control (3% corn oil diet), high corn oil (20%) or high olive oil (17% olive oil + 3% corn oil) diets from weaning or after carcinogenic induction. Firstly, we analysed the clinical and histopathological features of the mammary tumours. Then, we analysed the expression and activity of the antioxidant enzymes superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), as well as the non enzymatic antioxidant capacity by means of reduced (GSH) and oxidized (GSSG) glutathione levels and the GSSG/GSH ratio. Levels of F2-isoprostane (F2-IsoP) were measured as markers of lipid peroxidation. To evaluate oxidative DNA damage we determined 8-oxo-2′-deoxyguanosine (8-oxo-dG) levels and DNA breaks levels by the comet assay. Results showed that the HCO diet increased oxidative stress in the mammary gland related with its clear promoting effect on the carcinogenesis. The HOO diet conferred to the adenocarcinomas a clinical behaviour and histopathological pattern compatible with a lower degree of malignancy in comparison with the HCO diet. However, these effects were also associated to an increase in some oxidative stress parameters in the mammary gland, although the different 8-oxo-dG and GSH levels suggest a differential influence compared to the HCO diet. Oxidative stress was higher in mammary adenocarcinomas compared to adjacent normal tissue irrespective of the diet and the timing of administration, as evidenced by the lower antioxidant capacity of tumours. This effect was more important in the high fat diet groups. Nevertheless, high fat diets did not clearly modify the oxidative status of mammary adenocarcinomas which would be more dependent on the neoplastic transformation. In conclusion, changes in the oxidative stress levels of the mammary gland may be a mechanism by which high fat diets influence the carcinogenesis but in tumours this status would be more independent on the dietetic factors such as dietary lipids.

879 Increased MGMT expression by dietary constituents may be involved in their chemopreventive effects

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Alkylating agents present in the diet or endogenously produced may be mutagenic and carcinogenic. There have been several reports, including our own unpublished observations, of dietary chemoprevention conferred against alkylating agents. O^6-Methylguanine (O^6MeG) is an important promutagenic DNA adduct produced by SN-1 methylating agents. If these methyl adducts are not repaired by the cell, specifically by methylguanine methyltransferase (MGMT), they are converted in O^6MeG:T mismatch, which can be repaired by the mismatch repair system (MMR). O^6MeG still remains in one of the template strands and this will cause the repair process to be repeated, creating a "futile repair loop". This loop will eventually result in toxic double-strand breaks and will induce apoptosis. When both of these systems fail to repair, O^6MeG results in point mutations that can possibly initiate the carcinogenic process. In the present study the possible role of dietary constituents in the induction of expression and activity of the enzyme MGMT in colon cell has been investigated. A modified version of the comet assay has been developed to assess MGMT repair activity.

UA, a dietary triterpenoid, increased MGMT expression in Caco-2 after 6 hour incubation. In the attempt to see if this increase corresponds to an increase of O^6MeG repair, two colon cell lines (Caco-2, MMR efficient, and HCT116, MMR defective) were treated with the MGMT inhibitor BG (2hrs) followed by MNU exposure, and the DNA damage was evaluated 1, 24, 48 and 72 hrs later, by the comet assay. In Caco-2, after 1 hr MNU induced DNA damage detected by comet assay. This damage decreased in a time-dependent manner, in which this decrease was most marked in cells without BG treatment. After 72 hrs, the cells treated with BG present significantly more damage than cells without BG. This difference corresponds to O^6MeG that were converted in strand breaks by the MMR system. To prove this idea, the same treatments were performed in HCT116 cells and we found that there were no differences between cells with and without BG, due to a deficient MMR system.

In conclusion, we showed that it is possible to quantify O^6MeG by the comet assay in MMR proficient cells. As we found that UA increased MGMT expression, we can now use the comet assay to investigate if this increase corresponds to an increase in O^6MeG repair by MGMT. Evaluation of the effects of UA on damage induced by therapeutic alkylating agents should also be done.

