Influence of porcine genotype on the abundance of thyroid hormones and leptin in sow milk and its impact on growth, metabolism and expression of key adipose tissue genes in offspring

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Abstract

Neonatal mortality is greater in commercial porcine genotypes, compared with the ancient Meishan breed that rapidly lay down adipose tissue; this may be related to hormones, such as triiodothyronine (T₃) or leptin. Leptin is present in maternal milk; however, the extent to which this supply provides the neonate with leptin is unknown, but may play a role in growth and development. We investigated whether thyroid hormones and leptin concentrations in maternal milk differed between genotypes; and whether this influenced piglet concentrations or expression of genes involved in adipose tissue regulation. Eight Meishan and six commercial sows were entered into the study and milk samples from the day of parturition to day 4 postpartum was taken daily. The median birth weight piglet in each litter had a daily venous blood sample taken and was euthanised on day 4. Gene expressions of IGF-I, IGF-binding protein 3 (IGFBP-3), peroxisome proliferators activated receptor (PPAR) γ and glucocorticoid receptor (GR) were measured in adipose tissue

using real-time PCR. T₃ was increased in Meishan milk, but not in piglet plasma. Milk thyroxine was similar between breeds but commercial piglet levels were significantly higher. Leptin was higher in commercial sow milk throughout the study. Milk leptin was strongly correlated to plasma leptin during the first postnatal days and also to organ and body weight in Meishan piglets that also had significantly higher expression of GR, but not IGF-I, IGFBP-3 or PPAR γ . In conclusion, we have found a significant disparity in the provision of thyroid hormones in Meishan and commercial sow's milk. These changes are not always translated to plasma concentrations of hormone in the piglet. Leptin appears to have a stronger role in growth and development in the Meishan genotype compared with commercial; along with the increased GR expression, this may also represent a potential mechanism behind the rapid accumulation of adipose tissue in Meishan piglets.

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Introduction

It is well documented that Meishan, an ancient oriental breed of pig, have significantly lower neonatal mortality rates than commercial breeds (i.e. meat producing; Le Dividich *et al.* 1991), despite being smaller at birth and having larger litters (Le Dividich *et al.* 1991, Herpin *et al.* 1993). Meishan sows are obese and produce milk with a higher fat content than commercial sows (Alston-Mills *et al.* 2000), which may be one factor responsible for the apparent resistance to hypoglycaemia and hypothermia observed in their piglets. Our recent studies have established that there is a significant disparity in the expression of the mitochondrial uncoupling proteins (UCP)2 and 3 in adipose tissue and skeletal muscle between these breeds, as well as significantly higher plasma triiodothyronine (T₃) in the commercial breed on the first day

of life, but this does not appear to improve their ability to thermoregulate (Mostyn *et al.* 2004).

Adequate provision of milk to newborn piglets is critical in ensuring provision of nutrients, not only to negate hypoglycaemia, but also to provide energy for temperature regulation. Colostrum also supplies the newborn piglet with a source of immunoglobulins (Norcross 1982). Sow milk is known to contain a number of hormones, but it is unknown whether these are a major source to the piglet. Piglets, like most mammalian neonates, possess very little adipose tissue at birth (1% body weight; Mount 1968) and are, therefore, extremely sensitive to the decrease in environmental temperature (15–20 °C) experienced during the transition from the intra- to extra-uterine environment. This drop in ambient temperature results in a rapid fall in body temperature usually from 39 to 37 °C (Herpin *et al.* 2002).

In order to adapt to this large change in environmental temperature, most mammalian species possess brown adipose tissue (Nedergaard & Cannon 1992); a tissue specialised for heat production via mitochondrial UCP1, which is unique to brown adipose tissue. However, pigs are one of the few species that are established to date to lack brown adipose tissue (Trayhurn *et al.* 1989). Consequently, piglets utilise other strategies of heat production and conservation, including muscular shivering, in order to maintain homeothermy. These alternative strategies require a good supply of nutrients to the piglet, in particular, lipids for skeletal muscle energy utilisation; milk is, therefore, key in providing lipid for this purpose (Le Dividich & Noblet 1981).

