6/Omega-3 ratio. To our knowledge, this is the first study to investigate the potential influence of sex and/or age on stress-related changes in individual FAs profile. Increases in the

FAs among older individuals may reflect a predisposition towards greater mobilization of FAs, including Omega-3, from their fat reserves into the blood stream when exposed to demanding life situations. According to Hibbeln and Salem (1995), repeated/chronic mobilization of Omega-3 in response to stress in daily life could eventually lead to depletion of Omega-3 reserves. Given the importance of polyunsaturated FAs, especially Omega-3 for cardiovascular health (Delgado-Lista et al., 2012; Farvid et al., 2014; Yokoyama et al., 2007), greater mobilization of FAs (and subsequent depletion of reserves) in older adults may represent one mechanism through which stress procures increased risk for cardiovascular diseases among older individuals. In contrast, among younger individuals, circulating levels may have been sufficient to respond to the needs invoked by stress. Mobilization of FAs resources might not have been required, leading only to decreases in their existing circulating levels.

Individual differences in acute stress responses in FAs may reflect stress-related changes occurring in other physiological systems. For instance, increased inflammatory activity of Il-6, Il-1β and C-reactive protein has been shown to occur in response to stress (Steptoe et al., 2007) though studies suggest that sex and/or age differences also exist in this response (Edwards, Burns, Ring, & Caroll, 2006; Girard et al., 2016; Steptoe, 2002). Importantly, as precursors in the synthesis of eicosanoids (molecules modulating the inflammatory response), polyunsaturated FAs are involved in the inflammatory response (Galli & Calder, 2009). More particularly, Omega-6 FAs are thought to be the substrate of pro-inflammatory molecules and Omega-3 of anti-inflammatory molecules (Calder, 2009). One study (Ferrucci et al., 2006) examined the relationship between polyunsaturated FAs and inflammatory markers in 1123 men and women aged from 20 to 98 years old and found that higher total plasma Omega-3 to be associated with lower IL-6, IL-1ra, TNF-α, and higher soluble IL-6r. Inversely, TNF-α is also thought to be an important regulator in the metabolism of lipid, notably by inducing FAs lipolysis (Chen, Xun, Chen, & Wang, 2009). The autonomic nervous system, through the effects of catecholamines, is also an important regulator of FAs liberation (Holm, 2003). Post hoc analyses in the current sample suggest that FAs, inflammatory, and autonomic activity are indeed related. More specifically, increases in systemic inflammation (as measured via CRP) in response to stress were correlated with increases in total FAs and Omega-6. Parasympathetic withdrawal in response to stress (as

measured via HRV-HF) was associated with increases in Omega-3, polyunsaturated FAs and decreases in the Omega-6/Omega-3 ratio. However, controlling for stress-induced changes in inflammatory or parasympathetic activity did not significantly alter the results. It is unlikely, then, that changes in autonomic, immune, or endocrine activity were responsible for the changes observed.

It may be argued that the results may reflect age and sex differences in body fat. Men and older individuals tend towards greater visceral fat (Kuk, Saunders, Davidson, & Ross, 2009), which has been shown to be more responsive to lipolysis (Lafontan & Langin, 2009; Nielsen, Guo, Johnson, Hensrud, & Jensen, 2004). However, BMI was introduced as a covariate in the current analyses (when relevant). It is thus unlikely that that the observed age and sex differences were related to differences in body mass.

As hypothesized, participants' basal Omega-3 levels influenced their FA response to acute stress, particularly with regards to Omega-3, Omega-6, and the saturated FAs. More specifically, lower levels of basal Omega-3 were associated with an increase (or a larger increase) in plasma FAs levels post-stress while higher levels of basal Omega-3 were either associated with a decrease in FAs or a smaller increase. For saturated FAs, a decrease was observed in participants with higher basal Omega-3, and an increase was observed for those with lower basal Omega-3. Omega-3 reserves depend mostly on diet (Tvrzicka et al., 2011). However, Omega-3 consumption is typically insufficient in the western diet (Simopoulos ,1999). While speculative, the differential FAs response to stress observed in those with low versus higher basal Omega-3 in the current study may suggest health benefits of greater Omega-3 consumption. More specifically, individuals with lower baseline Omega-3 values may require mobilization of FAs from adipose tissue into the blood stream to a greater extent compared to those with higher baseline Omega-3 values, in whom circulating levels may be sufficient to respond to the needs of the challenge. This would be consistent with the results of two prior studies reporting that a diet rich in Omega-3 reduced stress reactivity across total free FAs and other parameters in healthy young adults (Carter et al., 2013; Delarue et al., 2003). More specifically, the stress-induced increases in total free FAs was no longer observed following Omega-3 supplementation.

