Dietary saturated fat and monounsaturated fat have reversible effects on brain function and the secretion of pro-inflammatory cytokines in young women.

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Running Head: Dietary fatty acids and brain function

List of abbreviations:; FA, fatty acid; fMRI, functional magnetic resonance imaging; HOA, low PA, high OA diet; HPA, high PA diet; IL, interleukin; LPS, lipopolysaccharide; MUFA, monounsaturated fatty acids; NLRP3, Nucleotide Oligomerization Domain (NOD)-Like Receptor Protein; OA, oleic acid; PA, palmitic acid; SFA, saturated fatty acids; TLR4, toll-like receptor-4; TNFα, tumor necrosis factor-α.

This study has been registered at http://www.clinicaltrials.gov/ as University of Vermont Protocol Record R01DK082803.

Abstract

Background: Previous literature suggests that a higher ratio of palmitic acid (PA)/oleic acid (OA) in the diet induces inflammation, which may result in deficient brain insulin signaling, and, secondarily, impaired physical activity, sleep efficiency, and cognitive functioning.

Objective: We hypothesized that lowering the typical dietary PA/OA would affect the activation of relevant brain networks during a working memory task and would also lower secretion of pro-inflammatory cytokines.

Design: In 12 female subjects participating in a randomized, cross-over trial comparing 3-week high PA diet (HPA) and low PA and a high OA diet (HOA), we evaluated functional magnetic resonance imaging (fMRI) using an N-back test of working memory, cytokine secretion by lipopolysaccharide(LPS)-stimulated peripheral blood mononuclear cells (PBMC), and plasma cytokine concentrations.

Results: Brain activation during the HPA diet compared to the HOA diet was increased in regions of the basal ganglia including the caudate and putamen ($p<0.005$). In addition, compared to the HOA diet, during the HPA diet, the plasma concentrations of IL-6 ($p= 0.04$) and IL-1β ($p= 0.05$) were higher, and there was a higher secretion of IL-18 ($p=0.015$) and a trend for higher IL-1β secretion ($p=.066$) from LPS-stimulated PBMCs.

Conclusions: The HPA diet resulted in increased brain activation in the basal ganglia compared to the HOA diet as well as increased secretion of pro-inflammatory cytokines. These data provide evidence that short-term (2 week) diet interventions impact brain network activation during a working memory task and that these effects are reversible since the order of the study diets was randomized. These data are consistent with the hypothesis that lowering the dietary PA content via substitution with OA also could affect cognition.

Key Words:

palmitic acid

oleic acid

functional magnetic resonance imaging

cytokines

brain activation

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1. Introduction

Cognition comprises a number of brain processes including memory, attention, problem-solving, and decision-making. Neuroscientists and physicians as well as lay people are interested in environmental factors that might enhance or impair cognition at any age, including diet, physical exercise, and sleep efficiency. Variations in dietary content of the saturated fatty acid (SFA), palmitic acid (PA; C16:0) and the monounsaturated FA (MUFA), oleic acid (OA; C18:1 n-9 or ω-9) have been linked to alterations in cognitive function in humans[1,2].

Specifically, Samieri et al.[1] reported the results of a sub-study conducted as part of a double-blind, placebo-controlled factorial trial of low dose aspirin and vitamin E supplements for the primary prevention of cardiovascular disease and cancer in women (Women's Health Study). Subjects aged ≥65 years underwent initial cognitive testing and then again at two follow-up points at approximately two-year intervals[1]. Based on the trajectory of change in cognitive function, global cognition and verbal memory were enhanced as a function of the MUFA/SFA ratio, particularly when comparing the extreme quintiles, respectively 0.9 and 1.3[1].

In our previously published studies, we reported the results of a diet history obtained from our young adult volunteers in two separate cohorts during screening. Our subjects' habitual" intake resulted in a MUFA/SFA ratio of 0.83 (Cohort 1) and 1.08 (Cohort 2)[3], similar to the lowest three quintiles of the Samieri study[1]. In two separate trials, we used the same, cross-over study paradigm in which we markedly lowered the PA intake by substituting OA, resulting in two highly contrasting MUFA/SFA ratios, one, the "HPA diet", (0.88) similar to the lowest quintile in the study by Samieri et al.[1] and to our subjects' "habitual diet" and a low PA/high OA diet ("HOA") with a ratio (10.1)[3,4] much higher than those in the fifth quintile of participants in the Women's Health Study. In these two distinct cohorts of young adults, we have studied the

effects of lowering the PA intake on a number of outcome variables including insulin sensitivity, inflammation, physical activity, mood, muscle gene expression, and blood lipid profiles[3-6]. Notably, lowering the dietary PA/OA ratio increased physical activity and lowered mood disturbance [3].

