Science Digest

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Chocolate and its effects on pregnancy.

The intake of products from *Theobroma cacao* are considered to have potential health benefits resulting from the intake of flavonoids strongly associated with this plant. Chocolate, a product from *Theobroma cacao*, is a source of flavonoids that has been associated with beneficial effects for the cardiovascular system, reduction of insulin resistance, improving lipid profiles and anti-inflammatory effects. These beneficial effects are due primarily to the antioxidant effects of the flavonoids. A publication from Italy investigated the potential effects of chocolate (cacao content ≥70%) in pregnancy (1). After screening for potential pregnancy-related complications 90 women (46 in Group A and 44 in Group B) entered the trail. Group A received 30g of ≥70% chocolate per day and Group B were not given a daily chocolate supplement.

The trial was initiated at first clinic visit (11 to 13 weeks gestation) and continued until delivery at term. Group B received the equivalent energy supplements to Group A. Diets and additional chocolate consumption was monitored by a dietician and any additional chocolate was classified as >60% or < 60% cacao. While there were no significant changes in the haematological parameters in Group A, chocolate had a positive effect on glycaemic and liver parameters and was effective in reducing both blood pressure and cholesterol during the pregnancies. The authors concluded that a moderate amount of high cocoa content chocolate might be a valuable supplement during pregnancy.

What do you know about sunscreens?

Sunscreens are a prominent factor in helping to prevent UV exposure-induced skin cancers and have been strongly advocated for this preventative measure. The use of sunscreens varies and is usually related to the length of time an individual is exposed to UV in sunlight and the area of the body covered. While historically there have been concerns relating to the composition of certain sunscreens, it is generally accepted that the current over the counter (OTC) sunscreens are safe to use and are strongly advocated for reducing the risk of skin cancer. A recent publication from the USA has provided new research information relating to the absorption of photo-protective agents through the skin and at detectable concentrations in the blood (2).

In this research four OTC sunscreens (two sprays, one lotion and one cream from different manufacturers, but not named) containing the active chemicals, avobenzone, oxybenzone, octocrylene and ecamsule were tested. Four groups of volunteers (three men and three women in each group) used one of the four sunscreens applying a set amount every two hours over 75% of their body surface area outside of normal swim wear four times a day for four days. Sequential blood samples were collected for seven days. All blood samples were analysed by liquid chromatography and tandem mass spectrometry.

All four photo-protective chemicals were found in the blood samples. Plasma avobenzone was detected from all four products and exceeded 0.5ng/ml (see below) from day one of use usually within six hours of application. Plasma oxybenzone was identified in only three products and the use of the three containing oxybenzone exceeded 0.5ng/ml within two hours of application and peaked at around 100ng/ml for the duration of the trail and a slow decrease for three days after cessation of use. Plasma octocrylene exceeded 0.5ng/ml peaking at around 5ng/ml through to day seven. Only one product contained ecamsule and plasma concentrations peaked at 1.5ng/ml but generally remained below 1.0ng/ml. While the authors recognized the limitations of this relatively small trail the levels of the compounds detected exceeded the FDA guidelines to trigger a systematic testing of chemicals with health related issues (0.5ng/ml). This concentration was exceeded by day one of the trail. The health impact of these four compounds in this research is not known.

Does phototherapy for neonatal jaundice affect oxidant/antioxidant status?

Hyperbilirubinaemia is a common problem in neonatal care, which may result from many causes. However, because of the potential neurotoxicity of unconjugated bilirubin in neonates due to an immature blood brain barrier and due to the lipid solubility, it can be absorbed in the brain resulting in the most severe outcomes of yellow staining of the brain (kernicterus). Historically neonatal hyperbilirubinaemia has been shown to equate with poor neonatal/paediatric outcomes resulting in loss of hearing and failure to achieve milestones. Phototherapy is an effective, non-invasive, technique for treating what is often a transitory elevation of bilirubin. The principle of phototherapy is that bilirubin photo-oxidises at 420 to 490nm into water-soluble isomers that are excreted.

Recent research from Egypt has investigated the effect of phototherapy on neonatal oxidant/antioxidant status (3). In this research 120 neonates requiring phototherapy (but with normal full-term birth weights) were randomly divided into three groups of 40 and each exposed to one of three phototherapy techniques: conventional (CP), intensive (IP) and LED (LEDP). All neonates had blood taken before treatment and 24 hours post-treatment. Neonatal complications other than hyperbilirubinaemia were excluded from this trial. Besides the biophysical parameters being monitored and plasma unconjugated and conjugated bilirubin, the researchers measured malondialdehyde (MDA), nitric oxide (NO), total antioxidant capacity (TAC) as well as zinc, iron and copper, which all have the potential to mediate free radical reactions.