Increases in fatty acids in a subgroup of our participants are noteworthy. Indeed, adverse effects on cardiovascular health of acute FA elevations have been reported in several

studies (Pilz et al., 2006; Tripathy et al., 2003). For example, Pirro et al. (2002) found that elevated total free FA concentrations occurring in the context of an insulin resistance syndrome was associated with a two-fold increase in ischemic heart disease in men over a follow-up period of 5 years as compared with those with lower free FAs. Moreover, a single high fat meal, which results in increased circulating FAs, has been shown to impair endothelial function by reducing flow-mediated vasoactivity (Lacroix, Desrosiers, Tardif, & Nigam, 2012; Vogel, Correti, & Plotnick, 1997). Endothelial dysfunction can lead to the development of atherosclerosis and is predictive of future cardiovascular events (Bonetti, 2002). Higher baseline levels of Omega-3 FAs (likely resulting from differential nutritional habits) may minimize the need to mobilize additional FAs resources during psychological stress, in addition to minimizing the depletion of the fat storage in polyunsaturated FAs that have been theorised to occur following repeated stress (Hibbeln & Salem, 1995).

While interesting, several factors may limit the conclusions that can be drawn from the current results. Participants were mostly French–speaking and Caucasian, which may limit the extent to which findings can be generalized to other ethnic groups. Participants were healthy, which among the older age group, may have led to the inclusion of individuals highly resilient to stress. This may have served to underestimate the importance of stress-related changes in FAs, particularly among older individuals. Nonetheless, as observed here, and in other publications with this same sample (D'Antono, Moskowitz, & Nigam, 2013; Juster, Moskowitz, Lavoie, & D'Antono, 2013; Levesque et al., 2010), older age was, as expected, associated with greater reactivity (and cardiovascular risk) across multiple physiological systems. Of course, findings relate only to the immediate post-stress response. It is possible that repeated testing over a longer period of time, both during and following stress exposure, would have shown a different or more complex response pattern. Given the space limitations of the current article, it was not possible to examine whether psychological states or traits of the participants known to influence stress reactivity (such as depressive symptoms, personality traits, or coping strategies) also impacted the FAs response to stress. This could, however, be matter for future research.

On the other hand, to our knowledge, this is the first study to examine the impact of acute psychological stress on the individual FAs profile in humans. Compared to

previous studies, the larger sample size in this study provided greater power to evaluate individual (subgroup) differences in FA mobilization. Laboratory testing allowed a very controlled manipulation of stress exposure, and we controlled, via fasting and strict instructions regarding exercise, for several confounding variables (for example, exercise level, type and quantity of food or beverage consumed) that could have otherwise affected FA profile. The methodology was rigorous and recruitment was conducted to create a heterogeneous sample composed of healthy participants, reducing the possible confounding effect of disease on FA metabolism and stress response. Moreover, interpersonal stressors were chosen to ensure relevance for both men and women (al'Absi, Bongard, & Lovallo, 2000; D'Antono, Moskowitz, Miners, & Archambault, 2005).

Conclusion

In conclusion, acute stress influences FAs activity differentially according to individual characteristics. The stress-induced change in FAs observed in the present study seems to converge in the direction of a greater cardiovascular risk profile in older individuals, and particularly in older men. The results of this study further support recommendations to consume sufficient amounts of Omega-3 for the prevention of cardiovascular diseases. Further investigations are necessary to more fully understand the mechanisms by which different FAs change in response to psychological stress, as well as the clinical implications of these changes.

Conflict of interest statement

The authors have no conflict of interest to report.

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Secondary analysis results

Individual differences in baseline fatty acids levels

Men showed higher concentrations of C18 :3 n-6 ($F=4.353$, $p<0.05$). See table 6 for details.

Table 6

Correlations between basal fatty acids levels, age and basal Omega-3.

