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ORIGINAL CONTRIBUTION

Moderately increased maternal dietary energy intake delays foetal skeletal muscle differentiation and maturity in pigs

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Abstract
Objectives This study aimed to evaluate the effects of moderately increased maternal dietary energy intake during gestation on foetal skeletal muscle development and metabolism with pig as a model.
Methods Twelve primiparous purebred Large White sows (initial body weight 135.5 ± 1.6 kg) were allocated to one of two energy intake treatments: normal-energy-intake group (Con, 30.96 MJ DE/day) as recommended by the National Research Council (NRC; 2012) and high-energy-intake group (HE, 34.15 MJ DE/day). The nutritional treatments were introduced from mating to day 90 of gestation. On day 90 of gestation, foetuses were examined by morphological, biochemical and molecular analysis of the longissimus muscle. Umbilical vein serum hormones were measured.
Results Sow body weight was increased in HE group compared with Con group ($P < 0.05$), whereas foetal myofibre density was decreased ($P < 0.05$). Meanwhile, protein concentration, creatine kinase and lactate dehydrogenase activities and umbilical vein serum triiodothyronine (T3) concentration were decreased in HE foetuses ($P < 0.05$). Maternal HE diets decreased the mRNA abundance of muscle growth-related genes, myosin heavy-chain (MYH/MyHC) genes (MYH2 and MYH1) and insulin-like growth factor 1 and insulin growth factor-binding protein 5 ($P < 0.05$). Furthermore, the protein expressions of myogenic differentiation factor 1, myogenin and fast-MyHC isoforms were reduced in HE foetuses ($P < 0.05$).
Conclusion Our results suggest that moderately increased maternal dietary energy intake delays the differentiation and maturation in skeletal muscle of the foetus on day 90 of gestation.

Keywords Energy intake · Gestation · Longissimus · Foetus · Myogenesis · Pig

Introduction

Maternal nutrition during gestation is closely associated with offspring growth and development in humans and animals because it alters expression of the foetal genome and may have lifelong consequences [1–3]. Particularly, the consequences of maternal obesity and overnutrition in adult offspring gain more attention [4, 5]. This phenomenon, termed "foetal developmental programming", has been seen a major risk factor for chronic diseases in adulthood [6], although the mechanisms involved remain poorly defined.

Human diets high in fat and energy density are associated with a rapid increase in the incidence of obesity in the both western world and certain developing countries [7]. Recent data from the National Health and Nutrition Examination Survey (NHANES; 2009–2010) show that about 32 % of nonpregnant women aged 20–39 years in the USA are clinically obese [8]. Although epidemiological studies indicate that maternal obesity has long-term effect on later health resulting in offspring increased risk of obesity and metabolic diseases [9–11], the underlying mechanisms that are responsible for such a significant change are not well

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known. It is inevitable that there are some limitations for human data in dietary manipulation, the control of maternal pre-pregnancy status, and access to fresh tissue obtained at key time points of development [12]. Animal models of obesity and overnutrition such as sheep and rodents provide essential information into the underlying cellular and molecular mechanisms that contribute to this adverse influence [13–15]. However, the majority of animal studies have been carried out by exposing mothers to very high dietary intake. There is little information available in animal models challenged with a defined degree of increase in maternal energy intake. Here, we intended to evaluate whether moderately increased maternal energy intake above requirements during gestation could produce significant effects on offspring.

Among all the target tissues affected by maternal diets, skeletal muscle has received a considerable scientific interest, mainly because it composes about 40–50 % of body mass [16] and plays a key role in the regulation of glucose homoeostasis. Muscle fibre number is known to be an important determinant of muscle mass [17]. In mammals, muscle fibres are formed prenatally, and subsequent skeletal muscle development depends largely on muscle fibre hypertrophy [18, 19]. Therefore, foetal stage is crucial for skeletal muscle development and could potentially permanently influence postnatal skeletal muscle growth processes of the offspring, thereby producing long-lasting physiological effects. However, the effects of moderately increased maternal energy supply during gestation on basic events of foetal skeletal myogenesis in animal models have been poorly documented.

Compared with sheep and rodents, the pig serves as an excellent experimental model for the study of human nutrition because they are more similar to humans in physiological, behavioural and genomic [20–22]. In addition, in pig foetal development, muscle fibre hyperplasia begins at approximately day 35 of gestation and is mainly completed by day 90 of gestation [23, 24], and myogenesis during this stage is susceptible to environmental factors [5], so we chose the pig as the experimental animal. Our objective was to investigate the effect of moderately increased maternal energy intake from day 1 to 90 of gestation on foetal skeletal muscle developmental characteristics and muscle fibre composition in pig fetuses.

Materials and methods

Animals, diets and sample collection

The experimental protocols used in the present study were approved by the Animal Care and Use Committee of Sichuan Agricultural University. Twelve primiparous purebred Large White sows, with the body weight of 135.5 ± 1.6 kg in average, were artificially inseminated three times with the semen of purebred Large White boars at the third observation of oestrus. The day of the last insemination constituted the first day of gestation. Sows were randomly assigned at mating to one of two experimental groups differing in daily digestible energy (DE) intake: fed 2.4 kg/d with a control diet containing 12.90 MJ of DE/kg to achieve a target energy intake of 30.96 MJ DE/day following the National Research Council (NRC; 2012) recommendation for gestating sows (Con) and fed 2.4 kg/d with a high-energy diet containing 14.23 MJ of DE/kg to achieve a target energy intake of 34.15 MJ DE/day (HE). The HE diet was formulated to allow the sow to ingest 10 % more DE than the Con group. Sows were fed isoprotein corn–wheat bran–soya bean meal-based diets (Table 1). Although the additional dietary fibres were added to the Con diet to decrease energy concentration, the levels of crude fibre (CF) are still low, which is basically consistent with CF content in conventional diet of gestating sow. During the experimental period, sows were housed in individual feed stalls in a breeding facility. Sows were fed discretely twice daily at 0800 and 1400 h with 50 % of the daily ration each time and had free access to drinking water. After a 12-h overnight fast on day 90 of gestation, pregnant sows were weighed and four sows per diet group were anaesthetized with an intramuscular injection of Zoletil (Zoletil 50, Virbac; 4 mg/kg body weight). The maternal abdomen was opened, and the reproductive tracts were removed immediately. Umbilical vein blood was collected from each foetus and coagulated for 30 min, then centrifuged at 3500 × g for 10 min to separate serum which was stored at −20 °C until analysis. The weight of each foetus was recorded. Three female foetuses and three male foetuses, with weight close to the average level, were selected from each sow for muscle sample collection. The longissimus muscles (LMs) were collected from the selected foetuses, snap-frozen in liquid nitrogen and stored at −80 °C until subsequent analysis. LM samples for histological analysis were fixed in 4 % paraformaldehyde in a phosphate buffer. Since the difference between female and male foetuses was not significant on day 90 of gestation, data from female and male foetuses were pooled together for analysis.