While all three phototherapy techniques reduced circulating bilirubin to safe concentrations, the researchers found that they differed in the range of oxidant/antioxidant parameters measured. The generation of MDA, NO and TAC had the lowest response to LEDP exposure followed by CP, and IP was third. Copper and iron (pro-oxidants) were more responsive in the IP treatment, with zinc (anti-oxidant) being highest in the LEDP group. While the authors concluded that all phototherapy techniques induced oxidative stress to the neonates, the LEDP treatment had less overall effect than either CP or IP treatments.

Converting blood group A to blood group O.

The use of the ABO blood group O in blood transfusion is acknowledged as a “universal donor” in that conceptually it can be transfused to other recipients with dissimilar ABO blood groups i.e. A, B and AB. While the reverse is not true as group A contains antibodies against B antigen and group B contains antibodies against A antigen. The ability to use group O as a “universal donor” is due to the red blood cells (RBCs) lacking certain carbohydrate structures on their cell surface. Group A has α-1,3-linked-N-acetylgalactosamine (GalNAC) and group B galactose, which confer the specificity of the blood group system. Historically attempts to remove the carbohydrates using enzymes has demonstrated the possibility of converting

blood groups A and B to O. However, the technology is limiting and the quantities of enzymes required is large/unit (mg to gm), rendering this approach impractical.

Research from Canada has reported overcoming some of the major hurdles in conversion of blood groups A and B to O (4). The authors considered that that the gut microbiome should have enzymes capable of removing GalNAC as this carbohydrate is present in gut mucins as a bacterial protective agent. A meta-genomic library was established from faecal samples from a blood group AB positive male donor. Library screening and biochemical analysis identified eleven hits with A antigen cleaving activity and one with B antigen activity. The eleven fosmids were sequenced and the sequences identified the bacteria the fosmids were obtained from. The genes were cloned and expressed in *E. coli* and the resulting proteins purified and characterised.

Two highly active enzymes were identified from *Flavonifractor plautii* which cleaved both A and B antigens. Using bioinformatics analysis, FpGalNAC deacetylase preliminary structure was determined followed by x-ray crystallography, which provided the protein structure and the catalytic domain of the enzyme. When purified enzyme was incubated with group A RBCs the enzyme removed all A antigenicity at a concentration down to 3mg/ml. Using fluorescence activated cell sorting and conventional agglutination techniques the converted group A RBCs were tested against anti-A and anti-H (present on group O RBCs). A complete conversion of A antigens to H antigens was demonstrated. To further confirm this result, blood from 26 blood group A donors were treated with FpGalNAC deacetylase in-vitro and all were converted to H antigens. Finally the researchers demonstrated that the enzymes could be removed by washing the RBCs and centrifugation. They considered that the use of the enzymes demonstrated a possible cost-effective process that would fit current blood transfusion systems.

Evolutionary loss of a gene predisposes humans to atherosclerosis.

Humans are the most prone species to cardiovascular disease (CVD) and atherosclerosis and despite the identity of risk factors, approximately 15% of first time heart attacks occur in the absence of the risk factors. Other animal species with similar risk factors, e.g. carnivores, do not develop CVD. While there is no doubt about the multiplicity of risk factors in humans, the puzzle has been the lack of development in other animal species.

Research from the USA may have provided a new understanding on the biology of atherosclerosis (5). About 2 to 3 million years ago humans lost the ability to synthesis N-glycolylneuramic acid due to the loss of the enzyme cytidine monophosphate –N-acetylneuraminic acid (Neu5Ac) hydrolase (*CMAH*) which results in an excess of sialic acid (Neu5Ac), the precursor for Neu5Gc in the glycocalyx in human cells.

Deficiency of Neu5Gc is implicated in glucose intolerance and hyperactive macrophages. In addition Neu5Gc is obtained from red meat and once absorbed is incorporated into glycolipids and glycoproteins. Although the amounts are small they accumulate in the epithelium and endothelium, initiating a xeno-antigen response, which in turn promotes local chronic inflammation (most humans have circulating xeno-antigens to Neu5Gc).

Using a mouse model for human atherosclerosis the authors inactivated the *Cmah* gene and demonstrated development of atherosclerosis. They then gave the mice non-human Neu5Gc (equating with red meat consumption) and demonstrated a high immune response and a diabetic phenotype. The mice also demonstrated an increase in foam cells and a decrease of phagocytosis activity. The authors conclude that the lack of the *CMAH* gene in humans helps to explain the significant difference between humans and other species for the susceptibility to atherosclerosis and CVD but indicate that further research is required relating to the metabolism of extrinsic Neu5Gc and its potential for initiating an immune response.

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