Basal FA	Age	Basal Omega-3
C16:0	r=0.327***	r=0.429***
C18:0	r=0.240***	r=0.587***
C18:2	r=0.371***	r=0.314***
C18:3 n-3	r=0.100†	r=0.762***
C18:3 n-6	r=0.157*	r=0.575***
C20:3 n-6	r=0.144	r=0.268***
C20:4	r=0.291***	r=0.298***
C20:5	r=0.350***	r=0.849***
C22:6	r=0.356***	r=0.804***
SFA/PUFA	r=-0.015	r=0.083
EPA/DHA	r=0.136†	r=0.317***
LA/AA	r=-0.008	r=-0.070
ALA/EPA	r=-0.336***	r=-0.298***

Notes. p<0.05*, p<0.01**, p<0.001***, p<0.10 †

Stress-related changes in fatty acid levels

In the overall sample, exposure to the stress protocol led to a significant increase in C16:0 (palmitic acid). There was also a significant decrease in ALA/EPA ratio. In subgroup analyses, men showed a significant increase in C20:4 n-6 and a significant decrease in EPA\DHA and LA\AA ratios. The precursor/product ratio decreased significantly in women. See Table 7 for details.

Table 7

Stress-related changes in fatty acid levels for the whole sample and by sex

	Whole sample			Men			Women		
Fatty acids	Baseline (mean±SD)	Final (mean±SD)		Baseline (mean)	Final (mean)		Baseline (mean)	Final (mean)	
C16:0	2438.99 ± 668.94	2468.41 ± 687.05	*	2556.09 ± 2556.09	2584.27 ± 771.43		2355.04 ± 596.69	2385.35± 609.88	†
C18:0	1039.53 ± 173.90	1027.53 ± 171.28		1055.48 ± 189.51	1034.00 ± 186.28		1028.09 ± 161.75	1022.89± 160.41	
C18:2	3220.55 ± 630.61	3244.12 ± 662.70		3185.44 ± 719.61	3226.66 ± 761.75		3245.72 ± 560. 43	3256.63± 584.97	
C18:3 n-3	266.73 ± 78.00	257.05 ± 71.73	†	278.78 ± 83.58	261.46 ± 79.70	†	258.20 ±73.00	253.92 ± 65.72	
C18:3 n-6	80.03 ± 29.43	80.89 ± 51.18		84.90 ± 31.85	81.90 ± 28.36		76.54 ± 27.19	80.17 ± 62.77	
C20:3 n-6	185.61 ± 58.19	184.48 ± 55.53		185.92 ± 56.44	186.95 ± 60.39		185.39 ± 59.68	182.71 ± 51.99	
C20:4	675.56 ± 163.23	683.36 ± 169.57		697.83 ± 153.98	715.22 ± 163.05	**	659.60 ± 168.46	660.52 ± 153.77	
C20:5	175.43 ± 66.42	174.93 ± 68.54		177.14 ± 62.81	170.67 ± 60.43		174.20 ± 69.17	177.99 ± 73.94	
C22:6	250.29 ± 69.31	255.49 ± 66.95	†	240.67 ± 68.58	242.71 ± 59.31		257.10 ± 69.33	264.53 ± 70.74	
SFA/PUFA	0.72 ± 0.11	0.72 ± 0.10		0.74 ± 0.11	0.74 ± 0.10		0.70 ± 0.10	0.70 ± 0.10	
EPA/DHA	0.707 ± 0.20	0.687 ± 0.19	†	0.75 ± 0.18	0.71 ± 0.19	**	0.68 ± 0.20	0.67 ± 0.20	
LA/AA	4.95 ± 1.18	4.91 ± 1.19	†	4.67 ± 1.01	4.61 ± 0.99	*	5.15 ± 1.25	5.13 ± 1.27	
ALA/EPA	1.27 ± 0.19	1.25 ± 0.19	**	1.29 ± 0.19	1.26 ± 0.19	†	1.26 ± 0.20	1.23 ± 0.19	*

Notes. Means and standard deviations (SD) are for non-transformed data for ease of interpretation. Results are expressed in umol/L. p<0.05*, p<0.01**, p<0.001***, p<0.10 †. SFA/PUFA: Saturated fatty acids/ polyunsaturated fatty acids; EPA/DHA: Eicosapentaenoic acid/ Docosahexaenoic acid; LA/AA: Linoleic acid/ Arachidonic acid; ALA/EPA: Alpha-linolenic/ Eicosapentaenoic acid

Moderation of stress-induced fatty acid changes by age, sex and baseline Omega-3 values

See table 8 for details.

