The effect of RN genotype on the formation of two carcinogenic heterocyclic amines, MeIQx and 4,8-DiMeIQx in fried pork

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Background
Carcinogenic heterocyclic amines (HCAs) are formed at ng/g levels during frying of meat. They are formed from creatine, glucose, free amino acids and some dipeptides (Jägerstad et al. 1983) via the Maillard reaction, and their formation depends on the cooking conditions and the type of meat. A large number of studies deal with the quantification HCAs in fried muscle foods of various origin. However, few of these studies take into consideration the natural variation of meat composition due to genetic and environmental factors. In order to make realistic and reliable estimations of the human intake of mutagenic/carcinogenic HCAs, it is important to study and perhaps control the variation of different muscle components that serve as precursors in the formation of HCAs.

Over the years, a particular major gene, the so-called RN^- allele, has been proven to have a significant influence on production and meat quality traits in the Swedish slaughter pig population. The dominant RN^- allele frequently occurs in Hampshire and Hampshire crosses. In the context of HCA precursors, it is most interesting to note that the RN^- allele causes significantly higher glycogen content in glycolytic muscles (Naveau 1986, Le Roy *et al.*, 1990).

Objectives
The main objectives of this study were to investigate the natural variation of glycogen/glucose, free amino acid and creatine levels in pig meat and to correlate these variations to genetic and environmental factors. Another objective was to study the formation of HCAs in fried patties in relation to meat composition.

Material and Methods
The animal material consisted of 240 Hampshire cross breeds, divided into four trial groups that were fed either conventional slaughter pig feed or feed that was composed after ecological principles, with or without the addition of hay. The pigs were all reared indoors in conventional pens. As previously reported, a number of technological and chemical meat quality parameters were determined in *M Longissimus dorsi* (LD) in subsamples of this larger material (Nilzén *et al.*, 1999).

RN genotype (n=99) was determined both by means of meat juice (concentration of glucose + glucose-6-phosphate (G-6-P)) from LD as described by Lundström and Enfält (1996) and as extracted from muscle tissue (concentration of glycogen and glucose + G-6-P) (Fernandez *et al.*, 1992). Creatine (n=33) was extracted from the meat and thereafter quantified by a spectrophotometric method (Wahlefeld *et al.*, 1974).

With the help of a Principal Component Analysis, 26 animals representing the different rearing regimes, sexes and RN genotypes and at the same time exhibiting "extreme" analytical results (in order to build in some variation in the model) for glycogen/glucose, creatine and other technological and chemical meat quality parameters measured, were picked out. The free amino acid (FAA) content in LD from these 26 animals was analysed with the assistance of David Eaker, Aminosyraanalyscentralen, BMC, Uppsala, Sweden.

A part of the LD from these 26 animals was minced, formed into patties and fried in a well-standardised way, at 200°C, 3 min per side. The outer layer (crust) was removed using a scalpel, and crusts were stored at -20°C until extraction two weeks later. Samples were extracted and purified according to the solid-phase extraction method of Gross and Grüter (1992) with some modifications (Fay *et al.*, 1997: Borgen unpublished data). HCAs were separated using reverse-phase HPLC (Gross *et al.*, 1992). The column (ODS 80™ TosoHaas, 250 x 4.6 mm i.d., 5µ) was eluted with acetonitrile and 0.01 M triethyl amine (pH adjusted to 3.6 with acetic acid). The flow rate was 1 ml/min and the injection volume was 90 µl. Chromatograms and spectra were obtained using a photodiode array UV detector (Varian 9065, Polychrome). Peaks were identified and quantified using retention times and the spectra from reference samples of known concentrations, run under the same conditions.

Results and discussion
The concentrations of glycogen and glucose + G-6-P varied considerably due to whether the pigs were carriers or non-carriers of the RN allele, 20 vs. 64 µmol/g, meat. The mean creatine value in the 33 samples analysed was 5.0 mg/g meat (SD 0.47), ranging from 3.8 to 5.8 mg/g meat. The levels of creatine were affected by an interaction between sex and RN genotype.
When studying the free amino acids in the meat ($n = 26$) it was discovered that the level of anserine, ornithine, glycine, taurine and the dipeptide carnosine was affected by the RN genotype. β-alanine, citrulline and ornithine concentrations were affected by feeding regime, ecological or conventional.

The mean percentage of crust, measured as the darker, harder parts peeled of the surface of the fried patties was 30.7% (SD 8.9). Mutagenic/carcinogenic MeIQx and 4,8-DiMeIQx were detected in all the samples in the range of < 0,1 ng/ g meat to 2,4 ng/g meat

(wet weight). There was no correlation between the concentrations of glycogen/glucose + G-6-P and MeIQx but 4,8-DiMeIQx levels were negatively correlated (- 0.456) to glycogen/glucose + G-6-P concentrations (p= 0.038). A one way analysis of variance revealed no significant effect of RN genotype on the levels of MeIQx, on the other hand there was a tendency (p=0.062) of higher concentrations of 4,8-DiMeIQx in fried meat of the non-carriers of the RN$^-$ allele (Table 1). This might be explained with the fact that the formation of HCAs is influenced both by the levels of precursors as well as the ratio between them. Thus, in this experiment the ratio between the precursors might have been more favourable in the meat from the non-carriers of the RN allele.

Conclusions

The precursors of HCAs were all affected by, primarily, RN genotype but also by feeding regime, ecological or conventional. The RN genotype, or more directly, the concentration of glycogen/glucose in the meat, did not influence the yield of the mutagenic/carcinogenic HCA, MeIQx. Levels of 4,8-DiMeIQx were on the other hand negatively correlated to glucogen/glucose+ G-6-P concentration and there was a tendency towards higher levels of 4,8-DiMeIQx in fried meat of the non-carriers of the RN$^-$ allele.

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Table 1. *The content of MeIQx and 4,8-DiMeIQx in fried pork from animals of different RN genotype, least-squares means ± standard error*

		RN genotype		
Heterocyclic amine	n	rn+/rn+	RN$^-$/-	p-value
MeIQx, ng/g meat (wet weight)	22	1.4 ± 0.7	1.3 ± 0.8	0.659
4,8-diMeIQx, ng/g meat	20	0.4 ± 0.2	0.2 ± 0.2	0.062

Figure 1. *MeIQx and 4,8-DiMeIQx in a spectrum of an unspiked sample.*