Prior studies also have shown links between MUFA/SFA changes and behavioral and cognitive outcomes. Sartorius et al.[7] showed that a high SFA diet in mice as well as acute intraventricular injection of PA decreased activation of insulin signaling in the brain, decreased locomotor activity in response to acute intraventricular injection of insulin, and disrupted normal wakefulness and sleep behavior compared to a high MUFA diet. Blocking the activity of interleukin (IL)-6 in mice fed the high SFA diet enhanced physical activity[8]. Hanson et al.[2] found that feeding older adults a single meal high in both SFA and high glycemic load carbohydrates improved and impaired cognition acutely, respectively, in those with or without cognitive impairment. Other studies suggest that a high SFA diet adversely affects the hippocampus and memory in rats, possibly via induction of inflammatory pathways[9]. It is relevant to specifically emphasize our findings that lowering the habitual dietary PA/OA ratio (same as raising the MUFA/SFA ratio) was associated with lower circulating concentrations of IL-6 and tumor necrosis factor-α (TNFα) and lower secretion of IL-1β, IL-18, and TNFα by lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs)[4,5].

In view of evidence that shifts in the dietary MUFA/SFA ratio affect cognition in the general population[1] and our own data relating to the reversible effects of this ratio on physical activity behavior and mood[3], we hypothesized that diets high or low in PA would differentially activate brain networks associated with working memory using functional magnetic resonance imaging (fMRI) as well as affect systemic inflammation.

2. Material and Methods

2.1 Subjects, screening, and design.

This study was approved by the University of Vermont (UVM) Institutional Review Board (IRB). The clinical aspects were managed at the UVM Clinical Research Center (CRC) and the imaging was completed at the UVM MRI Center for Biomedical Imaging. The subjects were derived from a sub-set of young adults participating in a randomized, double-masked, cross-over study of lean and obese adults in order to determine how dietary PA intake affected PA oxidation, insulin sensitivity, and inflammatory signaling ("parent protocol"), but our priorities for recruitment of women for the larger study necessitated our studying only women with respect to this sub-study using fMRI[5,10]. **Supplementary Figure 1** depicts the consort diagram for this sub-study. Twelve, healthy, lean or obese, but non-diabetic women aged 18 - 40 years were recruited (age range: 20-36 years, mean ± SEM = 26.5±1.3 years; body mass index >18<25, n=7, or >30, n=5). Exclusion criteria were similar to our previous studies[5,10,11].

As previously reported[6], we used two dietary history techniques to assess our subjects' habitual intake. In the cohort of subjects reported here, the habitual intake was as follows (% kcal): protein, 15.8; carbohydrate, 49.7; total fat, 36.6; saturated fat, 13.6; and monounsaturated fat, 11.8 (MUFA/SFA ratio, 0.93) respectively. The SFA intake is higher than is usually recommended for optimal cardiovascular health[12]. After screening to rule out relevant health problems, all subjects ingested a low fat/low-PA, baseline/control diet for seven days (Protein, 19.7 % kcal; Carbohydrate, 51.6% kcal; Fat, 28.4% kcal; PA, 5.3% kcal; OA, 15.9% kcal)[5,10]. This diet was patterned after the Therapeutic Lifestyles Diet[12]. Then, the subject participated in a cross-over study of two, 3-week experimental, low glycemic load diets, administered in random order: a diet high in PA (HPA; Fat, 40.4% kcal; PA, 16.0% kcal; OA,16.2% kcal; linoleic acid,

