Cell Stress and Chaperones (2019) 24:635–645
https://doi.org/10.1007/s12192-019-01002-1

ORIGINAL PAPER

Maternal heat stress regulates the early fat deposition partly through modification of m^{6}A RNA methylation in neonatal piglets

Jinghui Heng[1] · Min Tian[1] · Wenfei Zhang[1] · Fang Chen[1] · Wutai Guan[1,2] · Shihai Zhang[1,2]

Received: 26 March 2019 / Revised: 18 April 2019 / Accepted: 25 April 2019 / Published online: 8 May 2019

Abstract

It is known that heat stress induces various physiological challenges in livestock production including changes in lipid metabolism. However, the molecular mechanism of how heat stress regulates lipid metabolism at the mRNA level is still largely unknown. N^6-methyl-adenosine (m^{6}A) is the most common and abundant modification on RNA molecules present in eukaryotes, which affects almost all aspects of RNA metabolism and thus gives us the hint that it may participate in changes of gene expression of lipid metabolism during heat stress. Therefore, the purpose of the present study was to investigate the effect of heat stress on fat metabolism in 21-day Large White × Landrace piglets from sows challenged by heat stress from day 85 of gestation until day 21 of lactation. We measured the expression of heat shock proteins (HSPs), genes associated with lipid metabolism, m^{6}A-related enzymes, and m^{6}A levels in abdominal fat and liver of offspring piglets. Our results showed that high ambient temperature significantly increased the expression of HSP70 ($P < 0.01$) in both liver and abdominal fat and upregulated HSP27 in the liver ($P < 0.05$). Additionally, genes involved in fat metabolism such as ACACA, FASN, DGAT1, PPAR-γ, SREBP-1c, and FABP4 were upregulated in abdominal fat in the experimental group challenged by high ambient temperature. In the liver, heat stress increased the mRNA expression of DGAT1, SREBP-1c, and CD36 and decreased ATGL and CPT1A expression ($P < 0.05$). The m^{6}A level was higher in the heat stress group compared with the control group in the liver and abdominal fat of offspring piglets ($P < 0.01$). Notably, heat stress also increased gene expression of METTL14, WTAP, FTO, and YTHDF2 ($P < 0.05$) in both abdominal fat and liver. The protein abundances of METTL3, METTL14, and FTO were upregulated after heat stress in abdominal fat ($P < 0.05$) but not in the liver. Although there was no difference in the protein abundance of YTHDF2 in abdominal fat, its level was increased in the liver ($P < 0.05$). In conclusion, our findings showed that heat stress increased expression of genes involved in lipogenesis, which provided scientific evidence to the observation of increased fatness in pigs under heat stress. We also demonstrated a possible mechanism that m^{6}A RNA modification may be associated with these changes in lipid metabolism upon heat stress.

Keywords Heat stress · Fat deposition · Sows · m^{6}A RNA methylation

✉ Wutai Guan
wutaiguan1963@163.com

✉ Shihai Zhang
zhangshihai@scau.edu.cn

[1] Guangdong Province Key Laboratory of Animal Nutrition Control, College of Animal Science, South China Agricultural University, Wushan Avenue, Tianhe District, Guangzhou 510642, China

[2] College of Animal Science and National Engineering Research Center for Breeding Swine Industry, South China Agricultural University, Wushan Avenue, Tianhe District, Guangzhou 510642, China

Introduction

High ambient temperature has been considered as one of the major challenges affecting pig production (Kouba et al. 1999, 2001), resulting in the reduction of feed intake and poor antioxidant capacity (Collin et al. 2001; Gan et al. 2014). Furthermore, lipid metabolism has been reported to be widely regulated by heat stress in animals (Baziz et al. 1996). Under heat stress, greater fat deposition has not only been observed in pigs, but also in rodents (Katsumata et al. 1990) and poultry (Geraert et al. 1996; Lu et al. 2007). Kouba et al. (2001) reported that lipid metabolism in the liver and adipose tissue

was upregulated in growing pigs under high temperatures. Under heat stress, a large number of triglycerides were uptaken from blood and deposited in adipose tissue, leading to the increase of fatness (Holmes 1971; Lossec et al. 1998). Therefore, levels of plasma non-esterified fatty acids (NEFA) are significantly reduced in pigs during heat stress. Furthermore, long-term heat exposure affects the distribution of adipose tissue, which might increase heat loss and enhance adaptability (Lossec et al. 1998).