Biochemical and enzyme analysis

Homogenates of LM tissue (approximately 0.5 g) were used to quantify DNA, protein and activities of CK, lactate dehydrogenase (LDH), citrate synthase (CS) and isocitrate dehydrogenase (ICDH). The muscle homogenates were diluted to an appropriate concentration for further analysis. The DNA was determined fluorometrically

Table 1 Ingredients and composition of the experimental diets (as-fed basis)

Item	Con	HE
Ingredient, %		
Maize	45.00	45.00
Soya bean meal	13.60	13.60
Wheat bran	27.80	27.80
Soya bean oil	4.50	9.10
Wheat bran fibre	2.54	–
Soya bean fibre	1.10	–
Maize fibre	0.96	–
Salt	0.40	0.40
L-Lys·HCl, 75 %	0.10	0.10
L-Thr	0.10	0.10
Limestone	1.23	1.23
Monocalcium phosphate	1.99	1.99
Choline chloride, 50 %	0.14	0.14
Vitamin premix[a]	0.04	0.04
Mineral premix[b]	0.50	0.50
Total	100.00	100.00
Composition		
Digestible energy (MJ/kg)	12.90	14.23
Crude protein (%)	13.92	13.49
Crude fibre (%)	4.97	3.41
Lys (%)	0.69	0.69
Met + Cys (%)	0.35	0.35
Thr (%)	0.46	0.46
Ca (%)	0.96	0.96
Available *P* (%)	0.48	0.48
Total *P* (%)	0.79	0.79

[a] Provided the following per kg diet: vitamin A, 1.2 mg; vitamin D_3, 0.02 mg; vitamin E, 44 mg; vitamin K_3, 0.5 mg; thiamin, 1 mg; riboflavin, 3.75 mg; pyridoxine, 1 mg; vitamin B_{12}, 0.015 mg; niacin, 10 mg; pantothenic acid, 12 mg; folic acid, 1.3 mg; biotin, 0.2 mg

[b] Provided the following per kg diet: Fe, 80 mg; Cu, 10 mg; Zn, 100 mg; Mn, 25 mg; Se, 0.15 mg; I, 0.14 mg

using Hoechst 33258 (Beyotime Biotechnology) with calf thymus DNA (Sigma-Aldrich) as a standard. The detailed procedures have been reported by Rehfeldt and Walther [25]. The protein concentration was measured according to a previously described method [26]. The activities of CK and LDH were determined by assay kits purchased from Nanjing Jiancheng Institute of Bioengineering and expressed as on a per-milligram protein basis. CS and ICDH activities were measured using colorimetric methods with spectrophotometer according to the instructions of the kits (GenMed Scientifics Inc.) and expressed as μmol of substrate transformed per minute per-gram protein.

Histology of skeletal muscles

LM samples were fixed in 4 % paraformaldehyde in a phosphate buffer, embedded in paraffin, sliced at a thickness of 5 μm using a rotary microtome (RM2135, Leica) and stained with haematoxylin and eosin (H&E). At least ten different microscopic fields of each section and five sections per foetus were randomly chosen to determine the cross-sectional area and density of foetal muscle fibres. Muscle fibre density was defined as number of myofibers per mm^2 of the LM cross-sectional area.

Serum hormone assays

Serum T3 and thyroxine (T4) were determined by radioimmunoassay using commercially available standard kits (Nanjing Jiancheng Institute of Bioengineering). The minimum detectable levels of T3 and T4 were 0.1 and 0.2 ng/mL, respectively.

Total RNA extraction and real-time PCR

Total RNA was extracted from frozen muscle tissues using RNAiso Plus reagent (TaKaRa). The concentration of RNA was estimated by UV/VIS spectrophotometer (A260/A280, Beckman Coulter DU800; Beckman Coulter Inc.). The integrity of RNA was checked by electrophoresis on a 1 % agarose gel stained with ethidium bromide. Following RNA isolation, total RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent kit (TaKaRa) according to the manufacturer's recommendations. Real-time quantitative PCR was carried out on the ABI Prism® 7900HT Sequence Detection System (Applied Biosystems) using SYBR® Premix Ex Taq™ II (TaKaRa). The reactions were performed in a final volume of 10 ul containing 5 ul of SYBR® Premix Ex Taq™ II, 0.8 ul of the primer pair, 0.2 ul of ROX Reference Dye, 1 ul of cDNA template and 3 ul of dH_2O. The thermal cycling parameters comprised an initial denaturation step at 95 °C for 30 s, 40 cycles of PCR at 95 °C for 5 s and 60 °C for 34 s, followed by a dissociation step at 95 °C for 15 s, 60 °C for 1 min and 95 °C for 15 s. To confirm specific product amplification, melt curve analysis was conducted. All measurements were taken in triplicate. Primers for individual genes are designed using Primer Express 3.0 (Applied Biosystems) and are given in Table 2. The relative mRNA abundance of analysed genes was calculated using the method of $2^{-\Delta\Delta Ct}$ as previously described [27]. For normalization, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was chosen as the reference gene since no variation in its expression was observed between treatments. The mRNA level of each target gene for Con group was set to 1.0.