Omega-3

C18: 3 n-3: No significant main or interaction effects were found.

C20: 5 n-3: A significant main effect of basal Omega-3 was observed. More specifically, participants with lower basal Omega-3 showed an increase while those with higher basal Omega-3 showed a decrease. No significant interaction emerged.

C22 :6 n-3 : Significant main effects of basal Omega-3 and age were observed. While both age groups showed an increase in C22: 6 n-3 post-stress, this was most notable among the older participants. Those with lower basal Omega-3 showed a large increase in this fatty acid post-stress while those with higher basal Omega-3 showed a much smaller decrease.

Omega-6

C18:2 n-6: Only a significant Sex by Age interaction emerged. All groups showed an increase in C18:2 n-6 post-stress, but this was most notable among older men (see figure 5).

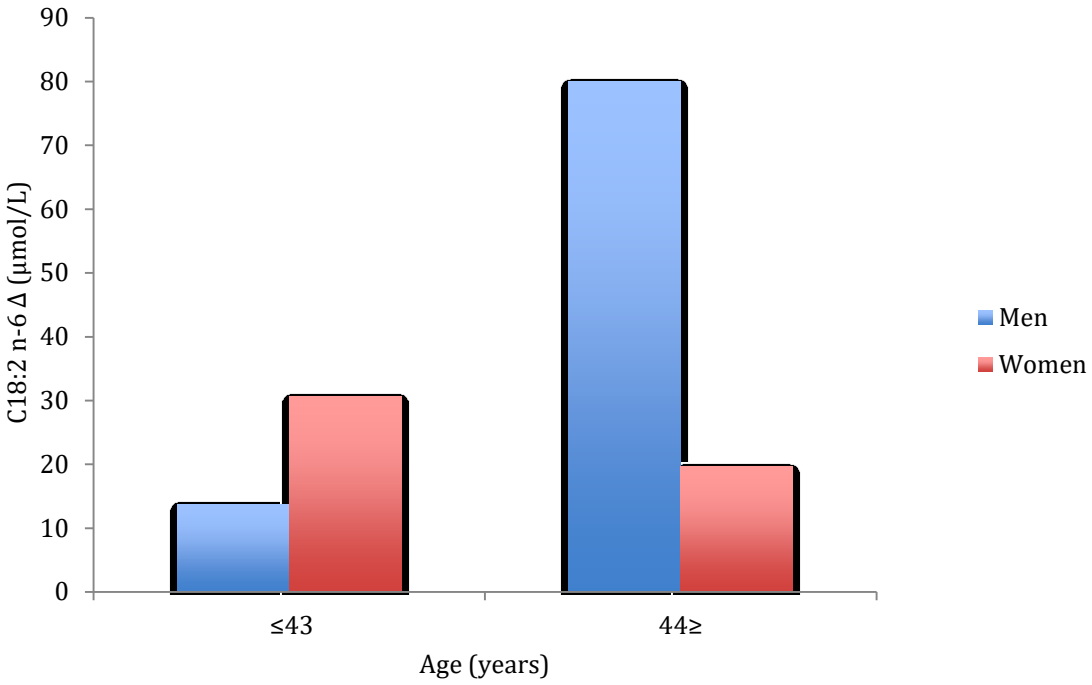

Figure 5. Change in C18:2 n-6 Following Stress is Sex and Age Dependent

C18:3 n-6: Only a significant basal Omega-3 by Age interaction emerged. Participants with a lower basal Omega-3 level presented with an increase in this fatty acid post-stress, while those with a higher basal omega-3 level showed a decrease post-stress (see figure 6). The latter was particularly notable among the younger individuals.

