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# Processed meat, added with nitrite and aerobically stored, promotes DMH-induced mucin depleted foci in the rat colon,

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## Abstract

Processed meat intake is associated with the risk of colorectal cancer. Our first aim was to support these epidemiological data with experimental data. Most processed meats are added with nitrite and cooked, producing free nitrosyl heme that may be more toxic than red meat myoglobin. Our second aim was to assess the effect of meat processes on carcinogenesis promotion.

- DESIGN:

We designed 3 experimental studies.

#1- An exploratory short-term study was designed with 16 models of cured meat, in a 2x2x2x2 design. Four factors were crossed: heme level in muscle, added nitrite, cooking temperature and anaerobic packaging. These meat products were analyzed for nitrosyl heme, hexanal, oxidative stability and pH. Sixteen groups of F344 rats were fed a low calcium diet containing a cured meat (55% dry mater) for 14 days, and biological samples collected. We measured fecal and urinary biomarkers (cytotoxicity, lipid peroxidation) previously associated with heme-induced promotion.

#2- Promoting effect of 4 model cured meats selected from study #1 was looked for in a 100-day study. Moist cured meats were included (47% dry matter) into low-calcium AIN76-based diets. Carcinogenesis endpoints were mucin depleted foci (MDF) and aberrant crypt foci (ACF) in the colon of dimethylhydrazine initiated F344 rats.

#3- Promoting effect of a 55% freeze-dried ham diet was looked for in a second 100-day rat study. It was a cooked cured ham obtained from a local supermarket. Carcinogenesis endpoints were MDF and ACF.

- RESULTS:

#1- Cytotoxicity and lipid peroxidation of fecal water, and a urinary marker of n-6 fat peroxidation (DHN-MA), dramatically increased in some groups but not all: the 4 tested processing factors modulated significantly the biomarkers in rats (all ANOVA $p<0.01$). A principal component analysis of data allowed us to select 4 cured meats out of 16, with typical patterns of biomarkers, for inclusion into study #2.

#2- The diet containing oxidized cooked nitrited high-heme meat significantly increased the number of MDF per colon compared to control diet (4.1 and 2.9 MDF/colon respectively, $p=0.04$). MDF were more abundant in those rats than in rats fed with similar non-nitrited meat or with similar non-oxidized meat (both $p<0.05$). The 4 cured meat increased ACF formation in rats (23 to 31% increase, all $p<0.05$).

#3- Freeze-drying strongly induced fat peroxidation in ham. Ham-fed rats and controls had 8.5 and 3.5 MDF/colon respectively ($p<0.0001$). Ham diet also increased ACF formation (+13%, $p<0.05$). Ham-induced promotion correlated with the above cited fecal and urinary biomarkers.

- CONCLUSION:

Freeze-dried cooked ham, and a model cured meat (similar to badly packaged cooked ham), promoted colon carcinogenesis in rats. Nitrite and oxidation independently played a part in this promotion. Results point to packaging and processing modifications toward healthier cured meat.


## WHY WAS THIS STUDY DONE ?

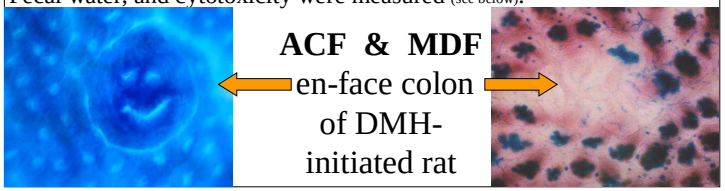
♦ Processed meat intake is associated with the risk of colorectal cancer in epidemiological studies. To reduce colorectal cancer risk, WCRF 2007 panel recommends: "**Avoid processed meat**".

♦ **Aim 1 :** To support epidemiological data with experimental data.
♦ **Aim 2 :** To compare different ways of processing meat: does it change promotion of chemically-induced colon carcinogenesis in rats?