Table 2 Primer sequences of the target and reference genes

Gene	Primer sequence (5′–3′)	Product (bp)	GenBank ID. or reference
Myostatin	Forward: TTTGGCAGAGCATTGATGTGA	131	NM_214435.2
	Reverse: TCCTGGTCCTGGGAAGGTTAC		
Pax7	Forward: TGAAGGTCGGAGTGAACGGAT	74	NM_001206359.1
	Reverse: CACTTTGCCAGAGTTAAAAGCA		
MyoD1	Forward: TGCCCAAGGTGGAAATCCTG	266	NM_001002824.1
	Reverse: GCTGTAATAGGTGCCGTCGT		
Myogenin	Forward: GAAAACTACCTGCCCGTCCA	184	NM_001012406.1
	Reverse: CCACAGACACGGACTTCCTC		
Myf5	Forward: GAGTTCGGGGACGAGTTTGA	156	NM_001278775.1
	Reverse: TTTCCTCTTGCACGCTTTGC		
MRF4	Forward: CGCCATCAACTACATCGAGAGGT	189	DQ139775.1
	Reverse: ATCACGAGCCCCCTGGAAT		
CKM	Forward: CACCCCTTCATGTGGAACGA	122	NM_001129949.1
	Reverse: TCGAACTTGGGATGCTTGCT		
Desmin	Forward: ATGAGCCAGGCCTACTCGTC	170	NM_001001535.1
	Reverse: GACACCTGGTACACTCGGGA		
GHR	Forward: TGATTCTACCCCCAGTTCCAGTTC	187	NM_214254.2
	Reverse: TCAGTCTTTTCATCAGGGTCATCA		
IGF1	Forward: GCACATCACATCCTCTTCGC	121	XM_005664199.1
	Reverse: GAACTGAAGAGCGTCCACCA		
IGF1R	Forward: GATTCAGGCCACCTCTCTCTCC	139	NM_214172.1
	Reverse: CCCTCCTACTATCAACAGAACGGC		
IGF2	Forward: TCAGGCTAGTCTCTCCTCGG	111	NM_213883.2
	Reverse: CTTGGGGATAATTGGGGGCA		
IGFBP5	Forward: GTGTACCTGCCCAACTGTGA	158	NM_214099.1
	Reverse: AAGCTGTGGCACTGGAAGTC		
MYH3	Forward: CCCGGCTTTGGTCTGATTT	74	Maak et al. [56]
	Reverse: GGTGTCGGCTGAGAGTCA		
MYH8	Forward: CGAGCCCTCCTGCTTTATCTC	98	Maak et al. [56]
	Reverse: TGCCAGATGAAAATGCAGGTT		
MYH7	Forward: GTTTGCCAACTATGCTGGGG	95	NM_213855.1
	Reverse: TGTGCAGAGCTGACACAGTC		
MYH2	Forward: CTCTGAGTTCAGCAGCCATGA	127	AB025260
	Reverse: GATGTCTTGGCATCAAAGGGC		
MYH4	Forward: GAGGTACATCTAGTGCCCTGC	83	AB021561
	Reverse: GCAGCCTCCCCAAAAATAGC		
MYH1	Forward: TTGACTGGGCTGCCATCAAT	111	AB025262
	Reverse: GCCTCAATGCGCTCCTTTTC		
GAPDH	Forward: TGAAGGTCGGAGTGAACGGAT	74	NM_001206359.1
	Reverse: CACTTTGCCAGAGTTAAAAGCA		

Pax7 paired box gene 7, *MyoD1* myogenic differentiation factor 1, *Myf5* myogenic factor 5, *MRF4* muscle regulatory factor 4, *CKM* creatine kinase, muscle, *GHR* growth hormone receptor, *IGF1* insulin-like growth factor 1, *IGF1R* insulin-like growth factor 1 receptor, *IGF2* insulin-like growth factor 2, *IGFBP5* insulin growth factor-binding protein 5, *MYH* myosin heavy chain, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase

Immunoblotting analysis

Protein extracts were obtained by homogenizing muscle tissues with a total protein extraction kit (Beyotime Biotechnology) according to the manufacturer's guide. The protein content was measured using the bicinchoninic acid (BCA) protein assay kit (Pierce). Then, western blot analysis for MyoD, myogenin, slow MyHC and fast MyHC

was carried out as previously described [28]. The relative expression of target protein was normalized using GAPDH as the internal protein, and then, the normalized values were used for the comparison of the expression of target protein between Con and HE group. Primary antibodies used in this study are shown in Table 3.

Statistical analysis

Data were analysed using the MIXED procedure of Statistical Analysis System (version 8.1, SAS Institute, Inc.) with treatment as the fixed effect and with sow as the random effect. The least square means were separated using the PDIFF option with the Tukey adjustment. Results were presented as least square means with their standard errors. Statistical significance was considered as $P < 0.05$, and a tendency towards difference was considered as $P < 0.10$.

Table 3 The primary antibodies used in the present study

Primary antibody	Introduction and company	Purpose
MyoD1	SC-304, Santa Cruz, USA	Analysis of MyoD1
Myogenin	SC-576, Santa Cruz, USA	Analysis of myogenin
MYH7	Ab11083, Abcam, USA	Analysis of slow MyHC
MYH1/2	SC-53088, Santa Cruz, USA	Analysis of fast MyHC (type 2a/x)

MyoD1 myogenic differentiation factor 1, *MYH*/*MyHC* myosin heavy chain

Table 4 Sow BW, litter size, foetal weight and variation in foetal weight within a litter on day 90 of gestation

Item	Con	HE	SEM	*P* value
Sow BW (kg)				
At insemination	135.6	135.4	1.0	0.930
At day 90 of gestation	188.3	201.6	3.8	0.005
Litter size	12.0	10.5	0.8	0.183
Foetal weight (g)	737.3	703.3	15.3	0.125
CV, %	13.60	12.21	0.79	0.243

$n = 4$ for each group

Con maternal normal energy intake, *HE* maternal high energy intake, *BW* body weight, *CV* coefficient of variation, values determined from foetal weights within each litter

Table 5 Muscle fibre density, muscle fibre cross-sectional area (MCSA), biochemical characteristics and metabolic enzyme activities in foetal longissimus dorsi muscle of Con and HE sows

Item	Con	HE	SEM	*P* value
Muscle fibre density	3887	3343	191	0.048
MCSA (um^2)	90.23	94.80	5.79	0.576
Protein (mg/g)	57.8	53.3	1.5	0.046
DNA (μg/g)	1971	1908	88	0.621
DNA/protein (μg/mg)	34.5	36.2	1.8	0.519
CK (U/mg protein)	3.86	3.56	0.10	0.048
LDH (U/mg protein)	1.62	1.40	0.04	0.001
ICDH (μmol/min/g protein)	103.85	92.62	4.28	0.083
CS (μmol/min/g protein)	14.88	13.32	0.78	0.151

$n = 24$ in each group

Con maternal normal energy intake, *HE* maternal high energy intake, *CK* creatine kinase, *LDH* lactate dehydrogenase, *ICDH* isocitrate dehydrogenase, *CS* citrate synthase

Results

Gestation performance

On day 90 of gestation, sows fed the HE diet were heavier than sows fed the Con diet ($P < 0.01$; Table 4). However, no significant differences were observed for litter size, foetal weight and variation in foetal weight within a litter (as indicated by the coefficient of variation) between Con and HE groups ($P > 0.05$).