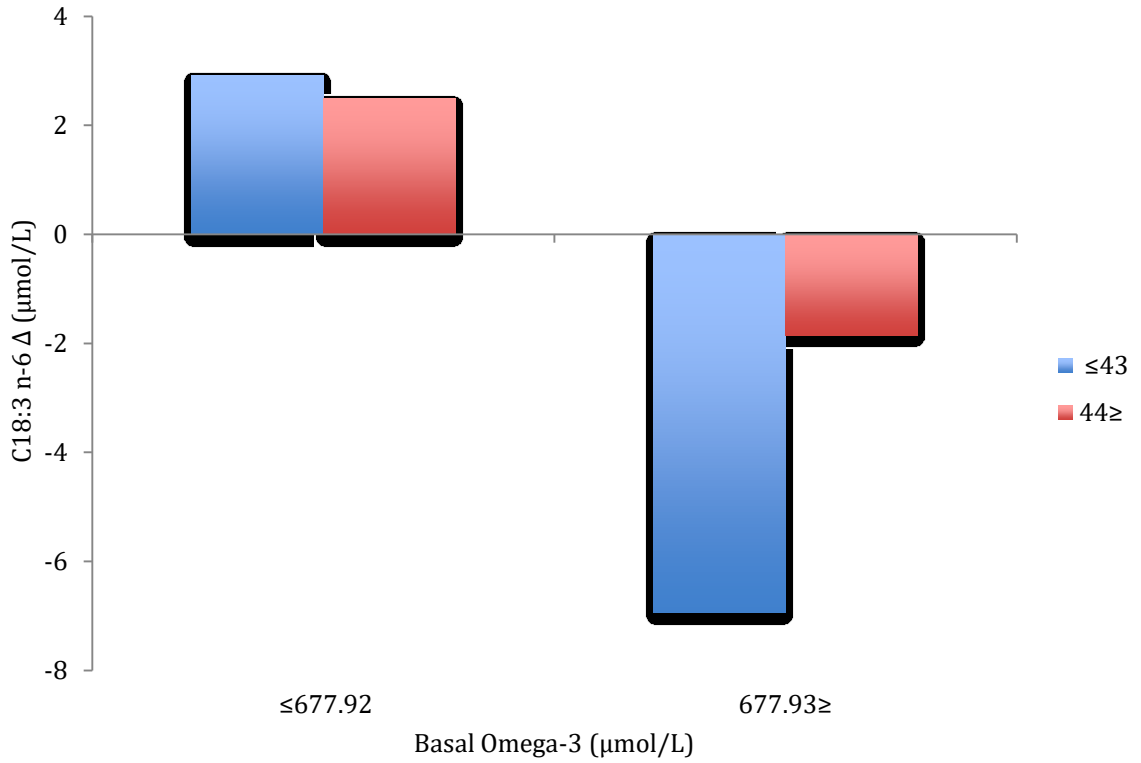

Figure 6. Change in C18:3 n-6 Following Stress is dependant on Age and Basal Omega-3 Levels

C20: 3 n-6: A main effect of basal Omega-3 levels was found. Participants with a lower basal Omega-3 level displayed a small increase, while those with higher basal Omega-3 levels showed the opposite response. No significant interaction was found.

C20: 4 n-6: A significant main effect of basal Omega-3 as well as a significant Sex by Age interaction were found. A lower basal Omega-3 level was associated with a large increase in C20: 4 n-6 post-stress compared to the small increase observed in those with a higher level of basal Omega-3. Evaluation of the interaction showed that while all groups displayed an increase in this fatty acid post-stress, the largest increase was observed in older men (see figure 7).