Leptin is a 16 kDa protein produced primarily in adipose tissue, which has roles in energy regulation and metabolism, food intake and reproduction (Pellemounter *et al.* 1995, Scarpace *et al.* 1997, Cunningham *et al.* 1999). Leptin is also present in the milk of humans and lactating sows and appears to be related to maternal adiposity in the human, but not in the sow (Houseknecht *et al.* 1997, Estienne *et al.* 2000). Milk-borne leptin may play a role in the maturation and development of the neonatal intestine (Wolinski *et al.* 2003). It is unknown whether leptin concentrations in sow's milk differ between commercial and Meishan breeds, which may in part explain differences in piglet physiology between the two breeds. Thyroxine (T_4) and T_3 are both present in colostrum and milk at concentrations that vary between species (Akasha & Anderson 1984). Thyroid hormones regulate energy metabolism by increasing respiration and energy expenditure and by lowering metabolic efficiency (Symonds *et al.* 1995) and exert their biological effects by promoting gene transcription (Bassett *et al.* 2003). A thyroid hormone deiodinating system exists in lactating mammary tissue of a number of species (Aceves & Valverde 1989, Slebodzinski *et al.* 1999) and this is thought to cause the comparatively low T_4 and high T_3 concentrations observed in milk compared with the maternal circulation. The precise physiological role of thyroid hormones in milk is unknown and it is unclear whether the amount received by the newborn is sufficient to cause any physiological effects.

Leptin is known to regulate and be regulated by a number of genes involved in growth and metabolism of adipose tissue, including insulin-like growth factor I (IGF-I), peroxisome proliferator activated receptor γ (PPAR γ) and glucocorticoid receptor (GR) (Gnanalingham *et al.* 2005, Kintscher & Law 2005, Trayhurn 2005). We have previously observed significantly higher plasma IGF-I in commercial compared with Meishan piglets (A Mostyn & JC Litten, unpublished observations; day 0, commercial, 33.2 ± 6.4 ; Meishan, 19.9 ± 2.9 ng/ml ($P < 0.05$)), it is not known whether these increased plasma concentrations are mirrored by adipose tissue expression of IGF-I or its binding protein, IGFBP-3. PPAR γ ligands, thiazolidinediones (TZDs), reduce leptin mRNA expression in adipose tissue and also antagonises the transactivation of the leptin promoter (Hammarstedt *et al.* 2005). Both actions would reduce circulating leptin and

action upon the leptin receptor. The GR is upregulated *in vitro* by chronic leptin administration, but is conversely downregulated following acute leptin administration (Gnanalingham *et al.* 2005). Breed differences in glucocorticoid activity have been observed in the placenta (Klemcke *et al.* 2003), brain, kidney and liver (Perreau *et al.* 1999) of Meishan and commercial pigs. Glucocorticoids have a key role in adipose tissue regulation, but the expression of GR in adipose tissue of Meishan and commercial piglets has not yet been explored.

The aim of this study was to investigate whether milk-borne leptin and thyroid hormones (T_3 and T_4) were differentially available in sow milk from two breeds of pig and whether the key adipose tissue genes were influenced by the availability of leptin or genotype. This study also aimed to establish the natural ontogeny of these hormones over the first 4 days of postnatal age in the milk of Meishan and commercial sows and relate these values to organ development and piglet growth parameters.

Materials and Methods

Eight Meishan and six commercial (Large White (75%) $\times$ Meishan (25%)) sows of similar mating date and parity were entered into the study. At around 4 h after birth, all piglets in each litter were weighed and those closest to the median weight were assigned to be blood sampled daily. At approximately 0930 h each day, colonic temperature was measured with a digital thermometer, piglets were weighed and venous blood sample was taken (as described by Litten *et al.* 2005). Milk was manually sampled from each sow and immediately frozen at -20°C . On days 0 and 4, morphometric measurements, including head circumference and crown to rump length (CRL), were made along with the measurement of total body electrical conductivity (TOBEC) as described previously (Mostyn *et al.* 2004). This is a non-invasive technique designed to estimate fat-free mass and thereby indirectly measures body fat in live animals (Bellinger & Williams 1993). TOBEC values are computed using the following formula, which takes into account body size:

$$\text{Lean mass} = \sqrt{\text{TOBEC} \times \text{CRL}}$$

On day 4, piglets were humanely euthanised with an overdose of barbiturate anaesthetic (200 mg/kg pentobarbital sodium:euthatal; RMB Animal Health, UK). All tissues, including a reproducible sample of s.c. adipose tissue (SAT), were rapidly dissected, weighed and stored at -80°C until further analysis. All operative procedures and experimental protocols had the required Home Office approval as designated by the Animals (Scientific Procedures) Act (1986).