Histological properties, biochemical characteristics and metabolic enzyme activities

As shown in Table 5, the number of muscle fibres per mm^2 was decreased ($P < 0.05$) in the foetal LM of HE group, whereas the muscle fibre cross-sectional area exhibited no significant differences ($P > 0.05$). Protein concentration and the activities of CK ($P < 0.05$) and LDH ($P < 0.01$) were significantly reduced ($P < 0.05$) in LMs of HE foetuses when compared with Con foetuses. Meanwhile, the activity of ICDH tended to be reduced ($P = 0.083$) in HE foetuses. No significant differences were observed for DNA concentration, DNA: protein ratio and CS activity between Con and HE groups ($P > 0.05$).

T3 and T4 concentrations in umbilical vein serum of foetuses

The differences of T3 and T4 concentrations in foetal umbilical vein serum between treatment groups on day 90 of gestation are shown in Fig. 1. Significantly lower T3 ($P < 0.05$) concentrations were found in umbilical vein

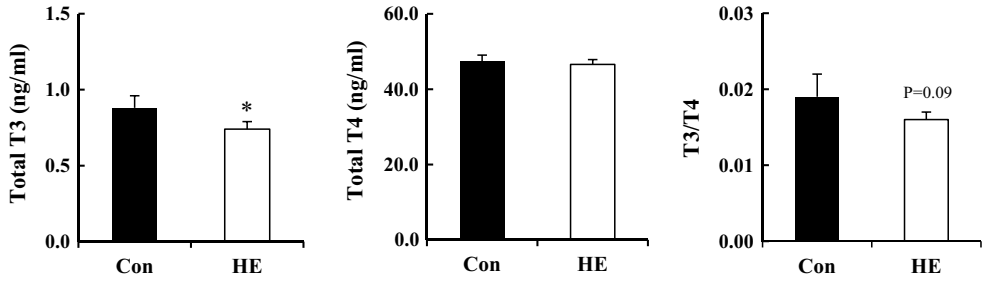

Fig. 1 Total T3 and T4 concentrations in foetal umbilical vein serum of Con and HE sows. Values are least squares means ± SEMs, $n = 24$. *Con* maternal normal energy intake, *HE* maternal high energy intake, *T3* triiodothyronine, *T4* thyroxine. *$P < 0.05$ compared with Con group

serum of HE foetuses compared with Con group. Furthermore, maternal HE diets tended to decrease ($P = 0.09$) the ratio of T3 to T4 in foetal umbilical vein serum. However, T4 concentration did not differ between Con and HE foetuses ($P > 0.05$).

Muscle growth-related genes expression

In Fig. 2a, the effects of maternal dietary treatment on the mRNA abundance of muscle growth-related genes are shown. The mRNA abundance of paired box gene (Pax7; $P < 0.01$), MyoD1 ($P < 0.01$), myogenin ($P < 0.01$), myogenic factor 5 (Myf5; $P < 0.01$), muscle regulatory factor 4 (MRF4; $P < 0.05$) and muscle creatine kinase (CKM; $P < 0.05$) was decreased in LM of HE foetuses relative to Con foetuses. However, moderately increased maternal energy intake did not significantly affect the mRNA abundance of myostatin and desmin in foetal LM ($P > 0.05$). Since MyoD1 and myogenin are key regulators of myogenesis, we further analysed the protein expression of MyoD1 and myogenin (Fig. 2b). Western blotting indicated that MyoD1 ($P < 0.01$) and myogenin ($P < 0.05$) protein expression were both significantly decreased in HE foetuses, consistent with their mRNA expression.

Gene expression of the IGFs system

As shown in Fig. 3, maternal dietary treatment had a significant effect on the mRNA abundance of IGF-1 ($P < 0.05$) and IGFBP-5 ($P < 0.05$) in LM of foetuses, which were lower in HE group than in Con group. In addition, the mRNA abundance of insulin-like growth factor 1 receptor (IGF-1R) also tended to be decreased ($P = 0.06$) in HE foetuses. Energy intake during gestation had no effect on growth hormone receptor (GHR) and IGF-2 mRNA expression ($P > 0.05$).

Myosin heavy-chain (MyHC) isoform genes expression

In Fig. 4a, the effects of maternal dietary treatment on the mRNA abundance of MyHC isoforms in LMs of foetuses are shown. The mRNA abundance of MYH2 ($P < 0.05$) and MYH1 ($P < 0.01$) was decreased in LM of HE foetuses relative to Con foetuses. No significant differences were observed for the mRNA abundance of MYH3, MYH8, MYH7 and MYH4 between Con and HE groups ($P > 0.05$). Western blotting further indicated that the protein expression of fast-MyHC (MYH1/2) isoform was lower ($P < 0.05$) in HE foetuses than in Con foetuses (Fig. 4b). No significant difference was observed for protein expression of slow-MyHC (MYH7) isoform between Con and HE groups ($P > 0.05$).