5.0% kcal; MUFA/SFA = 0.88); a diet low in PA and high in OA (HOA; Fat, 40.1% kcal; PA, 2.4% kcal; OA, 28.8% kcal; linoleic acid, 6.4% kcal; MUFA/SFA = 10.1)(based on analysis at Covance Laboratories, Madison, WI)[5,10]. For all 3 diets, FA composition was varied by adding oil blends to the six precisely formulated meals comprising the control diet (breakfast, lunch, and dinner, for two days) and nine meals comprising the experimental diets (meals for 3 days). The foods, including chicken and turkey (the only sources of meat) were all very low in fat. Thus, FA were mainly provided by vegetable oil blends appropriate to each diet (Natural Oils International, Inc., Simi Valley, California). The HPA and HOA diets otherwise contained the exact same foods with a three-day rotating menu. These oils, at room temperature, were mixed with food that had been warmed; thus, these oils were not used for cooking. The oil blend for the control diet consisted of palm oil (36.9%), high oleic sunflower oil (19.3%), and hazelnut oil (43.8%). The HPA oil blend consisted of palm oil (89%), peanut oil (6.75%), and virgin olive oil (4.25%), and the HOA "blend" consisted only of hazelnut oil. The HOA and HPA diets had identical, low glycemic loads (10.7, average of the three days of menus)[3].

All food and drink, except water, were provided by the CRC, and body weight remained stable throughout the study since we adjusted the energy intake as required to maintain a constant body weight over the 8 weeks of the study[4]. The subjects reported to the CRC in the morning, Monday – Friday, during each of the Control and Experimental Diet periods. The subjects ate their breakfast there on those days and were given instructions regarding convenient ways to add the oils to food items on each of the menus. In addition, the subjects received advice and support regarding the requirements of the study during these visits to the CRC[3,4,6]. Subjects also were given instructions to use spatulas, provided by the CRC, to help scrape all oil from its container. Each day, the subjects completed and signed a questionnaire attesting to or

168 commenting about their having eaten all the food (and food oil) and to not having consumed any
169 food or drink, except water, not on the menu. In addition, all food and oil containers were
170 inspected each day to be sure all food and oil were consumed. Any food or dietary oil that was
171 left over in the containers was weighed, and the data used to construct a modified food intake for
172 that day. Generally, we have only encountered occasional failure to eat all the food each day. In
173 one previous study[4], we found that the average number of days (out of 56) when some food was
174 returned during the HPA and HOA diets was 1.33 and 1.67, and the average daily consumption
175 of the oil for the HPA and HOA diets as a percentage of total oil administered was 99.9% and
176 99.2% (127.8 and 127.6 g/d).
177 The primary outcome was the blood oxygen level dependent measure from the fMRI during a
178 working memory task. fMRI studies were completed in the fasted state, on day 16 of each
179 experimental diet (after 15 days of diet). On day 8 of the Control/baseline diet and on the 22nd
180 day of each experimental diet (HPA and HOA), blood was collected in the fasted state for
181 measurement of cytokines in plasma and from LPS-stimulated PBMCs.
182
183 **2.2 fMRI working memory task and analysis.**
184 All subjects were imaged on a Philips Achieva 3.0 Tesla MRI. fMRI was performed
185 using EpiBOLD (echoplanar blood oxygenation level dependent) imaging using a single-shot
186 sequence (TR 2500 ms, TE 35 ms, flip angle 90 degrees, 1 NSA for 197 volumes). Resolution
187 was 2.5 mm x 2.8 mm x 4 mm. Thirty-four contiguous slices 4 mm thick with no gap were
188 obtained in the axial oblique plane parallel to the AC-PC plane using a FOV of 240 mm and a
189 matrix size of 128 x 96. Field map correction for magnetic inhomogeneities was accomplished

by acquiring images with offset TE at the end of the functional series. fMRI acquisition and preprocessing procedures were similar to our prior studies [11].

The fMRI task was a visually presented verbal N-back used to probe working memory circuitry. Participants saw a string of consonants (except L, W, and Y), presented in upper case letters, one every three seconds. Four conditions were presented: 0-back, 1-back, 2-back, and 3-back. The 0-back control condition had a minimal working memory load; participants were asked to decide if the current letter matched a single target letter that was specified before the epoch began. In the 1-, 2-, and 3-back conditions, participants indicated whether the current letter on the screen matched a letter that was either 1, 2 or 3 back in the sequence.

The 0-, 1-, 2-, and 3-back conditions were repeated three times in a counterbalanced order such that the same condition was not repeated two times in a row. In this block design task, participants responded to nine items in each block that took 27 seconds. A rest break followed with a plus sign (+) fixation for 12 seconds. The total time of the task was 8 minutes 12 seconds. Participants practiced the N-back task before the scanning session to ensure that they understood task instructions.