In sows, heat stress not only reduces appetite, but also decreases milk production and body reserve mobilization in order to limit heat production (Prunier et al. 1997; Quiniou and Noblet 1999). In addition, heat stress during pregnancy can modify offspring's metabolism through fetal programming. Recently, in utero heat stress was found to regulate the postnatal body composition (carcass characteristics) in pigs with increased deposition of adipose tissue and decreased skeletal muscle (Boddicker et al. 2014; Johnson et al. 2015; Lucy and Safranski 2017). Adipose tissue is controlled by lipolysis and lipogenesis through the regulation of key metabolic enzymes. Acetyl-CoA carboxylase (ACC) and fatty acid synthetase (FAS) are two critical lipogenic enzymes participating in de novo lipogenesis (Ameer et al. 2014), whereas hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) are mainly involved in lipolysis (Zimmermann et al. 2004). Fat metabolism can also be regulated by peroxisome proliferator-activated receptors (PPARs), which play a critical role in the regulation of lipid and lipoprotein metabolism and glucose homeostasis (Chinetti et al. 2000). Even though it has been demonstrated that heat stress increased the fatness in pigs, the underlying mechanism is largely unknown. Thus, whether heat stress during maternal gestation affects the de novo lipogenesis and lipolysis of offspring still needs further investigation.

Fat mass and obesity-associated protein (FTO) is highly expressed in the hypothalamus, fat, and skeletal muscle and is associated with the incidence of obesity in humans (Dina et al. 2007; Frayling et al. 2007; Grunnet et al. 2009). In pigs, FTO is also found to be associated with intramuscular fat content through regulation of preadipocyte proliferation and differentiation (Fan et al. 2009; Chen et al. 2016). Interestingly, a recent study demonstrated that FTO functioned as a demethylase that facilitated the removal of N^6-methyladenosine (m^6A), which was widely present in eukaryotes (Zhao et al. 2017). To date, m^6A is the most abundant internal mRNA modification (Fu et al. 2014; Wu et al. 2016) and is highly enriched near stop codons and in the 3′ untranslated regions (3′UTR) of transcripts (Meyer et al. 2012; Yue et al. 2018). It is methylated by methyltransferase complex consisting of Wilms' tumor 1-associating protein (WTAP), methyltransferase like 3 (METTL3), and methyltransferase like 14 (METTL14) (Liu et al. 2014), while demethylases including FTO and α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5) facilitate the removal of methyl groups from m^6A (Jia et al. 2011; Zheng et al. 2013). m^6A on mRNA can be recognized by m^6A-binding proteins such as YTH domain family (YTHDF) proteins 1, 2, and 3, and YTH domain containing (YTHDC) protein 1, which modulate mRNA metabolism, including mRNA maturation, translation, and decay (Zhao et al. 2017).

Links between RNA modifications and temperature have been recognized for a long time. Scientists hold a hypothesis that RNA methylations might play a vital role in thermal stabilization during heat stress (Baldridge and Contreras 2014). One study conducted by Zhou et al. (2015) demonstrated that in response to heat shock stress, the nuclear YTHDF2 preserved 5′UTR methylation of heat stress-induced transcripts by limiting the FTO-mediated demethylation. A more recent study found that, in HepG2 cells, the m^6A RNA methylation on heat shock proteins (HSPs) upon heat shock stress was through the decrease and increase of the mRNA expression of METTL3 and YTHDF2, respectively (Yu et al. 2018). The abovementioned evidence strongly indicates that high ambient temperature-related changes in fat metabolism may be regulated by m^6A mRNA modification. Therefore, the focus of our study was to determine the effect of heat exposure on lipogenesis and lipolysis in piglets from sows challenged by heat stress during late gestation and lactation as well as the contribution of m^6A mRNA modification to the changes of the expression of genes involved in lipid metabolism.

Materials and methods

Experimental design and sample collection

All animal procedures in this research were conducted under the protocol (SCAU-AEC-2010-0416) approved by the South China Agricultural University Animal Care and Use Committee. The trial was conducted in Wencun Pig Farm (Jiangmen, Guangdong Province, 21° 86′ N, 112° 49′ E, subtropical climate) from August 2018 to September 2018. Forty multiparous crossbred sows (Landrace × Yorkshire) of similar parity were selected on day 85 of gestation (the entire gestation period for sows is about 114 days) and were randomly assigned to the control rearing room or the heat stress rearing room and maintained until day 21 of lactation. Heat stress rearing rooms are conventional rearing rooms, which is a semi-opened building with a natural ventilation system, while control rearing rooms contain pad curtain with cycling water on one end of a completely closed room and large fans on the wall of the

opposite end. The amount of feed intake during late gestation was 3.5 kg per sow, while after gestation sows had ad libitum access to feed until the end of the experiment. During experiment, the temperature (T) and relative humidity (RH) were recorded every hour using an auto temperature and humidity recorder (W-series, Wangyunshan, Fujian, China). While temperature-humidity index (THI) was calculated according to the formula of Wegner et al. (2014): THI = [(1.8 T)]+ 32 − [0.55(RH/100)] × [((1.8 T) + 32) − 58]. At the end of the experiment, six piglets from sows within each treatment (1 piglet per pen) were randomly selected and sacrificed for samples. Adipose and liver tissues were dissected and stored in liquid nitrogen for further RNA and protein extraction and analysis.

