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Transcript levels of genes implicated in steroidogenesis in the testes and fat tissue in relation to androstenone accumulation in fat of pubertal pigs

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ABSTRACT

The present study was performed to measure messenger RNA levels of steroidogenic enzymes in testes and fat tissue and determine whether they are related to fat androstenone level. Real-time polymerase chain reaction experiments were performed on 26 testes and 12 adipose tissue samples from pubertal boars using 21 genes. The absence of significant correlations between fat androstenone and the transcriptional activity of the *SRD5A2* and *SRD5A3* genes but the high correlation coefficient with that of the *SRD5A1* gene ($r = 0.62$, $P < 0.05$) suggests that the enzyme coded by *SRD5A1* is mainly responsible for the last step of androstenone synthesis. The testicular transcriptional activities of *CYP17, CYP11A1, CYP19A, AKR1C-pig6, SRD5A1, LHCGR*, and *AR* were significantly correlated. Only transcriptional levels of *CYP17, CYP11A1, CYP19A, SRD5A1*, and *AKR1C-pig6* were correlated with the fat concentration of androstenone ($0.57 < r < 0.70$, $P < 0.05$) confirming that the amount of androstenone stored in fat is related to the production in testes of androstenone and more generally to all sex steroids. Altogether, our data are in favor of a preponderant role of *AKR1C-pig6* instead of *HSD17B3* for testicular synthesis of steroids. Concerning fat tissue, our data do not support a significant de novo biosynthesis of steroids in porcine adipose tissues. The presence of transcripts coding for steroid enzymes, especially those of *AKR1C-pig6*, suggests that steroids can be transformed. None of transcript abundance was related to androstenone accumulation ($P > 0.1$). Therefore, steroids synthesized elsewhere can be transformed in fat tissue but synthesis of androstenone is unlikely.

1. Introduction

Boar taint is defined as an unpleasant flavor and odor of porcine meat. The accumulation of 2 main compounds, 3-methylindole (=skatole) and androstenone, in fat is at its origin. This is the main reason for castration of male pigs reared for meat production. This practice is widespread in European countries [1] but generates severe physiological and behavioral signs of pain [2]. Therefore, surgical castration in male pigs is highly debated in Europe and, finding solutions to rear entire males without boar taint is of major interest. Androstenone, as other sex steroids, is produced essentially by the Leydig cells, and its level of production increases during pubertal development [3]. The pathways and genes involved in steroidogenesis, and hence in androstenone synthesis, have been addressed in the domestic pig testis but are not fully known [4,5]. Such knowledge will help to define a strategy of genetic selection sufficient to avoid boar taint without reducing reproductive performance, which is a major concern of pig breeders [6–8].

It is well-accepted that the level of expression of proteins is controlled at both transcriptional and posttranscriptional

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levels. Therefore, one approach to determine whether the expression of 1 gene candidate is implied in the variability of fat androstenone would be to determine whether the level of its protein expression is related to the fat androstenone level. However, specific antibodies against pig proteins controlling the reproductive function, including steroidogenic enzymes, are not commercialized, whereas messenger RNA (mRNA) can be easily designed for quantitative measure of transcript expression. Therefore, comparison of mRNA levels in pigs differing in fat androstenone level has been widely performed (eg, in [9–11]). This approach is supported by data from Gunawan et al [12,13] that described a positive relationship for variations in mRNA and protein expression of the same genes between different tissues or groups of males varying for fertility and sperm quality.

Contrary to humans, boar testes are able to produce large amounts of 5α-reduced steroids (epiandrosterone, androstanediol, and androstenone). Nevertheless, the 5α reduction of testosterone to dihydrotestosterone (DHT) has never been described in porcine testes [14]. In humans, the 5α-reduction of testosterone to DHT is catalyzed by a 5-alpha reductase (5α-R) encoded by *SRD5A* genes and occurs mainly in male accessory sex glands including seminal vesicles, epididymes, and the prostate [15]. In humans, *SRD5A2* is the predominant form in male reproductive tissues, whereas *SRD5A1* is expressed in peripheral tissues [16,17]. In fact, 3 genes (*SRD5A1*, *SRD5A2*, and *SRD5A3*) code for 3 isoenzymes of the 5α-R. Although only 5α-R1 and 5α-R2 appear to be involved in human steroid metabolism [16,17], the involvement of 5α-R3 cannot be excluded in pigs.