Statistical analyses involved deriving one mean image per individual for the contrast of interest in the activation task (e.g., 2-back minus 0-back) after accounting for the hemodynamic response function. These contrast images were then used for the second level paired *t*-test to examine diet effects on brain functioning. To correct for multiple comparisons, we used a gray matter mask generated from the current data and the cluster-level statistical threshold estimator from Brain Voyager QX to estimate a minimum cluster size threshold based on the approach of Forman et al.[13] that estimated a minimum cluster size of 12 voxels in functional space (3x3x3) at α=0.005.

213

214 **2.3 Metabolic assays.**

215 The FA composition of serum phospholipids (phosphatidylcholine,
216 phosphatidylethanolamine, and cardiolipin) was analyzed by flame ionization detector gas
217 chromatography[4,5].

218

219 **2.4 Measurement of cytokines in plasma and secreted by PBMCs.**

220 Plasma cytokines were measured from all 12 subjects[5]; cytokine secretion by PBMCs was
221 measured on only 11/12 subjects because of technical issues[5].

222

223 **2.5 Data analysis.**

224 This study employed a two-treatment, two-period, two-sequence cross-over design. Diet
225 effects were analyzed using a repeated measures analysis of variance, including sequence,
226 period, and treatment effects, with the baseline value as a covariate, when available[4,5].

227

3. Results

3.1 Working memory-related brain activation and performance.

The working memory task showed the expected [14] bilateral frontal, parietal, cerebellar, anterior cingulate, and basal ganglia network activation. There was activation for the 2-back minus 0-back contrast during the HPA diet compared to the HOA diet in the right caudate nucleus and left putamen in the basal ganglia (Figure 1; Table 1).

3.2 FA composition of serum phospholipids.

During the HPA diet, the PA/OA ratio was 67-69% higher in serum phosphatidylcholine ($p<0.0001$), phosphatidylethanolamine ($p=0.005$), and cardiolipin ($p<0.0001$) compared to the HOA diet (Figure 2). These data provide evidence that the diets were ingested as intended and had the anticipated effects on cellular lipids [3,4,6].

3.3 Secretion of cytokines by LPS-stimulated PBMCs and plasma cytokine concentration.

Compared to the HOA diet, during the HPA diet, there was a higher secretion of IL-18 ($p=0.015$) and a trend for higher IL-1β secretion ($p=.066$) from LPS-stimulated PBMCs, consistent with enhanced activation of the Nucleotide Oligomerization Domain (NOD)-Like Receptor Protein (NLRP3) inflammasome (Figure 3A). The HPA diet also was associated with higher plasma concentrations of IL-6 ($p=0.04$) and IL-1β ($p=0.05$), indicative of activation of both toll-like receptor-4 (TLR4) and the NLRP3 inflammasome *in vivo* (Figure 3B). The plasma concentration of TNFα trended upward (36%) during HPA ($p=0.09$; Figure 3B). However, we observed no statistically significant correlations between diet-change in plasma concentration of cytokines or

PBMC secretion of cytokines and respective diet-change in activation of those brain networks responsive to the working memory task.

4. Discussion

This study is the first to examine effects of varying the dietary PA/OA ratio on human brain functioning. Specifically, working memory-related brain activation was increased in the caudate and putamen of the basal ganglia after two weeks of a diet high in PA compared to a diet low in PA and high in OA. Additionally, we confirmed previous data[5] suggesting that the HPA diet resulted in relatively increased secretion of some cytokines modulated by TLR4 and the NLRP3 inflammasome.

While it is recognized that glucose and amino acids alter brain function (e.g.[15,16]), our study provides an important indication that another class of macronutrients, FAs, appear to alter brain activation during a cognitive stimulus. These data add to an emerging concept that, as with other sources of dietary energy, FAs, with their own unique chemical properties, affect neuronal activity, perhaps via changes in inflammatory signaling[2,7,8,17]. Thus, in future considerations of the health effects of various food sources of dietary FAs, brain effects may need to be taken into account.

