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Differences between porcine *longissimus thoracis* and *semitendinosus* intramuscular fat content and the regulation of their preadipocytes during adipogenic differentiation

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ABSTRACT

Intramuscular fat (IMF) plays an important role in pork quality. However, differences in the adipogenic regulation of IMF content between pig *longissimus thoracis* (LT) and *semitendinosus* (ST) remain unclear. Here, we found that IMF content of 180-day-old pig LT was greater than that of pig ST. Furthermore, lipid accumulation was earlier and greater in LT intramuscular preadipocytes (L-IMA) than in ST intramuscular preadipocytes (S-IMA) during differentiation. Interestingly, glucose consumption was lower in L-IMA than in S-IMA. Moreover, monounsaturated fatty acid content was greater in L-IMA than in S-IMA, whereas polyunsaturated fatty acid content was lower. Levels of the expression of key adipogenic genes were higher in L-IMA than S-IMA. Compared with S-IMA, adipogenic signals were more activated in L-IMA after adipogenic induction. In conclusion, IMF deposition differences between pig LT and ST were due to different glucose consumption, fatty acid composition, expression of key adipogenic genes and level of activating adipogenic signals between S-IMA and L-IMA during adipogenesis.

1. Introduction

The continuing demand for increased pork marbling has generated many studies focused on intramuscular adipose tissue and adipocytes, as intramuscular fat (IMF) content is regarded as important in improving meat quality (Dodson et al., 2015; Hausman & Poulos, 2004; Wu et al., 2013). Although there are many biochemical and histological differences between the pig semitendinosus (ST), longissimus thoracis (LT) and longissimus lumborum (LL) muscles (Brackebusch, McKeith, Carr, & McLaren, 1991; Realini et al., 2013; Realini et al., 2013), differences in the IMF content and intramuscular preadipocytes (IMAs) between pig ST and *longissimus thoracis et lumborum* (LTL) remain unstudied.

Because porcine IMAs have been studied *in vitro* as a cellular model of intramuscular adipogenesis, the protocols of isolation, culture and induction on IMAs are established (Hausman & Poulos, 2004; Sun et al., 2013; Wang et al., 2013; Wu et al., 2013; Zhou et al., 2007). In previous studies, the muscle samples came from ST (Hausman & Poulos, 2004; Hausman & Poulos, 2005). However, more recent articles in which IMAs were isolated from LTL reported different results during adipogenesis (Chen et al., 2016; Jiang et al., 2015; Sun et al., 2016; Wang et al., 2013; Wang et al., 2015; Zhang et al., 2014; Zhao et al., 2016). ST is regarded as a typical muscle model to explore characteristics of muscle stromal-vascular (SV) cells (Hausman & Poulos, 2004). Because of the important economic value of porcine LTL, it was generally used though in studies of meat quality (Dodson et al., 2010; Warner,

Abbreviations: Akt, protein kinase B; DM, differentiation medium; *ERK*, extracellular regulated protein kinase; *FABP4*, fatty acid binding protein 4; *FASN*, fatty acid synthase; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *GLUT4*, glucose transporter type 4; HE, Haematoxylin-eosin; IMF, intramuscular fat; IMA, intramuscular adipocyte; *LEPR*, leptin receptor; LT, longissimus thoracis; LTL, longissimus thoracis et lumborum; L-IMA, LT intramuscular preadipocyte; MDI, growth medium including 0.5 mM IBMX, 1 nM DEX, and 5 ng/mL insulin; mTOR, mammalian target of rapamycin; MUFA, monounsaturated fatty acid; *PPARG*, peroxisome proliferators activated receptor γ; PUFA, polyunsaturated fatty acid; SCD, stearoyl-CoA desaturase; SNAP23, synaptosomal-associated protein 23; *SREBF1*, sterol regulatory element-binding transcription factor 1; ST, semitendinosus; S-IMA, ST intramuscular preadipocyte; SV, stromal-vascular; *TAG*, triacylglycerol

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Table 1
Primer sequences used for real time quantitative PCR.

Gene	Accession number	Forward (5′ → 3′)	Reverse (5′ → 3′)
PPARG	NM_214379	AGGACTACCAAAGTGCCATCAAA	GAGGCTTTATCCCCACAGACAC
SREBF1	NM_214157	GCGACGGTGCCTCTGGTAGT	CGCAAGACGGCGGATTTA
FASN	EF_589048	AGCCTAACTCCTCGCTGCAAT	TCCTTGGAACCGTCTGTGTTC
FABP4	HM_453202	GAGCACCATAACCTTAGATGGA	AAATTCTGGTAGCCGTGACA
GAPDH	KJ_786424.1	AGGTCGGAGTGAACGGATTTG	ACCATGTAGTGGAGGTCAATGAAG

PPARG = peroxisome proliferators activated receptor γ; *SREBF1* = Sterol regulatory element-binding transcription factor 1; *FASN* = fatty acid synthase; *FABP4* = fatty acid binding protein 4; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase.

