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Plasma omega 3 polyunsaturated fatty acid status and monounsaturated fatty acids are altered by chronic social stress and predict endocrine responses to acute stress in titi monkeys

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ABSTRACT

Disturbances in fatty acid (FA) metabolism may link chronic psychological stress, endocrine responsiveness, and psychopathology. Therefore, lipid metabolome-wide responses and their relationships with endocrine (cortisol, insulin, and adiponectin) responsiveness to acute stress (AS) were assessed in a primate model of chronic social stress (CS). Compared to controls (not exposed to CS), CS increased ($P \leq 0.05$) circulating triacylglycerol (TG) and phosphatidylethanolamine (PE) n-6/n-3 and reduced ($P \leq 0.05$) cholesterol ester (CE) 16:1n7 and phosphatidylcholine (PC) 18:1n7, suggesting lower omega-3 FA status and stearoyl-CoA desaturase activity, respectively. Cortisol responses to AS positively correlated with TG n-6/n-3 ($r=0.93$; $P=0.007$), but only in CS monkeys. The adiponectin response to AS inversely correlated with CE n-6/n3 ($r=-0.89$; $P=0.045$) and positively with TG 16:1n7 ($r=0.98$; $P=0.004$), only in CS monkeys. Our results are consistent with previously reported FA profiles in stress-related psychopathology and suggest that compositional changes of specific lipid FAs may form new functional markers of chronic psychological stress.

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1. Introduction

Chronic psychological stress and enhanced stress reactivity are linked to the development of CNS disease [1–3], which has been proposed to be related to dysregulation in the hypothalamic–pituitary–adrenal (HPA) axis [1]. Repeated or chronic exposure to psychological stress commonly results in the acute-stress-induced hyper-responsiveness of the HPA axis (measured as delta cortisol) [4]. Hyper-responsiveness of the HPA axis or increased cortisol reactivity is also typical of persons suffering from major depression and heightened anxiety, particularly in the context of social inhibition and vulnerability to social evaluative threat [5,6]. This regulatory shift in the responsiveness of this hormone to stress may in part explain the link between chronic stress and chronic diseases like depression. Furthermore, variability in the magnitude and direction of stress responsivity may in part explain individual differences in disease risk. While the underlying basis for this functional change in endocrine responsivity remains unknown, we previously proposed that shifts in metabolic function, particularly in fatty acid metabolism, influence central mechanisms that regulate responsiveness in individuals undergoing chronic psychological stress [7].

Neuropsychiatric and cognitive diseases have poorly understood etiologies. Fatty acid dysregulation and deficiency may be linked to the development of these central nervous system (CNS) diseases. CNS diseases ranging from mood disorders to attention deficit and hyperactivity disorder have been shown to be associated with fatty acid deficiencies [8] and with alterations in specific fatty acid metabolites [9] [10]. For example, depression has been associated with increased plasma ratios of arachidonate:eicosapentaenate (AA:EPA) and n-6:n-3 fatty acid ratios, and lower EPA and C22:6n-3 docosahexaenoic acid (DHA) in circulating phospholipids and cholesterol esters [11–13]. Social anxiety disorder (SAD) has been associated with decreases of DHA and EPA in circulating phospholipids of red blood cells [14], and DHA and AA have been reported to be lower in the red blood cell phospholipids of schizophrenic patients [15]. In addition to the link between endogenous fatty acid metabolism and CNS diseases, fatty acids consumed exogenously have been shown to modulate symptoms

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of CNS disease. Dietary fish oil and DHA have been shown to exert anti-stress effects [16,17] and reduce depressive symptoms [18], suggesting that intake of n-3 fatty acids, particularly EPA and DHA, may have protective effects in mood disorders like major and bipolar depression [19]. However, some studies have failed to find such protective effects of consuming n-3 PUFA, further testing is required (e.g., [20]).

In providing a physiological basis for the associations between fatty acid metabolism and CNS diseases, it has been suggested that fatty acid dysregulation and deficiency may lead to abnormal brain function and health by decreasing neuronal membrane stability [21,22], altering membrane composition (e.g., n-6/n-3 fatty acids in membrane phospholipids) [22], and disrupting secondary messenger activity and neuropeptide trafficking (e.g., dopamine, serotonin, CRF) [23,24]. However, the factors leading to alterations in fatty acid metabolism and balance in the first place remain unknown. Although dietary and genetic factors likely play a role, other factors such as chronic psychological stress may significantly contribute to linking alterations in fatty acid balance and the development of CNS disease.

Recently, Lamaziere et al. [25] demonstrated the utility of a lipidomic approach to examining in rats omega 3 incorporation in the brain. In this project, a lipidomic approach was used to: (1) assess lipid metabolome-wide effects of chronic social stress in a non-human primate model (titi monkey (*Callicebus cupreus*)); (2) determine whether chronic social stress results in fatty acid metabolite profiles (e.g., lower omega 3 status) parallel to those seen in CNS diseases like depression; (3) test whether omega 3 status and concentrations of other plasma fatty acid metabolites explain variation in HPA responsiveness to acute stress; and (4) examine the stress response of the metabolic hormones insulin and adiponectin, which have both been proposed to link chronic stress with metabolic dysfunction (e.g., metabolic syndrome) and depression [26].

