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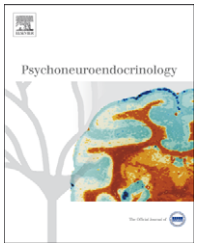

SHORT COMMUNICATION

# Effects of acute ovarian hormone suppression on the human brain: An *in vivo* $^{1}$H MRS study

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**Summary**
A previous proton magnetic resonance spectroscopy ($^{1}$H MRS) study carried out by our group indicated that post-menopausal women who started taking oestrogen therapy (ET) at or around the menopause had a significantly lower choline (Cho) concentration in the hippocampus and parietal lobe than those who were ET naïve, suggesting that long-term ET positively modulates neuronal/glial membrane turnover in older females. The objective of the current study was to determine whether neuronal membrane turnover is modulated by sex hormones in younger women following a pharmacologic challenge that induced acute ovarian hormone suppression. We carried out an *in vivo* $^{1}$H MRS study in a group of 10 premenopausal women pre- and post-8 weeks of acute ovarian suppression with a Gonadotrophin Releasing Hormone analogue (GnRHa) (two Zoladex® 3.6 mg implants). We report that young women, post-ovarian suppression, had a significant increase in Cho concentration (and Cho/Cr ratio) in the dorsolateral prefrontal cortex (DLPFC). They also showed a trend to a significant increase in Cho concentration in the hippocampus. This supports our previous findings and adds to the evidence that neuronal/glial membrane metabolism is affected by sex hormones in women.


## 1. Introduction

Despite increasing evidence that ovarian hormones affect cognitive function (see Craig and Murphy, 2007 for review),

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and the suggestion that the brain may be particularly sensitive to acute loss of ovarian function, the neurobiological mechanisms underpinning these phenomena remain poorly understood. Recent functional brain imaging studies suggest that acute ovarian suppression is associated with reduced brain activity in regions that include the dorsolateral prefrontal cortex (DLPFC), hippocampus and the inferior parietal lobe (Berman et al., 1997); and that acute treatment with oestrogen therapy (ET) increases left frontal activation (Shaywitz et al., 1999). However, the biological basis for the change in neuronal function following ovarian suppression, and replacement, is unknown.

Neuronal and glial function can be studied using proton magnetic resonance spectroscopy ($^1$H MRS). The main brain metabolites detectable by $^1$H MRS include *N*-acetylaspartate (NAA), a neuronal marker reflecting neuronal density and/or mitochondrial metabolism; choline (Cho) containing compounds (e.g., phosphocholine and glycerophosphocholine), which are proxy measures of membrane metabolism/turnover; creatine+phosphocreatine (Cr), which are associated with high-energy phosphate metabolism; myo-inositol (mI), a putative glial marker which affects neuronal signal transduction and cellular osmolarity; and glutamate+glutamine (Glx).

We previously reported that, in post-menopausal women, those who start taking ET at or around the menopause have a significantly lower Cho concentration in hippocampus and parietal lobe than those who were ET naive (Robertson et al., 2001). We therefore suggested that long-term ET positively modulates neuronal membrane turnover in older females. This finding raised the possibility that endogenous steroid hormones modulate neuronal membrane turnover in pre-menopausal women as well. To test this hypothesis we carried out an *in vivo* $^1$H MRS study in a group of younger women pre- and post-acute ovarian suppression using a Gonadotrophin Releasing Hormone analogue (GnRHa). Many premenopausal women experience acute ovarian suppression following surgical hysterectomy and oophorectomy. Furthermore, GnRHa is commonly used in clinical practice to manage a variety of gynaecological conditions, including *in vitro* fertilization and treatment of uterine myomas (fibroids), and as a treatment for advanced breast and prostate cancer. Study of the effects of acute loss of ovarian hormones in this population is therefore of significant clinical interest. We hypothesized, based on prior work, that acute ovarian steroid suppression would increase Cho concentration in the hippocampus and DLPFC.

## 2. Methods

We studied 10 young healthy premenopausal women (age range 26–47 years old) pre- and post-acute ovarian hormone suppression using a GnRHa (Zoladex® 3.6 mg). Women were excluded if they had any significant medical, psychiatric or neurological problems affecting brain function; or if they were taking regular prescribed medication. All subjects also underwent routine blood testing to confirm a normal haematological profile; and liver, renal, thyroid and ovarian function. All women had benign leiomyomata uteri (i.e., 'fibroids') and were prescribed GnRHa as part of their routine clinical management. Baseline scans (time 1) were carried out during normal menstrual functioning (i.e., during the follicular and menstrual phases of the cycle). They subsequently received two GnRHa implants (with a 4-week delay between implants) and had a repeat scan 8 weeks later when still taking GnRHa. All women signed informed consent as per the Ethics Committee Guidelines.