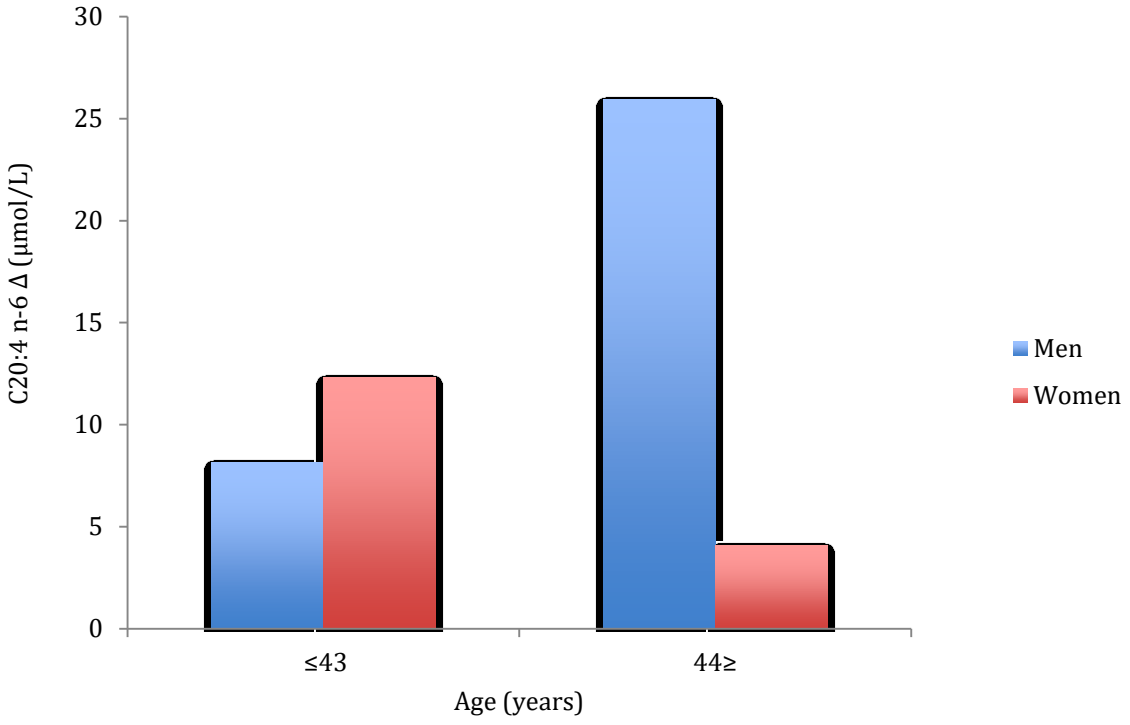

Figure 7. Change in 20:4 n-6 Following Stress is Sex and Age Dependent

Saturated Fatty Acids

C16:0: A main effect of basal Omega-3 level emerged. Participants with a lower level of basal Omega-3 showed a notable increase in C16:0 post-stress. This was not the case for those with a higher basal Omega-3 level. No significant interaction emerged.

C18:0: Significant main effects of basal Omega-3 and age emerged. Younger individuals showed a notable decrease in C18:0 post-stress. This was not the case among older participants. Those with a lower basal Omega-3 level showed an increase in C18:0 post-stress, while participants with a higher basal Omega-3 level showed a decrease. No significant interaction effect was found.

Ratios

Saturated/polyunsaturated FAs: No main effect emerged though a significant Sex by Age interaction was found. Change was minimal across the groups, with the exception of older men, who showed a notable decrease in the saturated/polyunsaturated FAs ratio post-stress (see figure 8).