Discussion

In the current trial, body weights of HE sows during gestation were enhanced compared with Con sows, but foetal weights were not significantly affected by gestation treatments, similar to other reports [29]. Muscle fibre number and muscle fibre cross-sectional area are known to be important determinants of postnatal muscle growth. Muscle fibre hyperplasia is completed prenatally and can be affected by maternal treatments during gestation, thereby influencing early postnatal muscle growth. In the present research, muscle fibre cross-sectional area of the foetuses was not markedly influenced by the sow treatments, but increased energy intake during gestation led to a smaller number of muscle fibres per mm^2 in the LM of HE foetuses compared with the foetuses of Con sows. These results are not consistent with the hypothesis that increasing the maternal nutrition during gestation has positive effects on muscle growth of the offspring. Some studies in pigs have shown that increased maternal feed allowance from day 25 to 50 of gestation caused an increase in the number of secondary fibres of the offspring [24, 30]. However, other studies in pigs have shown no consistent effects, and even disadvantageous effects, on muscle fibre development [18, 29, 31] and on growth performance [32, 33] when maternal feed intake was increased during early gestation. In more recent studies, the improvement in maternal vitamin

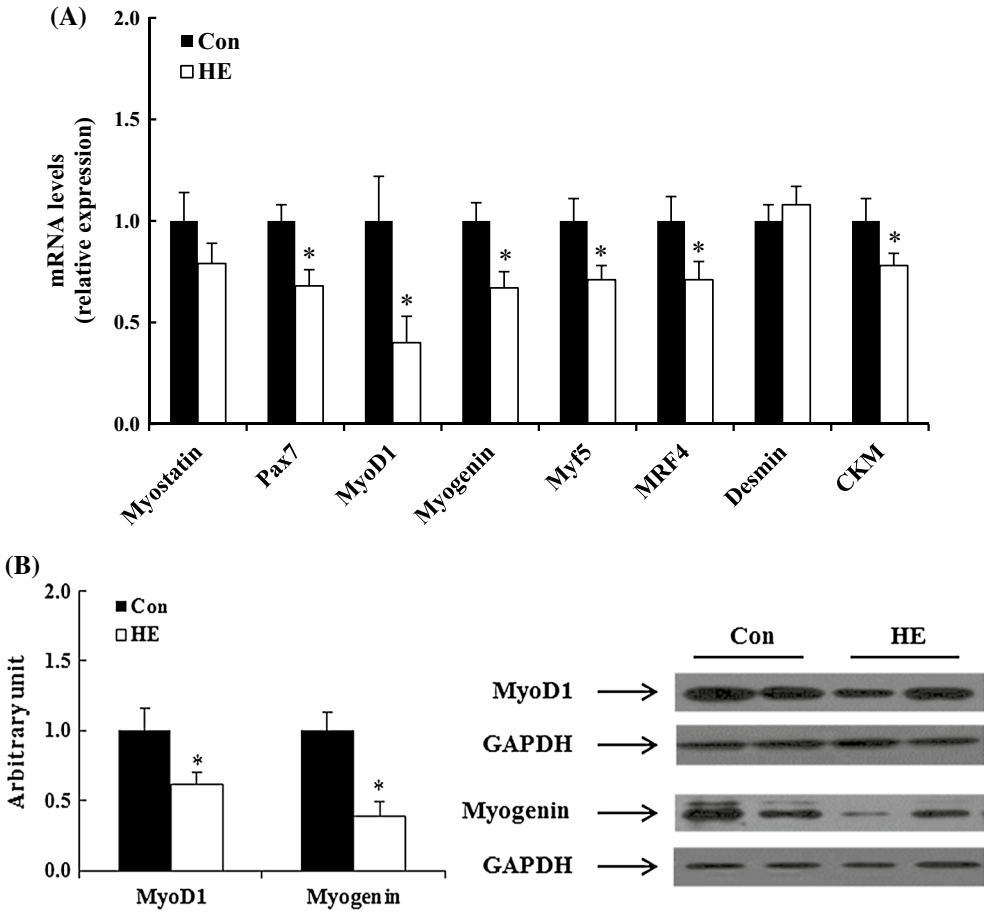

Fig. 2 The muscle growth-related genes mRNA expression (**a**) and MyoD1 and myogenin protein expression (**b**) in foetal longissimus dorsi muscle of Con and HE sows. Values are least squares means ± SEMs, $n = 24$. *Con* maternal normal energy intake, *HE* maternal high energy intake, *Pax7* paired box gene 7, *MyoD1* myogenic differentiation factor 1, *Myf5* myogenic factor 5, *MRF4* muscle regulatory factor 4, *CKM* creatine kinase, muscle, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase. *$P < 0.05$ compared with Con group

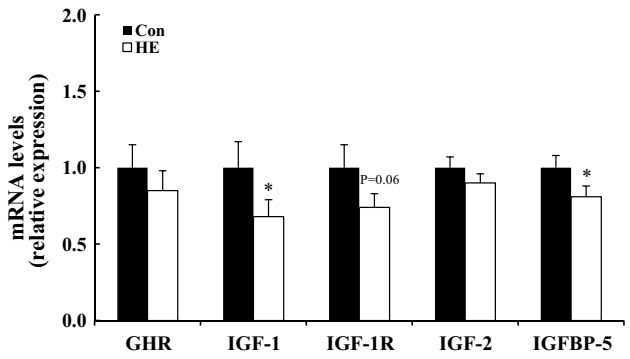

Fig. 3 GH-IGF system genes mRNA expression in foetal longissimus dorsi muscle of Con and HE sows. Values are least squares means ± SEMs, $n = 24$. *Con* maternal normal energy intake, *HE* maternal high energy intake, *GHR* growth hormone receptor, *IGF-1* insulin-like growth factor 1, *IGF-1R* insulin-like growth factor 1 receptor, *IGF-2* insulin-like growth factor 2, *IGFBP-5* insulin growth factor-binding protein 5. *$P < 0.05$ compared with Con group

D status beginning at day 43 before breeding through day 90 of gestation increased total muscle fibre number in LD muscle of foetal pigs [34], whereas dietary L-arginine supplementation to gilts during early gestation did not affect total muscle fibre number in foetal pigs [35]. Maternal treatment with porcine growth hormone in early gestation caused an increase in the number of muscle fibres only in low weight pigs [36, 37]. These findings indicate that the potential actions of increasing maternal nutrition in pregnant sows during gestation on skeletal muscle development of the offspring are highly controversial. Thus, there are contradictory results on how increased maternal nutrition during gestation affects myogenesis in the offspring. The time period and duration of treatment during gestation, the type and amount of nutrient changes involved in the treatment, the litter size and the muscle tested have been suggested as important factors resulting in variation between studies [18, 31].