$^1$H MRS data was obtained using a GE Signa 1.5 T HD TwinSpeed MRI scanner (General Electric, Milwaukee, WI, USA) at the Maudsley Hospital. A left DLPFC voxel of interest (VOI), measuring $16 \times 24 \times 20\,mm^3$, and a left hippocampal VOI, measuring $20 \times 20 \times 15\,mm^3$, were placed in standard locations using previously published methods (Robertson et al., 2001) and contained both grey and white matter. The hippocampal VOI also included the parahippocampal gyrus and the posterior portion of the amygdala. PRESS spectra (TE 30 ms, TR 3000 ms, 96 averages) were obtained from the VOIs after CHESS water suppression. In addition to peak area ratios of the metabolites NAA, Cr+PCr and Cho, institutional concentrations were determined using the LCModel software (Provencher, 1993). The metabolite concentrations reported by LCModel were divided by the fractional content of brain tissue (p[GM]+p[WM], where p[GM] and p[WM] represent the percentage of grey matter and white matter in the voxel, respectively) to correct for the relative proportions of grey and white matter, and cerebrospinal fluid (CSF) in the MRS voxel. These corrected concentrations were then calibrated to absolute molar units with respect to a phantom of known concentration, which was scanned in the same scanning session as the subject, using a PRESS acquisition with the same TE and TR.

## 3. Results

There were no structural abnormalities that precluded the inclusion of any of the subjects. We obtained reliable analysable data from the hippocampus in all 10 women and from DLPFC in 8 of the 10. After confirming that the data was normally distributed, we used simple paired *t*-tests to compare Cho and Cr concentrations in women at baseline and following 8 weeks of GnRHa. Measurement of plasma ovarian hormone levels at both time points confirmed that estradiol levels were within normal limits at entry into the study, and were suppressed following GnRHa administration ($p = 0.01$) (see Table 1).

All hormonal assays had a lower detectable limit leading to a skewed distribution of data. We therefore used (non-parametric) Wilcoxon rank tests for comparisons of plasma hormone concentrations across time. We found that women, post-ovarian suppression, had a significant increase in Cho concentration, and of Cho/Cr ratio in the DLPFC. Also we found a trend to a significant increase in the hippocampus ($p = 0.06$, two-tailed) (see Table 2). However, there was no difference in the concentration of any other metabolite pre- and post-suppression, or in regional brain volume.

## 4. Discussion

We investigated neuronal integrity in younger women pre- and post-acute ovarian hormone suppression using MRI and $^1$H MRS. Our results suggest that acute ovarian steroid suppression significantly increases Cho concentration (and

**Table 1** Variation in hormone concentration across time.

| Hormone concentration [median (25th, 75th quartile)] | Time 1 | Time 2 | *z* score | *p* |
|---|---|---|---|---|
| Estradiol (pmol/l) | 426 (215, 709.5) | 100 (62.25, 147.5) | −2.52 | 0.01 |
| Progesterone (nmol/l) | 5 (5, 36) | 5 (5, 5) | −1.6 | 0.11 |
| Testosterone (nmol/l) | 1.8 (0.8, 2.1) | 0.7 (0.7, 1.3) | −0.36 | 0.715 |
| Luteinizing hormone (μ/l) | 7.4 (4.5, 11.3) | 0.8 (0.8, 0.8) | −2.52 | 0.01 |
| Follicular stimulating hormone (μ/l) | 5.5 (3.75, 11.75) | 2.75 (1.35, 4.8) | −2.52 | 0.01 |

**Table 2** Variation in choline and choline/creatine concentration across time.

| | Concentration (SD) mM | | 95% confidence interval | | df | *p* |
|---|---|---|---|---|---|---|
| | Baseline | 8-week GnRHa | Lower | Upper | | |
| *Left hippocampus* | | | | | | |
| Cho | 1.17 (0.27) | 1.50 (0.34) | −0.67 | 0.02 | 9 | 0.06 |
| Cr | 4.30 (1.58) | 5.10 (0.30) | −2.12 | 0.53 | 9 | 0.21 |
| Cho/Cr | 0.29 (0.07) | 0.30 (0.06) | −0.07 | 0.03 | 9 | 0.52 |
| *Left dorsolateral prefrontal cortex* | | | | | | |
| Cho | 1.10 (0.21) | 1.27 (0.30) | −0.33 | −0.02 | 7 | 0.01 |
| Cr | 4.44 (0.78) | 4.37 (0.61) | −0.35 | 0.48 | 7 | 0.71 |
| Cho/Cr | 0.25 (0.05) | 0.29 (0.08) | −0.09 | −0.003 | 7 | 0.04 |

Cho/Cr ratio) in the DLPFC and possibly also the hippocampus. This supports our previous findings in a cross-sectional study into the effects of long-term ET in post-menopausal women (Robertson et al., 2001) and adds to the evidence that in women neuronal/glial membrane metabolism is affected by sex hormones.