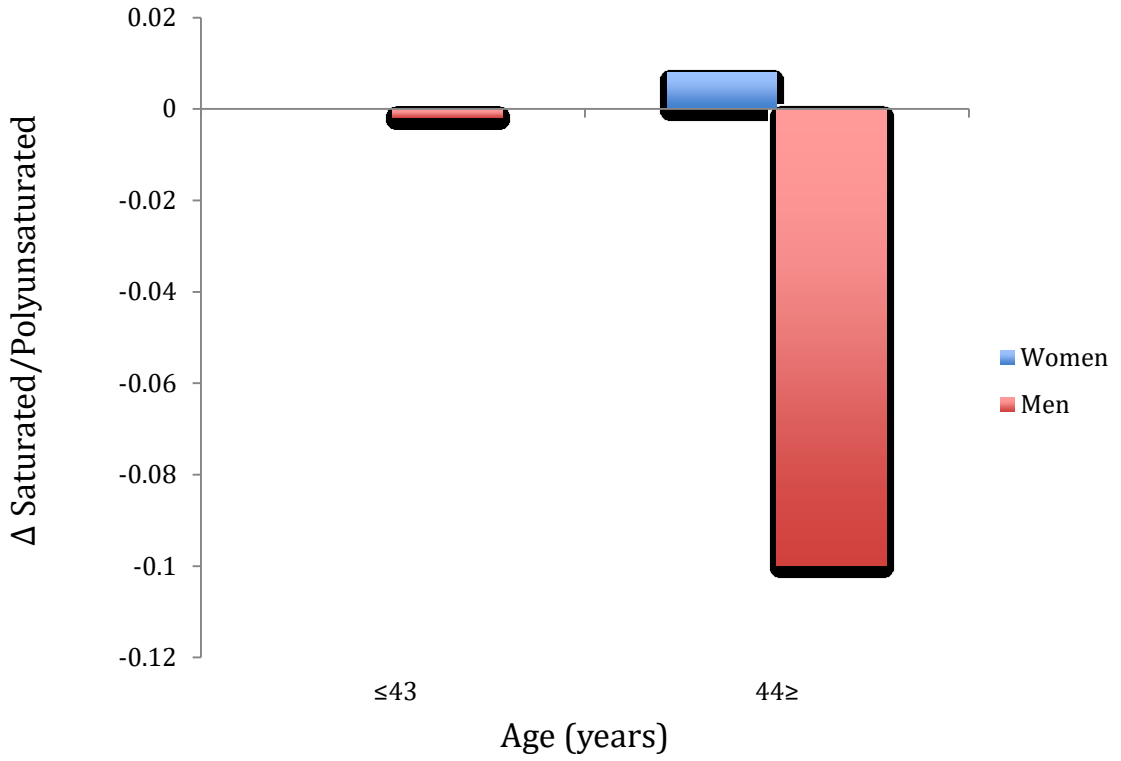

Figure 8. Change in Saturated/Polyunsaturated Fatty Acid Ratio Following Stress is Sex and Age Dependent

Precursor/product: Only a basal Omega-3 main effect emerged. In both groups, there was a decrease in the precursor/product ratio, but this was most notable among participants with lower basal Omega-3. No significant interaction was found.

EPA/DHA : A main effect of basal Omega-3 was found. Both group showed a decrease in EPA/DHA ratio post-stress, which was larger for those with higher basal Omega-3. No significant interaction emerged.

LA/AA: No significant main or interaction effects were found.

LNA/EPA: No significant main or interaction effects were found.

Table 8

Summary of results of the hierachichal regressions

		F	df	R2	Sex	Age	Omega-3 (basal)	Significant interaction
C16:0[1,7]	M1	3.043**	7,174	0.109	β=-0.052 T=-0.691	β=0.110 T=1.214	β=-0.205 T=-2.516*	
C18:0[3,5]	M1	6.982***	6,174	0.194	β=-0.10 T=-0.130	β=0.230 T=3.094**	β=-0.275 T=-3.168**	
	M1	4.811***	7,174	0.162	β=-0.070 T=-0.989	β=0.015 T=0.170	β=-0.095 T=-1.232	
C18:2[1,6,7]	M2	4.871***	8,173	0.184	β=-0.082 T= -1.153	β=0.013 T= 0.153	β=-0.084 T= -1.103	**Sex*age** β=-0.148 T=-2.143*
C18:3 n-3[3,5]	M1	7.742***	6,173	0.212	β=0.023 T= 0.317	β=0.084 T= 1.123	β=-0.031 T= -0.267	
	M1	4.637***	5, 176	0.116	β=-0.073 T= -0.984	β=0.148 T= 1.929†	β=-0.149 T= -1.630	
C18:3 n-6[3]	M2	5.229***	6,175	0.152	β=-0.086 T=-1.182	β=0.162 T= 2.143*	β=-0.178 T= -1.970*	**Omega-3*age** β=0.191 T=2.711**
C20:3 n-6[1,6]	M1	6.542***	6;175	0.183	β=-0.061 T= -0.865	β=0.136 T= 1.548	β=-0.230 T= -3.059**	
	M1	4.430***	8, 173	0.170	β=-0.121 T= -1.650	β=0.132 T= 1.653	β=-0.201 T=-2.642**	
C20:4 n-6[1,2,3,7]	M2	4.588***	9,172	0.194	β=-0.131 T= -1.807†	β=0.131 T= 1.654	β=-0.192 T= -2.552*	**Sex*age** T=-2.244* β=-0.155
C20:5[1,2,3,6]	M1	2.805**	8,173	0.115	β=0.074 T= 0.952	β=0.070 T= 0.855	β=-0.346 T= -2.456*	
C22:6[5,7]	M1	6.252***	6,173	0.178	β=-0.019 T= -0.262	β=0.244 T= 3.248***	β=-0.250 T= -2.105*	