Laboratory procedures

Milk samples were thawed and the whole milk was used for all the assays.

Plasma concentrations of glucose (coefficient of variation, CV=5.3%), triacylglyceride (TAG; CV=6.9%; Sigma Chemical) and non-esterified fatty acids (NEFA; CV=2.8%; Wako NEFA-C; Alpha Labs) were determined enzymatically. Total plasma and milk T₃ (CV=4.9), thyroxine (T₄; CV=8.4) and leptin (CV=2.6) concentrations were assessed using RIA (ICN Pharmaceuticals, Basingstoke, UK; leptin Linco, St Louis, MO, USA) as described previously by Litten *et al.* (2005).

Molecular biology procedures

Total RNA was extracted from SAT using the RNeasy kit (Qiagen Ltd) and was reverse transcribed (Moloney-murine leukaemia virus reverse transcriptase; Promega, UK). Real-time PCR was performed using a Quantica real-time thermocycler (Techne Incorporated, Barloworld Scientific Ltd, Stone, UK) with the Quantitect SYBR green PCR mix (Qiagen Ltd) in order to determine transcription levels of the following genes: IGF-I, IGFBP-3, PPAR γ and GR. 18S rRNA was used as a housekeeping gene. PCR primer sequences are shown in Table 1. Gene expressions were measured by the 2^{- Δ CT} method (Livak & Schmittgen 2001). Levels of gene expression were expressed as relative values using commercial pig values as a reference (1.0).

Statistical analyses

Power calculations using SamplePower 2 dictated that $n=6$ would have power of 92.4% to yield a statistically significant result. All statistical evaluations were performed by using SPSS 11.0 for windows (SPSS Inc., Chicago, IL, USA) using the General Linear Model procedure with correction for repeated measures if appropriate and Spearman's ρ -test. All values presented are means with their standard errors.

Results

Growth and morphology

Meishan piglets were weighed significantly ($P<0.05$) lower than commercial piglets (Fig. 1a), this was combined with a higher number of siblings in Meishan litters (Meishan, 12.8 ± 0.8 ; commercial, 7.8 ± 1.7 piglets per litter; $P<0.05$). They also had a lower fat-free mass ($P<0.05$) on days 1 and 4 of the study indicating that they were fatter (Fig. 1b).

Body composition

As expected, there were a number of differences between absolute organ weights between the breeds (heart, lung, brain and liver) due to the differences in body weight. When organ weights were expressed per kilogram/body weight, on day 4 of postnatal age only the spleen was heavier ($P<0.05$) in the Meishan piglets (Fig. 1c).

Plasma and milk hormones and metabolites

There was a trend for leptin in milk to be higher in samples from commercial sows, although this was only statistically significant on day 3 of piglet postnatal age (Fig. 2a). When the mean leptin concentration in milk was considered, there was a significant difference between genotypes with commercial sows producing milk containing more than twofold leptin (Fig. 2b). Plasma leptin concentrations were unaffected by age and similar between Meishan and commercial piglets throughout the study (e.g. day 0; Meishan, 2.0 ± 0.1 ; commercial, 2.0 ± 0.1 ng/ml).

T₃ concentrations were higher ($P<0.05$) in milk from Meishan compared with commercial sows (Fig. 3), although plasma T₃ was similar between genotypes on all days of the study (e.g. day 3; Meishan, 1.7 ± 0.2 ; commercial, 1.6 ± 0.4 ng/ml). In contrast, plasma T₄ concentrations were higher ($P<0.05$) in commercial compared with Meishan