The brain activation data showed differences between the HPA and HOA diets in the caudate and putamen during a working memory task. Working memory involves the active maintenance, manipulation, and updating of information in memory over a short period of time [18]. The striatum, which includes the caudate and putamen, has been shown to be involved in the updating of information in working memory [19,20]. Additionally, the striatum is involved in reward responses (e.g., [21]), normal eating behaviors [22], and voluntary motor control [23]. The current study showed that a high PA diet increased activation in the striatum and this effect was reversible with a diet

low in PA and high in OA. The mechanisms by which these FA interventions affected brain functioning, aside from the possible link to inflammatory signaling, remain to be determined.

One prior study employing resting-state MRI (no functional task) found that 12 weeks of a high saturated FA diet resulted in decreased intrinsic resting brain activity in the hippocampus and inferior parietal cortex, but there was no change in resting brain activation in subjects fed a diet enriched with monounsaturated FAs [7]. It is difficult to compare the results of a resting state study with a task-based study like the one described here, and further studies are needed to understand the influence of FAs on brain functioning.

The present study and two of our previous studies suggest that the HPA diet relatively increases inflammation and specifically IL-1β and IL-6 secretion [4,5]. Animal studies suggest that inflammation also might be a mechanism for altering normal brain function, where neuronal integrity is preserved [24]. Blocking TLR4 or the use of a neutralizing IL-6 antibody enhanced insulin signaling in the brain and improved brain function, including enhanced sleep efficiency and locomotion[8]; this observation could be relevant to our present findings as well our previous observation that the HPA diet was associated with higher circulating concentration of IL-6[4] and with lower physical activity[3].

It is possible that brain-derived neurotrophic factor (BDNF) is at the nexus where a high dietary PA/OA ratio, enhanced systemic inflammation, and alterations in brain function converge. BDNF is among a group of neurotrophins which support synaptic plasticity and is required for hippocampus-mediated learning, as well as acting as both a neurotransmitter and neuromodulator that affects the pre-synaptic release of other neurotransmitters[25]. Molteni et al.[26] showed dietary fatty acid effects on brain BDNF. They fed a high sugar and high SFA diet to rats; compared to controls, brain mRNA expression of BDNF was reduced and learning was impaired within two

298 months of the diet. The brain protein level of BDNF was also lower, when measured after 6
299 months of diet[26]. BDNF signals partially via the insulin receptor substrate-1, phosphatidylinositol
300 3-kinase, and Akt pathway, similar to insulin[27], but there also is evidence that
301 intracerebroventricular infusion of insulin increased BDNF protein level in the hippocampus of
302 young (4 months) but not older (24 months) rats[28] and that insulin signaling is required for normal
303 BDNF transport and hippocampal synaptogenesis [29].
304 There are obvious limitations to inferences about brain function in humans that can be drawn
305 from measurements of inflammation originating in the peripheral blood and the stochastic
306 variables of brain function such as those obtained from fMRI imaging. In addition, we cannot from
307 this small study determine the clinical significance of increased or decreased brain activation.
308 However, the present fMRI findings add to our previous report[3] implicating that brain function is
309 reversibly affected by a lower dietary PA/OA ratio, which, in turn, consistently is associated with
310 lower inflammation. Future studies might explore effects on cognitive performance, such as
311 episodic memory, and additional biomarkers for the effects of dietary FA composition, such as
312 circulating BDNF concentration.
313
314 **4.1 Conclusion**
315 This crossover study in young women revealed reversible effects on brain functioning and
316 cytokine production of a high PA diet and a low PA, high OA diet over a brief period. Diet may
317 be an intervention that can enhance or impair brain performance, possibly via a mechanistic
318 pathway that involves inflammation, brain insulin signaling, and perhaps neurotrophic effects
319 (e.g. brain levels of BDNF).

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6. References

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413 **Table 1**. Effects of HPA diet compared to the HOA diet for the 2-back minus 0-back contrast
414 including Talairach coordinates, cluster size (mm^3), region descriptions (Brodmann's areas, BA),
415 *t* values and uncorrected voxel-level *p* values.