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880 Diets high in corn oil or extra-virgin olive oil provided from weaning differentially modify sexual maturation and susceptibility to mammary carcinogenesis in rats

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Diverse evidence has shown the importance of early life events, including nutrition, in the modification of breast cancer risk. We investigated the effects of high-fat diets on sexual maturation, mammary gland development and its susceptibility to malignant transformation. Female Sprague-Dawley rats were fed a low fat (LF), a high corn oil (HCO) or a high extra-virgin olive oil (HOO) diet from weaning, gavaged with 7,12-dimethylbenz[a]anthracene (DMBA), and euthanized at 24, 36, 51, 100 and 246 days. We studied several parameters of growth and sexual maturation: body weight and mass index, vaginal opening, serum hormone levels, histological evaluation of the ovaries activity and morphological evaluation of mammary gland differentiation. Moreover, the expression of kisspeptin in hypothalamus as well as β-casein and estrogen and progesterone receptors in mammary glands was assessed by real time PCR. The clinical manifestation of carcinogenesis was studied by the tumour incidence (percentage of affected rats) and tumour yield (total number of tumours per group). The results showed that the body weight and body mass index increased in the HCO group in relation to the LF group. Vaginal opening was advanced in both high-fat groups, especially in the HCO group. Such group had also an increased body weight around puberty, higher number of corpora lutea at post-puberty, and tended to have higher mRNA levels of kisspeptin in hypothalamus. Both high-fat diets induced subtle modifications in the mammary gland morphology. No changes were found on serum hormone levels or the mRNA expression of β-casein and hormone receptors of the mammary gland among groups. The HCO diet had a clear stimulating effect of the carcinogenesis, inducing the highest tumour incidence and yield, whereas the HOO diet seem to have a weak enhancing effect, since only increased the tumour yield in comparison with the LF diet. Our data suggest differential effects depending on the oil that is consumed, showing that administration from pre-puberty with the HCO diet strongly advanced maturation and increased breast cancer risk, while the animals fed the HOO diet were more similar to the controls.

881 Effect of Pteridium aquilinum in gastric epithelial cells: potential synergistic effect with Helicobacter pylori infection

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Helicobacter pylori (Hp) is an important etiological factor in the development of gastric cancer. The molecular mechanisms of *Hp*-induced gastric carcinogenesis and the interplay with other environmental factors, such as toxins present in the human diet, are not fully understood. *Pteridium aquilinum* – bracken fern – is the only higher plant known to naturally cause cancer in animals. The carcinogenic toxin of bracken, ptaquiloside, was identified not only in the plant, but also in cow's milk and water. Moreover, epidemiological studies have consistently shown an association between bracken exposure and gastric cancer. The aim of the present work is to evaluate the involvement of *Pteridium aquilinum* in the gastric carcinogenesis process and its potential synergistic effect with *Hp* infection.

Bracken fern extracts damaging effects were investigated on *Hp* culture *in vitro*. Alterations associated with extract exposure were also analyzed on gastric epithelium either in the presence or absence of *Hp*. In addition, *Hp*-infected or not infected C57Bl/6 mice were also treated with *Pteridium aquilinum* in order to identify histological and molecular alterations in the gastric mucosa.

An inhibitory effect of bracken fern extracts was observed on *Hp* growth and survival *in vitro*. In gastric epithelial cells culture the bracken fern extract (20−40 mg extract/ml) decreased the number of viable cells in proliferation. Both γH2AX immunofluorescence and western blot analysis showed induction of DNA double strand breaks in cells exposed to extracts. *In vivo*, KI-67 immunostaining also indicated an increased proliferative index on gastric mucosa cells of mice exposed to bracken fern independently of the infection status. We further evaluated glycosylation alterations on the mice gastric mucosa upon *Pteridium aquilinum* exposure, and an increased expression of sialylated structures was observed.

Our *in vitro* results show the DNA damaging effects of *Ptequiridium aquilinum* extracts on gastric epithelial cells. In addition, results from the mouse model suggest that *Ptequiridium aquilinum* extract in association with *Hp* infection might have an important role in the human gastric carcinogenesis process.