Table 1 Primer sequences for genes used in real-time PCR

		Primer sequences	Product length (bp)
Gene			
IGF-I	Forward	5'-CTTCAGTTCGTGTGCGGAGAC-3'	134
	Reverse	5'-CTCCAGCCTCCTCAGATCACAG-3'	
PPAR γ	Forward	5'-ACGGGAAAGACGACAGACAAATC-3'	151
	Reverse	5'-CACGGAGCGAAACTGACACC-3'	
IGFBP-3	Forward	5'-CGGGTGCCTGACTCCAAATC-3'	122
	Reverse	5'-GTTCTGGGTGCCGTGCTCTG-3'	
GR	Forward	5'-GCTGCTGGTCTGCTGCTC-3'	82
	Reverse	5'-CCTTCACATTCGGCTGCTCTGG-3'	
18S	Forward	5'-GATCGCGCGCGCTATTCC-3'	125
	Reverse	5'-CTCCTGGTGGTGCCTCC-3'	

IGF-I, insulin like growth factor I; PPAR γ , peroxisome proliferators activated receptor γ ; UGF-BP3, insulin-like growth factor-binding protein 3; GR, glucocorticoid receptor; 18S, 18S ribosomal RNA.

Figure 1 (a) Mean body weight (in kilogram) in Meishan (■) and commercial (□) piglets over the first 4 days of postnatal age. Values are means with their standard errors. Significant differences between breeds at the same postnatal age is denoted by $*P<0.05$. (b) Mean TOBEC in Meishan (■) and commercial (□) piglets over days 0 and 4 of postnatal age. Values are means with their standard errors. Significant differences between groups at the same postnatal age is denoted by $*P<0.05$. (c) Body composition (g/kg) in Meishan (■) and commercial (□) piglets on day 4 of postnatal age. Values are means with their standard errors. Significant differences between breeds at the same postnatal age is denoted by $*P<0.05$.

piglets on all days for the study (Fig. 4). There were no significant differences in milk T_4 concentrations (e.g. day 1; Meishan, 15.3 ± 0.6 ; commercial, 15.9 ± 2.2 ng/ml).

Plasma glucose was found to increase in both genotypes during the study and was significantly higher in commercial piglets only on day 1 of postnatal age (Meishan, 5.0 ± 0.2 ; commercial, 6.8 ± 0.6 mM ($P<0.05$)). There were no significant differences in plasma NEFA or TAG between the genotypes during the study (data not shown).

A number of significant relationships were observed between the milk hormones and the plasma hormones and metabolites. Milk leptin was positively associated with plasma leptin in the Meishan group on days 0 and 1 of postnatal age, but on day 2 this relationship was reversed; thereafter, milk leptin was no longer associated with piglet plasma leptin concentrations (Table 2). On days 1 and 2 of postnatal age, milk leptin was significantly positively associated with plasma glucose levels in commercial piglets alone (Table 2); by day 3, this relationship had reversed (Table 2).

Figure 2 (a) Milk leptin concentrations in Meishan (■) and commercial (□) piglets over the first 4 days of postnatal age. Values are means with their standard errors. Significant differences between breeds at the same postnatal age is denoted by $*P<0.05$. (b) Average milk leptin concentrations in Meishan (■) and commercial (□) piglets over the first 4 days of postnatal age. Values are means with their standard errors. Significant differences between breeds at the same postnatal age is denoted by $*P<0.05$.

Figure 3 Sow milk T₃ concentrations in Meishan (■) and commercial (□) piglets over the first 4 days of postnatal age. Values are means with their standard errors. Significant differences between breeds at the same postnatal age is denoted by * $P < 0.05$.

The average milk concentration of leptin in sow milk over the study period was found to be significantly positively related to body weight, girth and growth rate, as well as gut, heart and spleen weight in Meishan piglets (Table 3). No growth parameters were associated with average milk leptin in the commercial piglets, although there was a significant positive relationship between lung weight and average plasma leptin ($R^2 = 0.68$, $P < 0.05$).

Gene expression of IGF-I, IGFBP-3, PPAR γ and GR

There were no differences in IGF-I, IGFBP-3 or PPAR γ gene expression in SAT of Meishan and commercial piglets on 4 days of age (Fig. 5; IGFBP-3 not shown). However, GR was significantly upregulated in SAT of Meishan piglets (Fig. 5). A significantly positive relationship was found between GR and day 4 TOBEC ($R^2 = 0.79$, $y = 4 \times 10^{-7}x + 1 \times 10^{-5}$; $P < 0.05$) in Meishan piglets.

Figure 4 Plasma T₄ concentrations in Meishan (■) and commercial (□) piglets over the first 4 days of postnatal age. Values are means with their standard errors. Significant differences between breeds at the same postnatal age is denoted by * $P < 0.05$.