**Hypothesis** : Ham and hot-dogs are added with nitrite and cooked. It produces free nitrosyl heme. We suggest it would be more toxic than native heme, bound inside red meat myoglobin.

## Carcinogenesis Endpoints: ACF & MDF

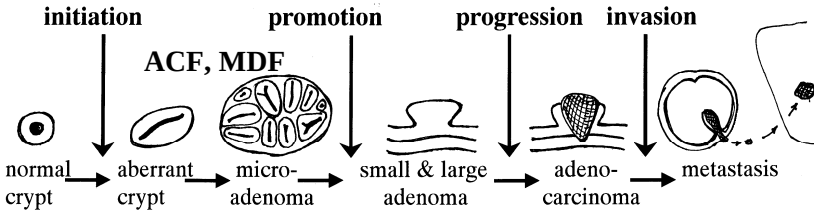
Rats were given an injection of dimethylhydrazine (DMH) and 7 days later randomized to various diets with processed meat for 100 days. Colon carcinogenesis was assessed by counting aberrant crypt foci (ACF) and mucin depleted foci (MDF), considered as precancer lesions. Fecal water, and cytotoxicity were measured (see below).




## Several fecal and urinary biomarkers correlate with heme-induced promotion

In fecal and urine samples from rats given red meat or hemin, TBARs, DHN-MA, and cytotoxicity measures after 14 d on diet, correlate with ACF and MDF promotion after 100 d on diet. *(Pierre et al., 2003; 2004; 2008)*

- Cytotoxicity of fecal water on apc+/+, apc +/-, and CMT93 cells
- Lipoperoxidation of fecal water, assessed by TBARs assay
- A urinary marker of n-6 fat peroxidation: 4-hydroxynonenal (HNE), measured as its urinary metabolite, DHN-MA

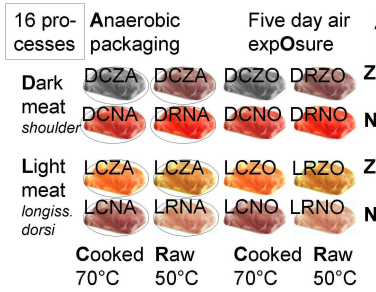



## Study #1 Design - 16 Models of Cured Meat

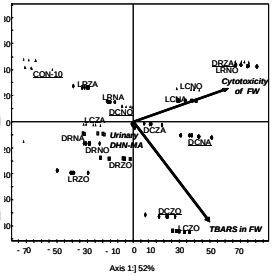
An exploratory short-term study was designed with 16 models of cured meat, in a 2x2x2x2 design. Four factors were crossed: **heme** level in muscle (L, light/D, Dark), **cooking** temperature (R, Raw/C, Cooked), added **nitrite** (Z, zero nitrite/N, Nitrite), and anaerobic **packaging** (A, anaerobic/O, oxidized. These 16 meat products were analyzed for nitrosyl heme, hexanal, pH and oxidative stability. Sixteen groups of F344 rats were fed a low calcium diet containing a cured meat (55% dry mater) for 14 d, and biological samples collected. They were compared with a control group given a 10% fat diet with no meat (CON). We measured fecal and urinary biomarkers previously associated with heme-induced promotion (cytotoxicity, lipid peroxides).

## Study #1 Results

Cytotoxicity and lipid peroxidation of fecal water, and a urinary marker of n-6 fat peroxidation (DHN-MA), dramatically increased in some groups but not all: the 4 tested processing factors modulated significantly these biomarkers in rats (all ANOVA $p<0.01$). A principal component analysis (PCA) of data allowed us to select 4 cured meats out of 16, with typical patterns of biomarkers, for inclusion into study #2: Model meats DCNO, DCZO, DCNA, and DRZA were selected.




**Design to make sixteen processed meat models**




**Principal Composant Analysis of biomarkers in 16 groups of rats given 16 cured meat models**