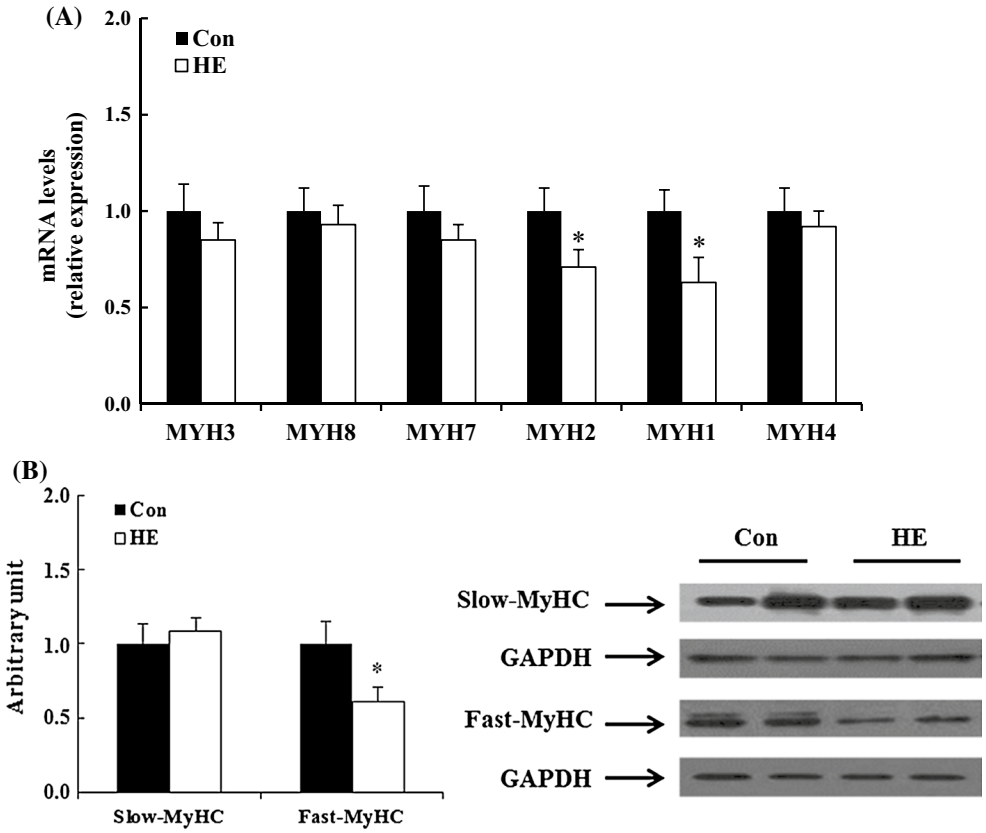

Fig. 4 Myosin heavy-chain (MyHC) isoform genes mRNA expression (**a**) and slow-MyHC and fast-MyHC protein expression (**b**) in foetal longissimus dorsi muscle of Con and HE sows. Values are least squares means ± SEMs, $n = 24$. *Con* maternal normal energy intake, *HE* maternal high energy intake, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase. *$P < 0.05$ compared with Con group

The phenotypic changes in muscle characteristics are often linked to metabolic and functional alterations. Available evidence suggests that maternal nutritional status could cause foetal skeletal muscle metabolic changes and alterations of myogenic gene expression [38, 39]. Thus, in the present study, to clarify the mechanisms leading to increased muscle fibre density, various traits of foetal growth and differentiation were determined.

During skeletal muscle development, muscle-specific proteins are expressed with progressing differentiation. The enzyme CK and LDH, as markers of muscle-specific differentiation, play a crucial role in energy metabolism [36, 40]. The present results suggested that increased supply of energy during gestation reduced protein concentration and the CK and LDH enzyme activities in the skeletal muscle of HE foetuses. Similarly, hormonal treatment of pregnant sows in early gestation with porcine somatotropin caused reduction in protein concentration and CK activity of 37-day-old foetuses [36]. The foetuses derived from HE sows with their lower muscle fibre density in LM on day 90 of gestation combined with lower muscular protein concentration and the CK and LDH enzyme activities suggest that the muscles of these foetuses may be less differentiated and mature than their counterparts from Con sows. The delay in muscle differentiation was also observed previously in 75-day-old foetuses in response to dietary L-arginine supplementation to sows from day 14 to 28 of gestation [41].

The delay in muscle differentiation and maturity mentioned above is underlined by the results on the expression of the MyHC isoforms. The myofibre types are in part characterized by the expression of various MYH genes [42]. MYH3, MYH8 and MYH7, which encode the embryonic, perinatal and slow MyHCs, are the predominant isoforms in prenatal mammalian muscles, which are gradually replaced by the postnatal MyHC isoforms, including fast 2a, 2x and 2b MyHC encoded by MYH2, MYH1 and MYH4, respectively [43, 44]. Studies in pigs have shown that postnatal fast-MyHC isoforms were expressed much earlier (day 35 of gestation) than were previously reported in small mammals [44]. In the present study, foetuses from HE sows exhibited lower mRNA expression of MYH2 and MYH1 and protein expression of fast 2a and 2 × isoforms. These results are similar to results obtained by Cerisuelo et al. [31] who reported that increasing the maternal feed amount during midgestation (from day 45 to 85 of gestation) led to fewer estimated glycolytic fibres (type 2b) in

the longissimus thoracis muscle of progeny compared with the progeny of control sows, but maternal treatment did not affect the number of type I fibre. The decreased fast-MyHC isoform in HE foetuses was also linked to the reduced LDH activity in this study. Together with the data on muscle fibre density, muscular protein concentration and CK and LDH enzyme activities, the lower expression of the fast-MyHC isoforms in LM of HE foetuses indicates that increased maternal energy intake caused a delay in muscle differentiation resulting in a lower degree of muscular maturity on day 90 of gestation.

Moreover, foetal skeletal muscle development is under the control of a wide variety of hormones and growth factors [45]. It is here shown that concentrations of T3 in umbilical veins serum of HE foetuses were decreased on day 90 of gestation compared with the Con foetuses. Because thyroid hormone, a major determinant of myofibre composition, is known to be involved in regulating the transformation from embryonic or prenatal MyHC isoform to adult fast-MyHC isoforms [46, 47], a lower degree of muscular differentiation and maturation in HE foetuses could be related, at least partly, to the decreased level of umbilical vein serum T3.