RNA extraction and RT-PCR

Total RNA from adipose and liver tissues was isolated using TRIZOL reagent (Takara, Dalian, Liaoning, China) according to the manufacturer's instructions, and mRNA concentration was measured by NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Ratios of absorption (260/280 nm) of all preparations were between 1.6 and 1.8. Aliquots of RNA samples were subjected to electrophoresis in 1% ethidium bromide-stained agarose gel to verify their integrity. For reverse transcriptase-polymerase chain reaction (RT-PCR), 2 μg of total RNA was used to synthesize cDNA in a 20 μL reaction mixture using HiScript II Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme, Nanjing, China) according to the manufacturer's instructions. The PCR products were confirmed by electrophoresis on 3% agarose gel.

Real-time PCR

Real-time PCR was conducted on the ABI StepOnePlus™ Real-Time PCR systems. The real-time PCR amplification was performed in a 20 μL reaction volume, containing 10 μL Real-Time PCR Master Mix (SYBR Green, Takara, Dalian, Liaoning, China), 2 μL cDNA, 0.8 μL of each PCR primer (10 μM), 0.4 μL ROX (10 u/μL, Dalian, Liaoning, China), and 6 μL diethyl pyrocarbonate water. The following thermal profile was used for qRT-PCR: heating at 94 °C for 5 min for initial enzyme activation, followed by 40 cycles of 94 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 20 s. Each sample was measured in triplicate. Relative gene expression was calculated according to the $2^{-\Delta\Delta Ct}$ method and β-actin was used to normalize variations in the amount of starting material. Gene-specific primer sequences of the reference and target genes are listed in Table 1 and were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China).

Western blotting analysis

Proteins from frozen adipose and liver samples were homogenized on ice in 800 μL RIPA Lysis Buffer (Beyotime, Shanghai, China) and total protein concentrations were detected using the BCA Protein Assay Kit (Beyotime, Shanghai, China) according to the manufacturer's recommendations. Next, equal amounts of protein were separated on 10% SDS-PAGE gels followed by electrotransfer to polyvinylidenedifluoride membranes (PVDF). After blocking with 6% skim milk powder in TBST, the membranes were incubated overnight with the following primary antibodies at 1:1000 dilution: METTL3 (Abcam, Cambridge, MA, USA), METTL14 (Abcam, Cambridge, MA, USA), FTO (Santa Cruz Biotechnology, USA), YTHDF2 (Millipore, Bedford, MA, USA), and β-actin (Santa Cruz Biotechnology, USA). Subsequently, the resultant blots were washed five times for 5 min each with TBST buffer. Then, the membranes were probed with a secondary antibody (1:5000 dilution) (Thermo Fisher Scientific, Rockford, IL, USA) for 1.5 h at room temperature. After washing five times for 5 min with TBST buffer, proteins on membrane were quantified by an image processing software (Image Pro Plus 6.0) (Rockville, MD, USA).

Measurement of m^{6}A content

The total content of m^{6}A in adipose and liver tissues was determined using an EpiQuik™ m^{6}A RNA methylation quantification kit (EpiGentek Group Inc., USA) according to the manufacturer's instructions. In this assay, total RNA is bound to strip wells using RNA high-binding solution. m^{6}A is detected using capture and detection antibodies and the detected signal is enhanced and then quantified colorimetrically by reading the absorbance in a microplate spectrophotometer. The amount of m^{6}A is proportional to the OD intensity measured. To determine the relative m^{6}A RNA methylation status of two different RNA samples, a simple calculation for the percentage of m^{6}A in total RNA can be carried out using the following formula:

$$\mathrm{m6A}\% = \frac{(\text{Sample OD}-\text{NC OD})/S}{(\text{PC OD}-\text{NC OD})/P} \times 100\%$$

Note: *S* is the amount of input sample RNA in nanograms. *P* is the amount of input positive control (PC) in nanograms.