Discussion

Our study has demonstrated for the first time a significant disparity in the provision of leptin and T₃ in milk from Meishan and commercial sows. Plasma concentrations of the respective hormones in piglets remained similar between breeds, despite this there were potential effects on organ and body growth between breeds. The differences in growth and adiposity of the piglet genotypes may be attributed to disparity in adipose tissue GR expression.

Leptin in sows milk

A role for leptin in maternal milk has yet to be defined, but may be involved in maturation of the small intestine or neonatal growth (Wolinski *et al.* 2003). These authors have demonstrated that piglets fed with milk formula had delayed small intestine mucosal development compared with feeding with sow's milk. Importantly, this effect could be reversed when the formula was supplemented with exogenous leptin (Wolinski *et al.* 2003). Interestingly, a strong positive relationship between gut weight and average milk leptin was observed in the Meishan piglets in this study (Table 3). Studies by Casabiell *et al.* (1997) have shown, in the rat, that leptin passes through the pups' digestive system in its intact form and can be detected in neonatal serum. Average milk leptin was positively related to piglet growth rate, girth, body, gut, heart and spleen weight in the Meishan group only, suggesting a growth promoting effect of milk-derived leptin. The positive relationship between spleen weight and leptin may represent an immunological effect. Leptin treatment has been suggested to increase proliferation of splenocytes and improve immune function after starvation (Mito *et al.* 2004); however, this would require further investigation. The present results complement the previous work, which found that chronic (6 days) leptin treatment of newborn piglets improved growth rate and altered body composition in the Meishan (Litten *et al.* 2005). From the present data, it seems unlikely that leptin from maternal milk has a large influence on neonatal plasma leptin concentrations, but may act to specifically promote tissue and body growth.

Previous studies have demonstrated that leptin is found at significantly higher concentrations in sows milk compared with the circulation (Estienne *et al.* 2000). This is in contrast to human milk, which contains leptin at a significantly lower concentration than that of maternal plasma (Houseknecht *et al.* 1997). In the human, specific hormones and growth factors, e.g. prolactin are concentrated in milk by the breast but leptin does not appear to be under the same regulation, this could be different in the pig. It is more likely that local production accounts for a high percentage of the leptin in sow's milk given that the concentrations are much higher than those found in maternal plasma (i.e. two- to sixfold higher depending on breed). The breast is composed of a high percentage of adipose tissue and it is possible that this depot

Table 2 Significant associations between milk and plasma hormones in measured during the study

	Breed	X-axis	Y-axis	R ²	Equation	n	P value
Age							
0	M	Milk leptin	Plasma leptin	0.98	$y=0.05x+1.6$	4	0.001
0	C	Milk leptin	Plasma NEFA	0.46	$y=0.008x+0.5$	6	0.008
0	C	Milk T ₃	Milk T ₄	0.98	$y=6.0x+13.1$	4	0.001
1	M	Milk leptin	Plasma leptin	0.94	$y=0.04x+1.6$	5	0.001
1	M	Milk leptin	Plasma NEFA	0.39	$y=0.07x+0.4$	5	0.037
1	C	Milk leptin	Plasma glucose	0.83	$y=0.06x+5.5$	4	0.001
2	M	Milk T ₃	Plasma T ₃	0.95	$y=-0.14x+3.0$	4	0.001
2	M	Milk leptin	Plasma leptin	0.56	$y=-0.008x+2.1$	4	0.001
2	C	Milk leptin	Plasma glucose	0.46	$y=0.06x+6.2$	5	0.001
2	C	Milk leptin	Plasma NEFA	0.47	$y=0.08x+0.2$	5	0.037
2	C	Milk T ₄	Milk T ₃	0.87	$y=-5.8x+21.9$	5	0.037
3	M	Milk leptin	Plasma T ₄	0.91	$y=1.4x+19.4$	5	0.037
3	C	Milk leptin	Plasma glucose	0.57	$y=-0.09x+9.8$	5	0.037
4	M	Milk leptin	Plasma TAG	0.47	$y=0.04x+0.6$	5	0.037
4	M	Milk T ₃	Plasma leptin	0.88	$y=0.24x+1.6$	5	0.037
4	C	Milk T ₃	Plasma NEFA	0.87	$y=1.3x+0.07$	4	0.001
4	C	Milk T ₃	Plasma TAG	0.97	$y=0.7x-0.08$	4	0.001