Prenatal myogenesis is a well-organized and balanced process, which includes commitment of stem cells to the myogenic lineages, myoblast proliferation, cell cycle withdrawal, differentiation and fusion of myoblast and finally the maturation of muscle fibres [48]. The complex process is regulated by a sequential series of transcription factors, including Pax7, a marker of myogenic precursor cells, and the family of myogenic regulatory factors (MRFs) which consists of MyoD1, Myf5, myogenin and MRF4 [49, 50]. The expression of MRFs is induced by Pax7, which leads to myogenic differentiation [5, 50]. It has been demonstrated that MyoD1 is essential for the myoblast formation, while myogenin plays a dominant role in myoblast fusion and myotube formation [51]. The present results showed that the reduction in muscle fibre density in HE foetuses was accompanied by a lower expression of the myogenic regulatory factors MyoD1 and myogenin at both the mRNA and protein levels, suggesting that a moderately increased maternal energy intake during gestation downregulated the myogenesis. Because the muscle-specific CKM gene is transcriptionally induced when skeletal muscle myoblasts differentiate into myocytes [52], the downregulation of myogenesis in HE foetuses was also reflected by a decreased mRNA expression. Previous studies have found that maternal obesity coupled with high-energy diets led to low-grade inflammation in foetal muscle tissue, which attenuated myogenesis [5, 38]. In this study, whether such a high energy intake can induce inflammatory responses in HE sows, which changes foetal skeletal muscle development by downregulating myogenesis, is not known, but the sows did gain lots of weight.

In addition, the IGFs system is involved in the regulation of skeletal muscle growth and metabolism [53]. The biological actions of most IGFs are mediated through the IGF-1R and are modulated by the IGFBPs in local tissues [54]. IGFBP-5 is the major IGFBP secreted by skeletal muscle and has been shown to associate with myogenic differentiation [53, 55]. In this study, the downregulation of MRFs expression in LM of foetuses from HE sows was accompanied by simultaneous marked changes in both IGF-1 and IGFBP-5 at the mRNA levels, suggesting that extra energy supplementation to sows during gestation did not result in promoting foetal muscle development and did not trigger the up-regulation of muscle growth-related genes on day 90 of gestation.

This study provides important new information describing the effects of a moderate increase in energy intake in pregnant sows on foetal skeletal muscle development and metabolism during gestation. Results show that a high energy supply during gestation delays the differentiation and maturation in skeletal muscle of the foetus on day 90 of gestation in pigs. This finding may have important implications for meat animal production as well as human health. In addition, further studies are required to investigate whether developmental delay in muscle differentiation on day 90 of gestation will be compensated until birth and to how maternal HE diet affects the postnatal muscle growth of offspring.

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Compliance with ethical standards

Conflict of interest Tiande Zou, Dongting He, Bing Yu, Jie Yu, Xiangbing Mao, Ping Zheng, Jun He, Zhiqing Huang, Yan Shu, Yue Liu and Daiwen Chen declare no conflict of interest.

References

1. Godfrey KM (1998) Maternal regulation of fetal development and health in adult life. Eur J Obstet Gyn Reprod Biol 78:141–150
2. Wu G, Bazer FW, Cudd TA et al (2004) Maternal nutrition and fetal development. J Nutr 134:2169–2172
3. Metges CC, Görs S, Lang IS et al (2014) Low and high dietary protein: carbohydrate ratios during pregnancy affect maternofetal glucose metabolism in pigs. J Nutr 144:155–163
4. Drake AJ, Reynolds RM (2010) Impact of maternal obesity on offspring obesity and cardiometabolic disease risk. Reproduction 140:387–398