Contrast	Coordinates x y z	Cluster Extent	Region Description	*t* value	*p* value
2-back – 0-back					
HPA-HOA	5 16 6	605	Right caudate head	5.14	<0.001
	-25 37 9	1574	Left putamen	4.42	<0.001

416
417
418

Figure Legends

Figure 1. Greater brain activation was found after the HPA diet compared to the HOA diet for the 2-back minus 0-back conditions ($p<.005$; n=12) in the right caudate and left putamen during fMRI scanning. Orange colors represent activation that is greater for HPA diet compared to the HOA diet on the 2-back condition compared to the 0-back condition. Hash marks are centered on the right caudate head. The fMRI contrast images were analyzed with standard second level repeated measures ANOVA in Brain Voyager using diet as a within-subjects factor. To correct for multiple comparisons, we used a gray matter mask generated from the current data. We then used the cluster-level statistical threshold estimator from Brain Voyager QX to estimate a minimum cluster size threshold. Abbreviations used: fMRI, functional magnetic resonance; HOA, high oleic acid diet; HPA, high palmitic acid diet

Figure 2. The HPA diet was associated with higher PA/OA ratios in serum phosphatidylcholine (PC), phosphatidylethanolamine (PE), and cardiolipin (CL). Blood samples were collected from overnight-fasted subjects at the end of the baseline diet and each experimental diet (HPA, HOA). The fatty acid content of serum PC, PE, and CL was measured using thin layer chromatography followed by gas chromatography (see Methods). Results are mean ± SEM (n=12). * $p = 0.005$; ** $p \leq 0.0001$ for diet effects.

Figure 3. A higher PA/OA ratio in the diet enhanced LPS-stimulated cytokine production by PBMCs and increased plasma concentrations of pro-inflammatory cytokines. **A.** LPS-stimulated cytokine production by PBMCs after HPA and HOA diets (n=11). PBMCs were collected from overnight-fasted subjects at the end of the baseline diet and each experimental diet (HPA, HOA)

and stimulated *in vitro* for 24 hr. with 1 ng/ml lipopolysaccharide. Secreted cytokines were measured by BioPlex or ELISA (see Methods). In order to display all the cytokines in the same graph, actual cytokine concentrations were multiplied by the respective correction factors, shown on the abscissa. IL-1β trended upward during the HPA diet (p=0.066). **B.** Plasma concentrations of pro-inflammatory cytokines after the HPA and HOA diets (n=12). Results are mean ± SEM. * p<0.05 for diet effects.