NEFA, non-esterified fatty acids; T₃, triiodothyronine; T₄, thyroxine; TAG, triacylglycerol; M, Meishan; C, commercial.

provides a source of leptin; alternatively, studies have demonstrated that human mammary epithelial cells produce leptin (Smith-Kirwin *et al.* 1998). One further explanation for the high levels of leptin observed in sow's milk could be a lack of leptin-binding protein. A portion of leptin found in the circulation exists bound to a short form of the leptin receptor thought to be a binding protein (Huang *et al.* 2001). Although the long- and short-form of the leptin receptor are present in mammary tissue in a number of species (Laud *et al.* 1999), it is unknown if the binding protein is secreted into milk.

The concentration of leptin in sows milk were three- to tenfold higher than piglet plasma depending on genotype in this study. Estienne *et al.* (2000) reported leptin plasma concentrations of 3.7 ± 2 ng/ml in lactating sows; these are comparable to concentrations taken previously in lactating commercial sows in our unit (5.5 ng/ml within 24 h parturition). The role of leptin in sows milk may, therefore, be different from that of human milk. A number of studies have found no link between sow milk leptin concentrations and body composition/weight or sow plasma concentrations (Estienne *et al.* 2000) suggesting that the milk concentration of leptin in pigs is not indicative of body composition nor fat

content. In the present study, there was a strong trend for the concentration of leptin in milk to be higher in commercial sows (Fig. 2a and b), despite commercial sows being considerably leaner than the obese Meishan sows, in accordance with previous studies.

Despite a twofold greater concentration of leptin in commercial sow milk, there was no difference between circulating leptin levels in the offspring, suggesting that either leptin is broken down (a) from its intact form in the stomach, or does not pass through the gut wall or (b) through first pass metabolism in the pig in contrast to the rat (Casabiell *et al.* 1997). However, leptin receptors do exist in the stomach and it is possible that part of the relationships observed between milk leptin and metabolites may be due to leptins' action through these receptors (Mix *et al.* 2000).

Thyroid hormones in sows milk

A number of studies have demonstrated the presence of thyroid hormones in human maternal milk at varying concentrations (T₄ 0–77 ng/ml, T₃ 0.1–4 ng/ml; Mizuta *et al.* 1983, van Wassenauer *et al.* 2002) that are comparable to

Table 3 Significant associations (all positive) between the mean milk leptin concentration (X-axis) over the first 4 days of postnatal age and growth and organ weights taken on day 4 of postnatal age in Meishan piglets

	R ²	Equation	n	P value
Y-axis				
Body weight	0.78	$y=0.08x+0.6$	7	0.014
Growth rate	0.74	$y=0.009x-0.006$	6	0.036
Girth	0.84	$y=1.5x-26.3$	7	0.019
Gut weight	0.86	$y=5.4x+40.1$	7	0.007
Heart weight	0.86	$y=0.8x+2.9$	7	0.023
Spleen weight	0.66	$y=0.3x+1.2$	7	0.016

Figure 5 (a) Glucocorticoid receptor (GR) expression in adipose tissue from Meishan (■) and commercial (□) piglets on day 4 of postnatal age. Values are means with their standard errors. Significant difference between breeds at the same postnatal age is denoted by $*P < 0.05$. (b) Insulin-like growth factor I (IGF-I) expression in adipose tissue from Meishan (■) and commercial (□) piglets on day 4 of postnatal age. Values are means with their standard errors. (c) Peroxisome proliferator activated receptor γ (PPAR γ) expression in adipose tissue from Meishan (■) and commercial (□) piglets on day 4 of postnatal age. Values are means with their standard errors.

those measured in the pig in the present study. T_3 was elevated in Meishan compared with commercial sows' milk, although a similar trend in piglet plasma T_3 was not observed. Although the significantly higher concentration of T_3 in Meishan milk did not appear to influence piglet plasma concentrations of T_3 ; it may have had a negative effect of plasma T_4 , which was considerably reduced in Meishan compared with commercial piglets throughout the study.