2. Methods and materials

2.1. Subjects and housing conditions

All experimental procedures were approved by the Animal Care and Use Committee of the University of California, Davis, and complied with National Institutes of Health ethical guidelines as set forth in the Guide for Lab Animal Care. All twelve male titi monkeys (*C. cupreus*) were laboratory-born and raised in stable family groups until they reached reproductive maturity (18 months). At random, 6 subjects were separated (chronic psychosocial stressor) from their families while the other 6 remained with their families (control). Animals were housed according to standard laboratory protocol that includes feeding twice daily (0700 and 1300 h) with water available ad libitum. All monkeys were provided the same diets which consisted of monkey chow (LabDiet 5045), cottage cheese, marmoset jelly (LabDiet 5041), apples, raisins, and baby carrots. Chow and all of the other food items were offered together, ad libitum, twice a day. The fatty acid composition of the total diet was of 34.4% saturated, 35.1% monounsaturated, 27.9% of n-6 polyunsaturated and 2.6% n-3 polyunsaturated fat as a percent of total fatty acids. Animals were maintained on a 12 h/12 h light dark cycle, with lights on at 0600. Skylights provide additional exposure to sunlight and natural variation in day-length. All blood draws were taken during the light cycle between 10:00 and 11:00 AM.

2.2. Experimental design

Chronic stress was introduced by capturing all family members from 6 randomly selected subjects. These chronically stressed monkeys were moved and singly housed for 4 months in new cages within the same colony room. The 6 control monkeys were moved to a new cage in the colony room and remained with their parents for 4 months. Four months after remaining in isolation, an acute stress was introduced to both chronically stressed and control monkeys. Starting at 10:00 AM, each subject was captured and removed from their respective cages followed by a baseline blood draw. After the baseline blood sample, monkeys were exposed to acute stress which involved placing each individual into transport cages as solitary confinement for 30 min at which time a second blood draw was taken (Fig. 1: the diagram of the study design). Immediately after the second blood draw, all monkeys were returned to their experimental home cages. Blood samples were collected at a designated and private location in the colony room within 3 min of entering the monkey's cage. One to two milliliters of blood was collected at each of the two collection times.

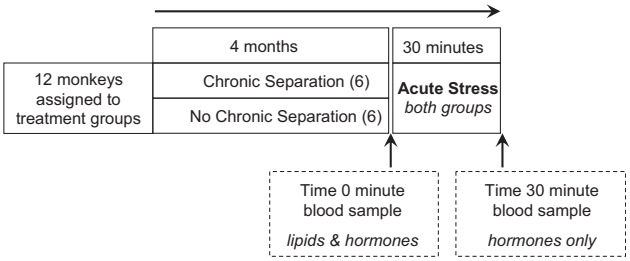

Fig. 1. Schematic of the study design.

2.3. Biochemical analyses

Blood was collected from all participants into EDTA evacuated tubes, centrifuged immediately (1300*g*, 10 min, 20 °C), portioned into aliquots, and stored at −80 °C until analyzed. Plasma cortisol concentrations were determined using a standard RIA kit (Diagnostics Products Corporation [27]). Plasma insulin was assayed using an ultra sensitive insulin ELISA kit having broad species cross reactivity, including human (Crystal Chem, Inc.), and plasma adiponectin concentrations were determined using a standard RIA kit (Millipore, Inc; previously developed by Linco, Inc.) designed to assess human adiponectin, but routinely used for non-human primates [28]. All plasma hormones were measured at 4 months just prior to acute stress (time 0) and 30 min after acute stress.

2.4. Analysis of the fatty acid composition of plasma lipids

Fatty acid analyses of circulating lipid classes were determined by high-throughput methods developed by Lipomics Technologies, Inc. (West Sacramento, CA). The lipids from plasma (200 μL) were extracted using a modified Folch extraction in chloroform:methanol (2:1 v-v) [29] in the presence of a panel of quantitative authentic internal standards. Extracted concentrated lipids were analyzed by HPLC for phospholipid separation, TLC for non-polar lipid classes. Lipid classes were trans-esterified in 3 mol/L methanolic HCl in sealed vials under a nitrogen atmosphere at 100 °C for 45 min. The resulting fatty acid methyl esters were extracted from the mixture with hexane separated and quantified by capillary gas chromatography using an Agilent 6890 gas chromatograph (Santa Clara, CA) equipped with a 30-m DB-88 capillary column (Agilent Technologies) and a flame-ionization detector [30]. Fatty acids of each lipid class were determined quantitatively (μmol/L) and expressed as a % of total fatty acids within that class (mol%). Fatty acids in which 20% of the data were missing or below the limit of quantification were dropped from the analyses and considered not determined in the results section. Lipid classes were abbreviated in