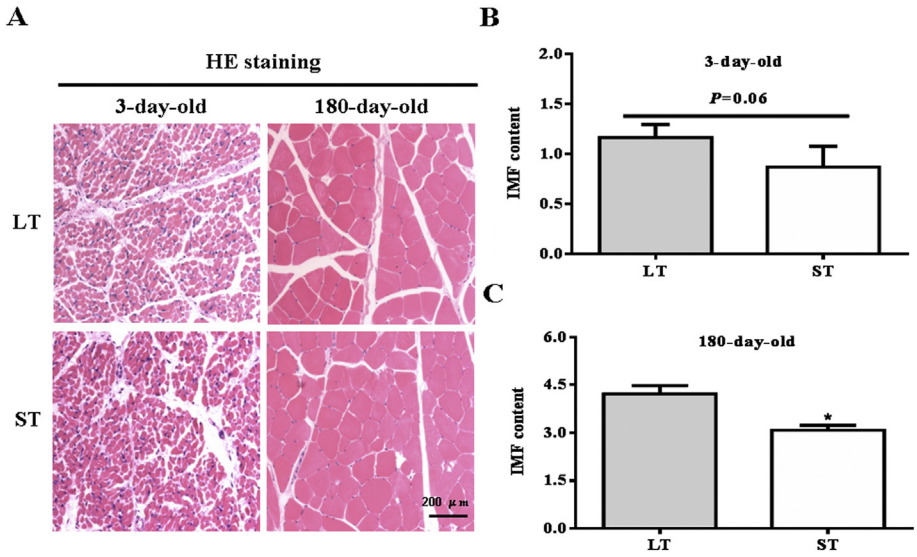

Fig. 1. Detection of IMF (intramuscular fat) content in *longissimus thoracis* (LT) and ST (*semitendinosus*) of 3-day-old and 180-day-old pigs. (A) HE (haematoxylin-eosin) staining of LT and ST sections. Scale bar: 200 μm; (B) IMF content in LT and ST of 3-day-old piglets; (C) IMF content (%) in LT and ST of 180-day-old pigs. Data represent means ± SEM (n = 4), *P < 0.05.

Kauffman, & Russel, 1993). Preadipocytes isolated from different fat depots of pig, bovine, mouse and human differ in size, response to insulin and dexamethasone agents, and lipid synthetic capacity (Arrighi et al., 2015; Hausman, Basu, Wei, Hausman, & Dodson, 2014; Ortiz-Colon, Grant, Doumit, & Buskirk, 2009; Rosen & Spiegelman, 2014; Siciliano et al., 2016; Wang et al., 2013; Wu et al., 2013; Zhang et al., 2014). Moreover, new candidate genes for porcine adipocyte size and fat content have been reported but not studied in depth. *SNAP23* (synaptosomal-associated protein 23) is implicated in the size of adipocytes derived from subcutaneous, visceral and longissimus dorsi muscle and is considered an interesting candidate gene in porcine adipose tissue growth (Kociucka, Jackowiak, Kamyczek, Szydlowski, & Szczerbal, 2016). *Perilipin 5*, *SCD* (stearoyl-CoA desaturase) and *LEPR* (leptin receptor) play crucial roles in the regulation of lipid deposition and fatty acid storage in porcine muscle and backfat (Pena, Ros-Freixedes, Tor, & Estany, 2016; Zappaterra, Mazzoni, Zambonelli, & Davoli, 2017). This gives rise to new questions: what are the adipogenic differences between IMAs isolated from skeletal muscles of pig ST and LTL and how are they regulated?

In the current study, we explore the differences between porcine LTL and ST IMF content and the regulation of the adipogenic differentiation of their preadipocytes. The IMF content, cellular and molecular events during fat deposition, IMA viability, adipogenic differentiation, triacylglycerol (TAG) content, platelet derived growth factor receptor α positive (*PDGFRα*$^+$) cell number, glucose consumption, key adipogenic gene expression, and important adipogenic signals of ST IMA (S-IMA) and LT IMA (L-IMA) were evaluated. Based on these factors, we found IMF deposition differences between porcine LT and ST and differential regulation of adipogenesis between L-IMA and S-IMA. These novel findings not only help us understand fat deposition characteristics in porcine LT and ST but also provide suitable cell models for the control of IMF content to improve pork marbling.

2. Materials and methods

2.1. Animals

Guanzhong Black pigs (Berkshire pig × Baimei pig) were from the Experimental Farm of Northwest A & F University (Yangling, Shaanxi Province, China) and handled in accordance with the guidelines (14–233, 10 December 2014) of Northwest A&F University Animal Care Committee. Animals were allowed access to feed and water *ad libitum* under the same feedstuff and management conditions and were humanely sacrificed at the end to ameliorate suffering. LT and ST samples were collected from six pigs at 3 and 180 days of age, respectively. For IMF content and haematoxylin-eosin (HE) staining, the samples were immediately conserved in liquid nitrogen.

2.2. Haematoxylin-eosin (HE) staining

The LT and ST samples were fixed with 4% paraformaldehyde at room temperature. Fixed tissues were dehydrated in 30% sucrose (*v*/v) and sectioned (10 μm) with a sliding microtome (Leica, Solms, Germany). Sections were stained with HE according to a previously published method (Wang et al., 2015).

2.3. Cell isolation and culture

ST and LT skeletal muscles from Guanzhong Black piglets (3 days of age) were aseptically isolated and all visible connective tissue was removed. After mincing to 2–3 mm in serum-free DMEM/F12, the finely minced tissues were digested at 37 °C for 120 min in a shaking water bath at 40 rpm and passed through sterile 178 and 74 mm steel mesh filters to isolate the separated cells. Cells were rinsed with serum-free DMEM/F12 medium, centrifuged twice at 1500 ×*g* for 10 min and