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Original article

Circulating antioxidant profile of pregnant women with asthma

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SUMMARY

Background & aims: One of the most prevalent complications of pregnancy is asthma which is associated with an increased incidence of intrauterine growth restriction. The mechanisms that affect fetal development in pregnancies complicated by asthma are not clearly defined. Antioxidants are particularly important during pregnancy due to their protective role against a state of high oxidative stress as gestation progresses. The current study was designed to characterise the circulating profile of tocopherols and carotenoids in pregnant women with asthma to determine whether asthma severity and dietary intake were associated with an altered antioxidant profile.

Methods: Maternal dietary intake and plasma and erythrocyte concentrations of tocopherols and carotenoids were examined in women with ($n = 84$) and without asthma ($n = 47$) at 18, 30 and 36 weeks gestation. Tocopherol and carotenoid levels were related to fetal and birth outcomes.

Results: Pregnant women with moderate/severe asthma were found to have increased plasma concentrations of total carotenoids ($P < 0.05$), lutein ($P < 0.05$ and α-tocopherol ($P < 0.02$) late in gestation compared to those women with mild asthma and healthy pregnant controls. Moderate/severe asthmatics had higher erythrocyte α-tocopherol quinone levels early in gestation relative to the controls ($P < 0.02$) but this marker of oxidative stress decreased as gestation progressed. Tocopherols and carotenoids were positively associated with birth weight centile ($P < 0.05$).

Conclusion: These findings suggest that the maternal system adjusts antioxidant pathways in response to the presence of a high oxidative load induced by asthma during pregnancy in an attempt to ensure continued fetal growth in an adverse environment.

1. Introduction

Dietary antioxidants such as tocopherols and carotenoids are likely to have a significant effect in modulating systemic oxidative stress. Tocopherols (vitamin E) are low molecular weight substances that act to break free radical chain reactions involved in lipid peroxidation.[1] In this way, they are thought to provide a protective mechanism against oxidative damage to the body.[2] The most active of the tocopherols is α-tocopherol which converts lipid peroxyl radicals and oxygen radicals found in lipid membranes to less reactive forms[2] in order to maintain the integrity of membrane fatty acids.[3] Carotenoids are tetraterpenoid organic pigments that are naturally occurring in the chloroplasts of plants. There are two classes, xanthophylls which include lutein and zeaxanthine and carotenes which include α-carotene, β-carotene and lycopene. Carotenoids (β-carotene, α-carotene, γ-carotene, and β-cryptoxanthin) primarily act as antioxidants by scavenging free radicals.

Normal pregnancy has been characterised as being a state of high oxidative stress.[4] Oxidative stress is defined as an imbalance between the cellular generation of Reactive Oxygen Species (ROS) and the capacity of antioxidants to prevent oxidative damage. The increased maternal and fetal utilisation of energy, as well as an increased oxygen intake as gestation progresses promotes oxidative stress and places huge demands on the maternal system to balance the generation of ROS via the up regulation of antioxidant mechanisms. Pregnancies complicated by inflammation and oxidative stress such as pre-eclampsia[5] have been reported to have higher levels of maternal circulating α-tocopherol.[6] Gamma (γ) tocopherol was found to be decreased in pre-eclamptic women relative to healthy pregnancies.[7] Levels of lycopene were found to be reduced in pre-eclamptic women.[8] These studies suggest that in a high oxidative stress environment antioxidant defences are

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compromised and in some cases there may be a compensatory increase in the circulation of some dietary antioxidants in an attempt to protect the maternal system from oxidative stress. However it is currently unknown whether antioxidant mechanisms are altered in pregnant asthmatic mothers.

We have previously reported that α-tocopherol levels are lower in non-pregnant asthmatics than healthy controls[9] and reduced circulating levels of antioxidants are associated with airway hyperresponsiveness.[10] Other studies reported reduced levels of circulating carotenoids in serum,[11] and whole blood[12] of non-pregnant asthmatic subjects. These studies suggest that worsening asthma disturbs the oxidant-antioxidant balance. Pregnancies complicated by maternal asthma are associated with an increased incidence of intrauterine growth restriction [13, 14], with significant implications for both the short and long term health of the offspring[15] Reduced fetal growth may be a consequence of high oxidative stress in pregnancies complicated by asthma. Cigarette use by pregnant women with asthma is a highly prevalent comorbidity of these pregnancies[14] that could also significantly lower antioxidant defences and impact on fetal development. It has been previously shown that cigarette use can reduce circulating antioxidants in non-pregnant subjects.[16] Since we have previously identified that tocopherols[9] and carotenoids[10] are altered in non-pregnant asthmatics, the current study was designed to assess if there are any differences in the circulating tocopherols and carotenoids of pregnant women with asthma compared to women without asthma and whether these differences were related to dietary intake, asthma treatment, asthma severity or cigarette use. It was hypothesised that maternal asthma during pregnancy would be associated with reduced circulating concentrations of tocopherols and carotenoids which would be further depleted by increased asthma severity and cigarette use. We also hypothesised that a reduction in circulating antioxidants would be associated with poor fetal outcomes.