The immediate precursor of androstenone is androstadienone, which itself derives from pregnenolone via androstadienol. Pathways leading to other sex steroids also use pregnenolone as a precursor and can be viewed as competing metabolic pathways. Production of androgens by Leydig cells is, at least in part, under the endocrine control of both gonadotropins (LH and FSH) and under the paracrine control of testosterone and estradiol [18]. The action of these hormones is mediated by specific receptors, LHCGR for LH, FSHR for FSH, ESR1 and ESR2 for estradiol, and AR for testosterone. In addition, both gonadotropins are under the negative feedback of hormones produced by the testes. These hormones include sex steroids and proteins such as follistatin and inhibin [18–21]. Follistatin is coded by the *FST* gene, whereas the *INHA* gene codes for the alpha subunit of both inhibin A and inhibin B.

In humans, adipose tissue is capable of both synthesis (essentially from steroid precursors such as dehydroepiandrosterone) and inactivation of active androgens [22]. In pigs, recent data on a limited number of animals, with a semi-quantitative measure of gene expression, suggested that the fat tissue is not able to synthesize steroids [5]. However, it is necessary to confirm these initial observations with a new set of data and a quantitative technique.

The aims of the present study were (1) to characterize the gene(s) from the 5α reductase family that is/are responsible for the final step of androstenone synthesis, (2) to evaluate, in testes and fat tissue from pubertal male pigs, the expression of 21 transcripts potentially involved in the synthesis and regulation of sex steroids, and (3) to determine how the expression of these genes relates (3a) to each other and (3b) to the concentration of fat androstenone.

2. Materials and methods

2.1. Animals, sample collection, and fat androstenone measurement

A subset of purebred Pietrain or crossbred Pietrain × Large White (n = 36) boars raised for a larger experiment on genetic and genomic values of entire male pigs [23,24] was used for the present experiment. They were raised from postweaning to slaughter (110 kg) in a testing station (Le Rheu, France) in different batches. They were housed in groups of 10 to 12 pigs on concrete semi-slatted floor (12 m^2/pen) and received natural light. They had free access to water and were fed ad libitum, at an electronic single-space feeder, with pellets containing 9.5 MJ net energy/kg; 163 g total proteins/kg, 0.94 g digestible lysine/MJ net energy, and 0.18 g digestible tryptophan/MJ net energy.

Animals were slaughtered at 157 ± 11 d of age and 112 ± 5 kg live weight (mean ± standard deviation) in the local abattoir (Cooperl, 35160, Montfort sur Meu). Twenty-six animals were collected for RNA preparation from testes with genital tracts collected immediately after evisceration. Twelve animals were used for RNA preparation from adipose tissue with fat samples (2–5 cm^3) collected in the neck area (between the cervical and first dorsal vertebrae) immediately after carcass splitting. Within 30 to 60 min of death, tissue samples were frozen in liquid nitrogen and stored at −80°C. The day after slaughter together with measures of meat quality used for other purposes, an additional sample of fat was collected for androstenone measure in all pigs (n = 38) in the same neck area as for mRNA analyses. This sample was stored at −20°C until analysis by HPLC as previously described [25].

2.2. Gene expression analysis

Samples for RNA preparation were disrupted, homogenized, and ground to a fine powder by rapid agitation for 1 min in a liquid nitrogen–cooled grinder with stainless steel beads before RNA extraction. Total RNA was extracted with trizol [26]. RNA concentration and quality were evaluated by a ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and an Agilent Bioanalyser 2100 (Agilent Technologies, Santa Clara, CA, USA). The RNA integrity numbers were 6.4 ± 0.6 for the adipose tissue and 8.9 ± 0.6 for the testicular tissue. Complementary DNA was synthesized using random primers and murine Moloney leukemia virus reverse transcriptase (Applied Biosystems, Foster City, CA, USA).

The transcriptional activity of essential genes for steroidogenesis in testis (*CYP11A1, CYP17, CYP19A, CYP21, HSD17B1, HSD17B3*, all *AKR1C* porcine genes: *AKR1C-pig1+pig3*, *AKR1C-pig4*, and *AKR1C-pig6*, *SRD5A1*, *SDR5A2*, and *SRDA3*) was evaluated. In addition, transcripts encoded by genes of hormone receptors essential for the control of Leydig cell activity (*FSHR, LHCGR, AR, ESR1*, and *ESR2*) and transcripts of genes involved in the control of Leydig cell