The Cho is a rate-limiting precursor in the synthesis of acetylcholine and a precursor to cell membrane phosphatidylcholine. The Cho $^{1}$H MRS peak mainly reflects the total amount of free acetylcholine, Cho, glycerophosphocholine, phosphocholine and phosphoethanolamine. Cr is often used as a physiologically stable reference metabolite with which other metabolites are compared. Some previous $^{1}$H MRS studies have reported elevated Cho or Cho/Cr ratios in patients with Alzheimer's dementia (AD) and other degenerative dementias (Kantarci et al., 2004). Furthermore, a higher Cho/Cr ratio (in supraventricular white matter) has recently been reported to be associated with an increased risk of developing dementia or AD within 4 years (den Heijer et al., 2006). However, other studies have not reported higher Cho or Cho/Cr ratio in people with AD (Huang et al., 2001). The reasons for the disparity between studies remain unclear but are likely to include; (i) methodological factors such as between group differences (e.g., gender differences) in many cross-sectional studies and differences in the region(s) of brain studied and (ii) technical factors such as the magnet strength used and whether or not chemical shift imaging (CSI) methods were employed. It has been suggested, for example, that measurement of Cho concentration is more likely to be accurate with increased magnet strength using single-voxel MRS in specific brain regions than prescribed by CSI (Valenzuela and Sachdev, 2001). Overall, however, current studies suggest that higher Cho or Cho/Cr ratios may be a marker for risk of developing and/or current AD and other forms of dementia.

The cause of increased free Cho concentration may be due to loss of cholinergic neurons (Ross et al., 1997), and/or an increase in cell membrane phosphatidylcholine catabolism to provide free Cho for the subsequent deficiency in acetylcholine production (Kantarci et al., 2004). We, and others, have previously reported that sex hormones upregulate, and reduce the ageing of cholinergic neurons and receptors (Norbury et al., 2007; van Amelsvoort et al., 2003).

Several recent studies have suggested that ET may exert a neuroprotective effect against the development of AD (LeBlanc et al., 2001). Further, *in vitro* and *in vivo* studies in animals and humans (Maki, 2005) suggest that the neuroprotective effects of ET may be limited to a 'window of opportunity' immediately post-menopause, whereas administration of ET after this time may have a neutral (or negative) effect. Although the findings of a rapid increase in Cho in healthy women following the acute phase of oestrogen withdrawal may be consistent with this hypothesis, larger studies are still required to test this theory further.

The current study is limited by a number of factors that include the small sample size and the limited number of brain regions studied (e.g., acute ovarian steroid suppression may also increase Cho in the parietal cortex). Although scanner drift[1] could potentially confound the quantification

[1]Scanner drift refers to changes in the MR signal over time resulting from events such as recalibrations of the scanner,

of metabolite concentrations, the use of calibration spectra derived from a stable $^{1}$H MRS phantom scanned in the same session as each subject should correct for any variation in metabolite levels due to scanner drift. Correcting the concentrations for the amount of CSF in the MRS voxel should also correct for any between-session differences in regional brain volume. The study would also have benefited from a control group who did not undergo acute ovarian suppression. However, the absence of a between-group comparison is unlikely to fully account for the significant within group differences reported. Finally, the absence of a statistically significant difference in the Cho/Cr ratio in the hippocampal voxel may have been partly due to technical difficulties in using MRI and $^{1}$H MRS in this brain region. In particular, hippocampal spectra can suffer from susceptibility artefacts due to surrounding structures (e.g., bone, CSF, etc.) leading to poor shimming which may degrade the quality of the spectra and increase the variability in MRS measurements (Dixon et al., 2002; Hammen et al., 2005). This is particularly problematic for Cr as the secondary resonance may be affected by water suppression pulses. Future studies are needed with larger samples, including a control group, and a wider analysis of brain regions reported to be involved in memory processing and aetiological processes involved in dementia.

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## Conflicts of interest

Dr. Craig is funded by a Medical Research Council (UK) Fellowship.

Dr. Michael Craig, Dr. Ruth O'Gorman, Dr. David Lythgoe, Dr. Andy Simmons and Dr. Ginny Ng have not received any money from any pharmaceutical companies for consulting fees or sitting on advisory boards, etc. and do not have any equity ownership/stock options in publicly or privately traded firms.

Dr. Janice Rymer has received less than $10,000 per year as a member of the Advisory Board for Wyeth-Ayerst and has received less than $10,000 per year in lecture fees from Organon. She does not have any equity ownership/stock options in publicly or privately traded firms.

Dr. Pauline Maki has received less than $10,000 per year as a member of the Advisory Board for Wyeth-Ayerst and has received less than $10,000 per year in lecture fees from Wyeth-Ayerst. She does not have any equity ownership/stock options in publicly or privately traded firms.

Professor Murphy has received funding from the Medical Research Council (UK), the Wellcome Trust, the Psychiatry Research Trust and Wyeth-Ayerst for research.

(*footnote continued*)
replacement of scanner parts following maintenance, variations in the temperature of scanner electronics or slow degradation of electrical components. The changes tend to be small, but can be important in challenge studies such as this.

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