Saturated/Polyunsaturated[1,2]	M1	2.669*	5,176	0.070	β=0.088 T=1.188	β=0.094 T=1.165	β=-0.012 T=-0.151	
	M2	2.996**	6,175	0.093	β=0.099 T= 1.339	β=0.093 T=1.161	β=-0.021 T= -0.276	**Sex*age** β=1.151 T=2.092*
EPA/DHA[3,6,7]	M1	4.306***	6,174	0.129	β=0.136 T=1.853†	β=-0.017 T= -0.188	β=-0.247 T=-3.180**	
LA/AA[1,2]	M1	1.756	5,176	0.048	β=0.013 T= 0.167	β=0.002 T= 0.019	β=0.135 T= 1.723†	
LNA/EPA[5,6]	M1	1.250	5,174	0.035	β=0.014 T= 0.173	β=0.117 T=1.244	β=-0.131 T=-1.613	

Notes. Covariates: [1]Body mass index, [2]Years of schooling, [3]Cups of coffee or tea/week, [4]Glasses of alcohol/week, [5]Hours of exercise/week, [6]Total cholesterol, [7]Cigarettes/week. $p<0.05$*, $p<0.01$**, $p<0.001$***, $p<0.10$ †. M1=model 1, M2=model 2

Posthoc analysis results

Table 9

Correlations of main dependant variables with change scores of stress other physiological systems

	CRP	Cortisol	HF (HRV)	LF/HF (HRV)	HR	IL6	SBP	TNF-α
Total FAs	**0.165***	0.011	-0.114	-0.009	-0.146†	0.007	0.081	0.004
Omega-3	-0.080	0.041	**-0.152***	0.090	-0.089	-0.034	-0.023	-0.026
Omega-6	**0.190****	-0.008	-0.125	0.036	-0.098	-0.006	0.098	0.012
Polyunsaturated	0.119	0.000	**-0.164***	0.011	-0.122	-0.003	0.066	0.011
Saturated	0.042	0.019	-0.103	0.001	-0.119	0.032	0.058	0.022
Omega-6/Omega-3	0.140†	-0.057	**0.155***	-0.126	0.041	0.011	0.059	0.047

Notes. p<0.05*, p<0.01**, p<0.001***, p<0.10 †

Table 10

Summary of posthoc hierarchical regressions

		F	df	R2	Sex	Age	Omega-3 (basal)	Significant interaction	CRP	HF(HRV)	HR
Omega-3[2,3,9]	M1	6.938***	6,172	0.195	β=0.027 T=0.381	β=0.201 T=2.701**	β=-0.392 T=-5.386***			β=-0.173 T=-2.468*	
Omega-6[1,2,6,7,10]	M1	3.579***	8,172	0.143	β=-0.076 T=-1.028	β=0.056 T=0.617	β=-0.167 T= -2.142*		β=0.054 T=0.589		
	M2	3.723***	9,171	0.164	β=-0.084 T=-1.153	β=0.056 T=0.627	β=-0.156 T= -2.027*	Sex*age β=-0.147 T=-2.079*			
Saturated[1,7]	M1	4.169***	6,175	0.125	β=-0.022 T=-0.295	β=0.210 T=2.648**	β=-0.276 T=-3.347***				
Polyunsaturated[1,2,6,7,9]	M1	5.862***	8,170	0.216	β=-0.057 T=-0.813	β=0.113 T=1.309	β=-0.341 T=-4.558***			β=-0.185 T=-2.653**	
	M2							Sex*age β=-0.107 T=-1.543			
Omega-6/Omega-3[1,3,5,9,10]	M1	6.327***	7,17	0.207	β=-0.102 T=-1.385	β=-0.199 T=-2.605**	β=0.384 T=5.282***		β=0.126 T=1.402	β=0.149 T=2.156*	

Oleic Acid (C18 :1 n-9) [6,7,8,10]	M1	5.293***	8,169	0.200	β=-0.022 T=-0.311	β=-0.018 T=-0.200	β=-0.149 T=-1.960†	β=0.133 T=1.494	β=-0.086 T=-1.204
Total FA[1,6,7,8,10]	M1	5.039***	8,169	0.193	β=-0.037 T=-0.516	β=0.081 T=0.915	β=-0.321 T=-4.197***	β=0.055 T=0.614	β=-0.083 T=-1.167

Notes.Covariates: [1]Body mass index, [2]Years of schooling, [3]Cups of coffee or tea/week, [4]Glasses of alcohol/week, [5]Hours of exercise/week, [6]Total cholesterol, [7]Cigarettes/week, [8]Heart rate, [9]Heart rate variability high frequency, [10]C-reactive protein. p<0.05*, p<0.01**, p<0.001***, p<0.10 †. M1=model 1, M2=model 2