Figure

Figure 1

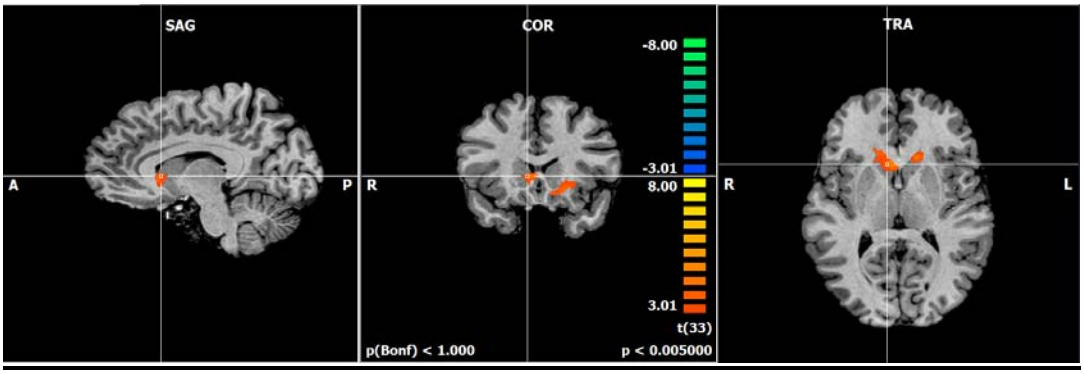


Figure 2

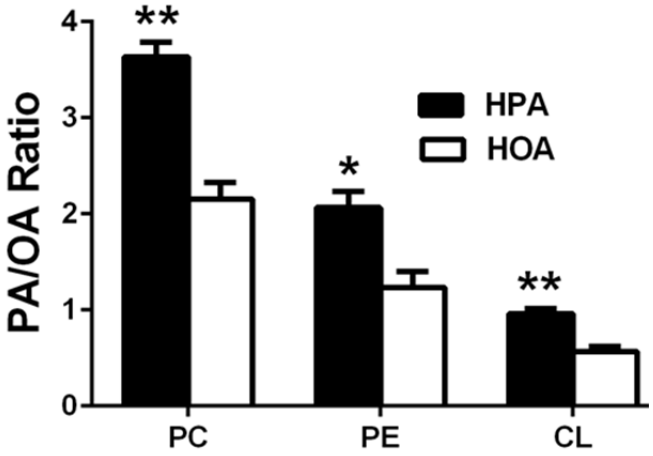
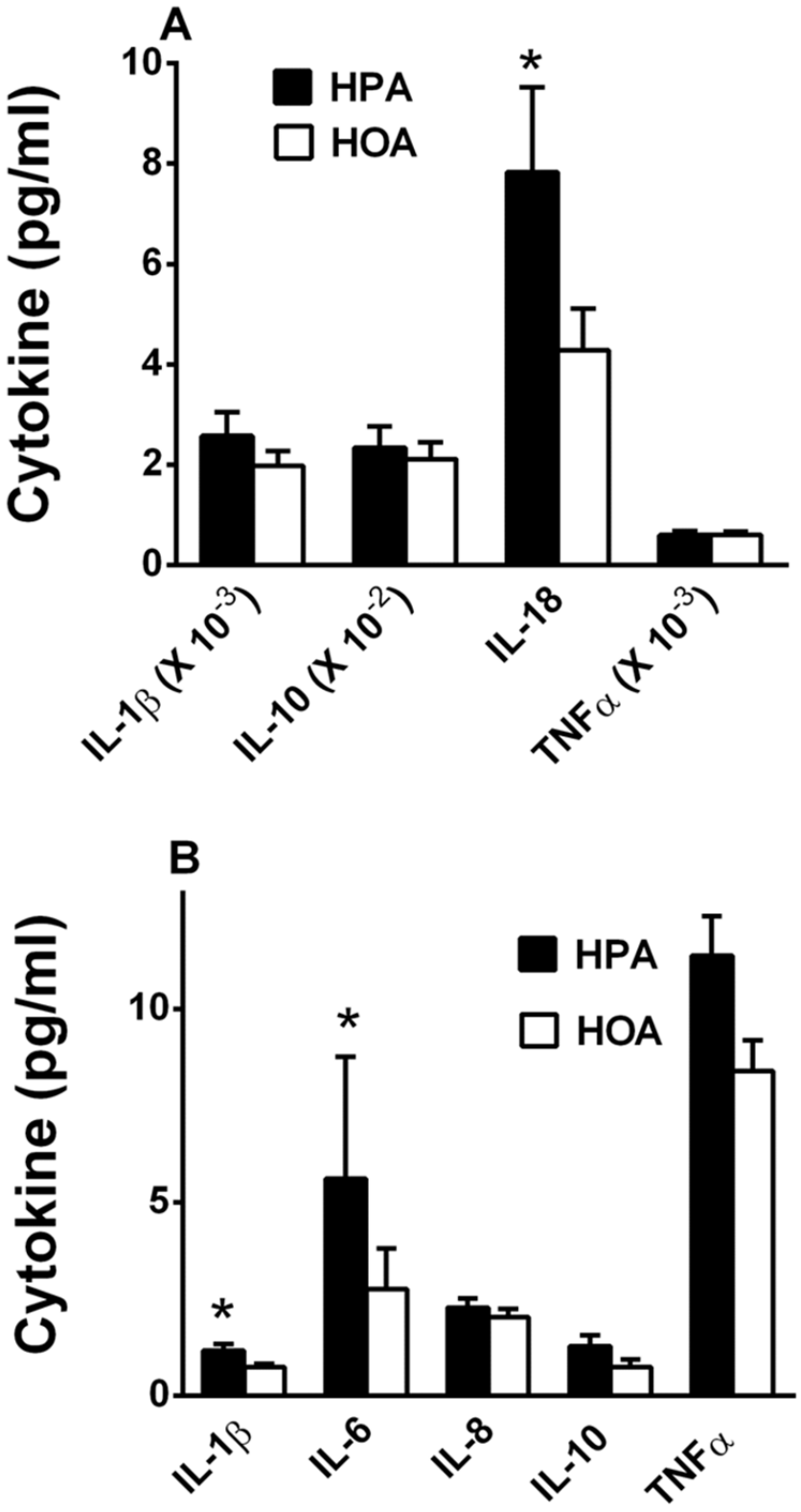


Figure 3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6
4
2
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HPA
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*
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IL-18
TNFα (X 10-3)
B
Cytokine (pg/ml)
10
5
0
HPA
HOA
*
*
IL-1β
IL-6
IL-8
IL-10
TNFα