2. Methods

2.1. Experimental subjects

The study was approved by the Hunter New England Area Health Service and University of Newcastle Human Research Ethics Committees. Pregnant women were recruited at the John Hunter Hospital antenatal clinic during the first trimester ($n = 135$; controls $n = 47$, and asthmatics $n = 84$) and provided written informed consent for participation. The protocol for this study has been described in detail previously.[17,18]. Using the smallest difference observed previously in our analyses of carotenoid levels in non-pregnant asthmatic and healthy control women,[12] a power calculation was performed allowing a-priori for analysis of asthma severity and treatment effects. To identify a true difference in means of 15 ug/l with standard deviation 12, we needed to recruit a minimum of 32 control women and 64 women with asthma (32 with mild asthma and 32 with mod-severe asthma) for power equal to 80% and a type 1 error rate of 0.05. The inclusion criteria for women with asthma were women with a doctor diagnosis of asthma who were less than 18 weeks pregnant at the time of consent. The inclusion criteria for control subjects were women with no pre-existing health problems who were less than 18 weeks at the time of consent. Smokers and obese women were included in the study as these are common co-morbidities associated with pregnancies complicated by asthma. Those women with complications other than asthma such as pre-eclampsia, gestational diabetes, infection or preterm delivery were excluded from the analysis retrospectively ($n = 4$). Birth weight and fetal sex were determined at birth.

Clinical asthma severity was rated as mild, moderate or severe using the integrated severity score described in the Australian Asthma Management Guidelines,[19] which closely approximate the National Heart, Lungs and Blood Institute Guidelines.[20] Proper inhaler use and compliance was assessed by the study research nurse.[21] Cumulative, inhaled corticosteroids (ICS) dose was calculated for each trimester, and summarised as the mean daily dose of beclomethasone dipropionate or equivalent used during pregnancy, where 1 μg BDP was considered equal to 1 μg budesonide or 0.5 μg fluticasone propionate.[22] For data analysis, the low, moderate and high ICS dose groups were combined. Some women ($n = 36$) were using the combination of a long acting β2 agonist with ICS. Asthmatic women in all groups, including the no glucocorticoid group, used the inhaled short acting β2 agonist, salbutamol for symptom relief when required. Current smoking status was assessed by direct questioning at recruitment. All women participating in the study underwent ultrasound assessment at 18, 30 and 36 weeks of gestation. Fetal biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) were measured. Birth weight, length and head circumference were recorded at delivery. Customised birth weight centiles were calculated using www.gestation.net, which accounts for maternal height and weight, ethnicity, parity, fetal sex and gestational age. Length and head circumferences were converted to centiles using John Hunter Hospital growth charts. Gestational age was determined by date of the last menstrual period and confirmed at 18 week ultrasound. Gestational age was not determined at a 12 week ultrasound as these scans are conducted in private clinics and the data was unavailable to the study.

2.2. Blood collection

Twenty mL of whole blood was collected into EDTA coated tubes from the median cubital vein at each visit (18, 30 and 36 weeks). Whole blood was centrifuged at 3000 rpm at 4 °C for 10 min. Plasma and erythrocytes were decanted into 500 μL aliquots and stored at −80 °C until required for analysis. Erythrocytes were used as a representative measure of antioxidant accumulation in tissues and provided a longer term measure of antioxidant status as well as an indication of the degree of protection of cell membranes from oxidation.

2.3. 24 hour food recall

At each gestational visit, participants completed a 24 h (hr) food recall questionnaire which has previously been shown to be a useful tool for analysing mean dietary intakes.[23] The questionnaire asked for detailed information on meals and snacks consumed 24 h prior to the clinic visit. For this study, the data was analysed from a subgroup of women (i.e. those who had each provided a blood sample and filled out the 24 h food recall questionnaire at each gestational time point), incorporating controls ($n = 32$) and asthmatics ($n = 57$). Data were entered into the Foodworks (Xyris, Brisbane) database, incorporating the AusFoods (Brands) and AusNut (All Foods; Food Standards Australia & New Zealand) were extracted.

3. Biochemical analyses

3.1. Analysis of tocopherols and carotenoids in plasma

Antioxidant analysis was performed based on an established method using high performance liquid chromatography. All work was carried out in the dark to prevent photooxidative degeneration and samples were kept on ice. 400 μL (μL) of plasma was transferred into polypropylene culture tubes to which 1 mL (mL) of