5. Du M, Yan X, Tong JF et al (2010) Maternal obesity, inflammation, and fetal skeletal muscle development. Biol Reprod 82:4–12
6. Harding JE (2001) The nutritional basis of the fetal origins of adult disease. Int J Epidemiol 30:15–23
7. Vernarelli JA, Mitchell DC, Rolls BJ et al (2014) Dietary energy density is associated with obesity and other biomarkers of chronic disease in US adults. Eur J Nutr 54:59–65
8. Flegal KM, Carroll MD, Kit BK et al (2012) Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010. JAMA 307:491–497
9. Bruce KD, Hanson MA (2010) The developmental origins, mechanisms, and implications of metabolic syndrome. J Nutr 140:648–652
10. Heerwagen MJ, Miller MR, Barbour LA et al (2010) Maternal obesity and fetal metabolic programming: a fertile epigenetic soil. Am J Physiol-Regul Integr Comp Physiol 299:R711–R722
11. Rkhzay-Jaf J, O'Dowd JF, Stocker CJ (2012) Maternal obesity and the fetal origins of the metabolic syndrome. Curr Cardiovasc Risk Rep 6:487–495
12. Schlabritz-Loutsevitch N, Ballesteros B, Dudley C et al (2007) Moderate maternal nutrient restriction, but not glucocorticoid administration, leads to placental morphological changes in the baboon (Papio sp.). Placenta 28:783–793
13. Samuelsson A-M, Matthews PA, Argenton M et al (2008) Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance a novel murine model of developmental programming. Hypertension 51:383–392
14. Zhu M, Du M, Nathanielsz P et al (2010) Maternal obesity up-regulates inflammatory signaling pathways and enhances cytokine expression in the mid-gestation sheep placenta. Placenta 31:387–391
15. Yan X, Huang Y, Zhao J-X et al (2011) Maternal obesity-impaired insulin signaling in sheep and induced lipid accumulation and fibrosis in skeletal muscle of offspring. Bio Reprod 85:172–178
16. Rooyackers OE, Nair KS (1997) Hormonal regulation of human muscle protein metabolism. Annu Rev Nutr 17:457–485
17. Rehfeldt C, Stickland NC, Fiedler I et al (1999) Environmental and genetic factors as sources of variation in skeletal muscle fibre number. Basic Appl Myol 9:235–254
18. Nissen P, Danielsen V, Jorgensen P et al (2003) Increased maternal nutrition of sows has no beneficial effects on muscle fiber number or postnatal growth and has no impact on the meat quality of the offspring. J Anim Sci 81:3018–3027
19. Du M, Zhao JX, Yan X et al (2011) Fetal muscle development, mesenchymal multipotent cell differentiation, and associated signaling pathways. J Anim Sci 89:583–590
20. Miller E, Ullrey D (1987) The pig as a model for human nutrition. Annu Rev Nutr 7:361–382
21. Humphray SJ, Scott CE, Clark R et al (2007) A high utility integrated map of the pig genome. Genome Biol 8:R139
22. Spurlock ME, Gabler NK (2008) The development of porcine models of obesity and the metabolic syndrome. J Nutr 138:397–402
23. Wigmore P, Stickland N (1983) Muscle development in large and small pig fetuses. J Anat 137:235
24. Dwyer C, Stickland N, Fletcher J (1994) The influence of maternal nutrition on muscle fiber number development in the porcine fetus and on subsequent postnatal growth. J Anim Sci 72:911–917
25. Rehfeldt C, Walther K (1997) A combined assay for DNA, protein, and the incorporation of [^{3}H] label in cultured muscle cells. Anal Biochem 251:294–297
26. Smith P, Krohn RI, Hermanson G et al (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85
27. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25:402–408
28. Zhu MJ, Ford SP, Means WJ et al (2006) Maternal nutrient restriction affects properties of skeletal muscle in offspring. J Physiol 575:241–250
29. Bee G (2004) Effect of early gestation feeding, birth weight, and gender of progeny on muscle fiber characteristics of pigs at slaughter. J Anim Sci 82:826–836
30. Gatford KL, Ekert JE, Blackmore K et al (2003) Variable maternal nutrition and growth hormone treatment in the second quarter of pregnancy in pigs alter semitendinosus muscle in adolescent progeny. Brit J Nutr 90:283–293
31. Cerisuelo A, Baucells M, Gasa J et al (2009) Increased sow nutrition during midgestation affects muscle fiber development and meat quality, with no consequences on growth performance. J Anim Sci 87:729–739
32. Heyer A, Andersson H, Lindberg J et al (2004) Effect of extra maternal feed supply in early gestation on sow and piglet performance and production and meat quality of growing/finishing pigs. Acta Agr Scand A-Anim Sci 54:44–55
33. Musser R, Davis D, Tokach M et al (2006) Effects of high feed intake during early gestation on sow performance and offspring growth and carcass characteristics. Anim Feed Sci Tech 127:187–199
34. Hines E, Coffey J, Starkey C et al (2013) Improvement of maternal vitamin D status with 25-hydroxycholecalciferol positively impacts porcine fetal skeletal muscle development and myoblast activity. J Anim Sci 91:4116–4122
35. Bérard J, Bee G (2010) Effects of dietary L-arginine supplementation to gilts during early gestation on foetal survival, growth and myofiber formation. Animal 4:1680–1687
36. Rehfeldt C, Kuhn G, Vanselow J et al (2001) Maternal treatment with somatotropin during early gestation affects basic events of myogenesis in pigs. Cell Tissue Res 306:429–440
37. Rehfeldt C, Nissen PM, Kuhn G et al (2004) Effects of maternal nutrition and porcine growth hormone (pGH) treatment during gestation on endocrine and metabolic factors in sows, fetuses and pigs, skeletal muscle development, and postnatal growth. Domest Anim Endocrin 27:267–285
38. Tong JF, Yan X, Zhu MJ et al (2009) Maternal obesity downregulates myogenesis and β-catenin signaling in fetal skeletal muscle. Am J Physiol Endocrinol Metab 296:E917–E924
39. Simar D, Chen H, Lambert K et al (2012) Interaction between maternal obesity and post-natal over-nutrition on skeletal muscle metabolism. Nutr Metab Cardiovas 22:269–276
40. Lefaucheur L, Ecolan P, Barzic Y-M et al (2003) Early postnatal food intake alters myofiber maturation in pig skeletal muscle. J Nutr 133:140–147
41. Kalbe C, Bérard J, Porm M et al (2013) Maternal L-arginine supplementation during early gestation affects foetal skeletal myogenesis in pigs. Livest Sci 157:322–329
42. Schiaffino S, Reggiani C (2011) Fiber types in mammalian skeletal muscles. Physiol Rev 91:1447–1531
43. Lefaucheur L, Edom F, Ecolan P et al (1995) Pattern of muscle fiber type formation in the pig. Dev Dynam 203:27–41
44. Da Costa N, McGillivray C, Chang KC (2003) Postnatal myosin heavy chain isoforms in prenatal porcine skeletal muscles: insights into temporal regulation. Anat Rec Part A 273:731–740
45. Florini JR, Ewton DZ, Magri KA (1991) Hormones, growth factors, and myogenic differentiation. Annu Rev Physiol 53:201–216
46. Finkelstein DI, Andrianakis P, Luff AR et al (1991) Effects of thyroidectomy on development of skeletal muscle in fetal sheep. Am J Physiol Regul Integr Comp Physiol 261:R1300–R1306
47. Zhang D, Wang X, Li Y et al (2014) Thyroid hormone regulates muscle fiber type conversion via miR-133a1. J Cell Biol 207:753–766

48. Charge SB, Rudnicki MA (2004) Cellular and molecular regulation of muscle regeneration. Physiol Rev 84:209–238
49. Pownall ME, Gustafsson MK, Emerson CP Jr (2002) Myogenic regulatory factors and the specification of muscle progenitors in vertebrate embryos. Annu Rev Cell Dev Biol 18:747–783
50. Relaix F, Rocancourt D, Mansouri A et al (2005) A Pax3/Pax7-dependent population of skeletal muscle progenitor cells. Nature 435:948–953
51. Sabourin LA, Rudnicki MA (2000) The molecular regulation of myogenesis. Clin Genet 57:16–25
52. Zhao J, Schmieg FI, Simmons DT et al (1994) Mouse p53 represses the rat brain creatine kinase gene but activates the rat muscle creatine kinase gene. Mol Cell Biol 14:8483–8492
53. Collett-Solberg PF, Cohen P (2000) Genetics, chemistry, and function of the IGF/IGFBP system. Endocrine 12:121–136
54. Duan C, Ren H, Gao S (2010) Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. Gen Comp Endocr 167:344–351
55. Ren H, Yin P, Duan C (2008) IGFBP-5 regulates muscle cell differentiation by binding to IGF-II and switching on the IGF-II auto-regulation loop. J Cell Biol 182:979–991
56. Maak S, Wicke M, Swalve HH (2005) Analysis of gene expression in specific muscles of swine and turkey. Arch Tierz Dummerstorf 48:135–140