The higher milk T_3 concentration observed in Meishan sows was unexpected as these sows have considerably more body fat than commercial sows and as such would be expected to have lower circulating T_3 . However, there was no difference, in the plasma T_3 concentrations of piglets. This raises two questions: (i) do milk thyroid hormone levels reflect maternal circulating concentrations? and (ii) what is happening to the high concentrations of T_3 provided to the Meishan piglets that is not observed in neonatal plasma? It seems likely that milk thyroid concentrations do not reflect maternal circulating levels; it has been demonstrated in lactating cows that the highest concentrations of T_3 in colostrum coincided with the lowest maternal plasma concentrations (Pezzi *et al.* 2003), and could be important in improving neonatal outcome (Symonds *et al.* 1989). Thyroid hormones can pass through the digestive system in their intact active form, suggesting that digestion is not causing the discrepancy between milk and plasma concentrations, and it is possible that T_3 is being bound to thyroid-binding protein. Only negative relationships between milk T_3 and plasma thyroid hormones were observed, confirming that the milk concentrations were not increasing plasma concentrations in piglets.

Thyroxine deiodinases are known to be present in the mammary gland of lactating sows (Slebodzinski *et al.* 1999), which is responsible for mammary production of T_3 . T_4 was present at a very low concentration in milk when compared with the levels of T_3 , which were similar to those observed in plasma (Figs 2 and 3), suggesting that deiodination was taking place.

Interestingly, the differences observed in plasma T_3 between Meishan and commercial piglets on day 0 in a previous study (Mostyn *et al.* 2004) were not apparent. A potential reason for this disparity was that the maternal diet was different in the present study. A commercially available diet suitable for lactating sows (ABN Supreme Lactation Pellets, ABN, Peterborough, UK) was provided to all pigs on the trial. The components of this diet had been modified to include extra phytase. Phytic acid is known to inhibit absorption of trace minerals (such as zinc) from the diet (Hurrell 2003). Zinc allows T_4 to enter cells and be converted into T_3 thus stimulating thyroid production; treatment with zinc causes an increase in both free and total plasma T_3 (Baltaci *et al.* 2004). These dietary changes could have influenced thyroid hormones in the sows and consequently, placental availability of thyroid hormones may have been altered during gestation leading to the discrepancy with the previous study (Mostyn *et al.* 2004).

Milk T_3 was strongly positively correlated with the lipid metabolites, NEFA and TAG on day 4 in the commercial group only, suggesting that this source of T_3 is beginning to act in a 'traditional' way, i.e. oxidation of lipids. This was not apparent in the Meishan group, thus it may be one of the mechanisms, which allow Meishan piglets to lay down vast amounts of adipose tissue during the postnatal period; alternatively, this may represent a potential 'sparing' of lipid stores, which relates to the increased neonatal survival rates observed in this breed.

Adipose tissue gene expression

The expression of PPAR γ , IGF-I and IGFBP-1 were found to be similar between the piglet genotypes. However, the significantly higher GR in Meishan adipose tissue may be the key to increase adiposity in this breed. Previous work has demonstrated that Meishan pigs have significantly higher plasma cortisol concentrations (Mormede *et al.* 1984, Sutherland *et al.* 2005) when compared with leaner, commercial breeds, such as Landrace and Yorkshire. These studies were investigating the role of stress and immune competency on cortisol concentrations and not adipose tissue regulation. Moreover, a study of dexamethasone perfusion during pregnancy in rat induced small birth weight offspring associated with GR upregulation in adipose tissue (Cleasby *et al.* 2003). These previous observations suggest that the differential cortisol levels in Meishan and commercial sows could program adipose tissue GR expression and fat mass growth. The strong relationship between GR and TOBEC on day 4 suggests that increased GR expression, which would promote lipid storage, along with the lower concentrations of maternally derived leptin, which would compel the piglet to feed, may be driving the increased adiposity observed in the Meishan piglets.

In conclusion, we have found a significant discrepancy between the provision of thyroid hormones and leptin found in Meishan and commercial sow's milk. These changes do not appear to be translated to piglet circulating concentrations of hormones. Milk-derived T_3 and leptin regulate piglet plasma hormones and metabolites, but only leptin appears to be linked to organ and body growth. The benefit of milk-derived leptin and thyroid hormones remains to be established.

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