Statistical analysis

Statistical analysis was performed using the statistical software IBM SPSS Version 25.0. Data were analyzed using a *T* test analysis and each sample was measured in triplicate. All results were shown as mean ± SEM. Differences were

Table 1 Primer sequences used in quantitative real-time PCR assay

Primers	Accession no.	Sequences (5′ → 3′)	Product size (bp)
PPAR-γ	NM_214379.1	F-AACATTTCACAAGAGGTGACCA R-GATCTCGTGGACGCCATACT	213
SREBP-1c	NM_214157.1	F-AGCGGACGGCTCACAATG R-CGCAAGACGGCGGATTTA	121
ACACA	NM_001114269.1	F-ACATCCCCACGCTAAACA R-AGCCCATCACTTCATCAAAG	186
FASN	NM_001099930.1	F-GCTTGTCCTGGGAAGAGTGTA R-AGGAACTCGGACATAGCGG	115
CD36	NM_001044622.1	F-ACCCTGAGACCCACACAGTC R-TACAGCTGCCACAGCCAGAT	119
FABP4	NM_001002817.1	F-TGAAAGGTGTCACGGCTAC R-TCGGGACAATACATCCAACAGAG	102
CPT-1A	XM_021091195.1	F-ATTACGACGGCAGGCTGTTGAAG R-AATAGGCTTGGCGACACTTGGC	133
HSL	NM_214315.3	F-GCCCGAGACGAGATTAGC R-ATGAAGGGATTCTTGACGATG	143
ATGL	No. EU373817	F-CGCCAGCATCATCGAGGTGTC R-GCAGCCACGGATGGTCTTCAC	94
DGAT1	NM_214051.1	F-GCTTCAGCCTTCTTCCACGAGTAC R-CGATGATGAGCGACAGCCACAC	163
METTL3	XM_003128580.5	F-CCAGCACAGCTTCAGCAGTTCC R-TGGAGATGGCAAGACGGATGGAG	129
METTL14	XM_003129231.6	F-GAGGAAGAGGTGGAACTTCTGCTG R-CTCCTCCACGGCCTCCTCTG	96
WTAP	NM_001244241.1	F-GCGGGAATAAGGCCTCCAAC R-TGTGAGTGGCGTGTGAGAGA	136
FTO	NM_001112692.1	F-GCAGAGCCGCCTACAACCTAAC R-ACCGCTGACCTGTCCACCAG	142
ALKBH5	XM_021067995.1	F-GCAAGGTGAAGAGCGGCATCC R-GTCCACCGTGTGCTCGTTGTAC	128
YTHDF1	XM_021078235.1	F-GTCTACCTGCTCTTCAGCGTCAAC R-AGATCCACTTCACGTCGAACTTGC	139
YTHDF2	XM_005665152.3	F-CCACCTCCACCACAGCCTACTC R-CCAGCCTGAGACTGTCCTACTCC	140
HSP27	NM_001007518.1	F-GGCGTCTCGGAGATCCAGCAG R-TGCTCATCCTGCCGCTCCTC	143
HSPA9	NM_004134.6	F-CAGATGTGCTGCTCCTGGATGTC R-TGGCTCTTCTTGGTTGGAATGGTG	112
β-actin	397,563	F-TGCGGGACATCAAGGAGAAG R-AGTTGAAGGTGGTCTCGTGG	176

considered very significant if $P \leq 0.01$, significant at $P \leq 0.05$, and not significant at $P > 0.10$.

Results

Environmental temperature-humidity index description

During the experimental period, environmental temperature and average hourly temperature-humidity index (THI) in the heat room and control room were shown in Fig. 1.

Effect of heat stress on body temperature of piglets and sows

The rectum temperature of sows was increased from 39.2 to 39.7 °C ($P < 0.05$), while the rectum temperature of piglets was slightly increased from 39.1 to 39.4 °C ($P < 0.10$).

Effect of heat stress on HSP expression

The expression of heat shock protein 70 (HSP70) and heat shock protein 27 (HSP27) was summarized in Fig. 2. Compared with the control group, the expression of HSP70 was significantly increased in abdominal adipose tissue ($P < 0.01$, Fig. 2a) and the liver ($P < 0.01$, Fig. 2b) under heat stress. Moreover, heat stress increased the expression of HSP27 in liver tissue compared with that in the control group ($P < 0.05$, Fig. 2b). However, no difference was found in the expression of HSP27 in abdominal fat between heat stress and control group in our study.

Effect of heat stress on genes involved in lipid metabolism

Figure 3 presented the relative expression of genes participated in lipogenesis, lipolysis, and lipid transportation. In abdominal fat, heat stress significantly upregulated the expression of

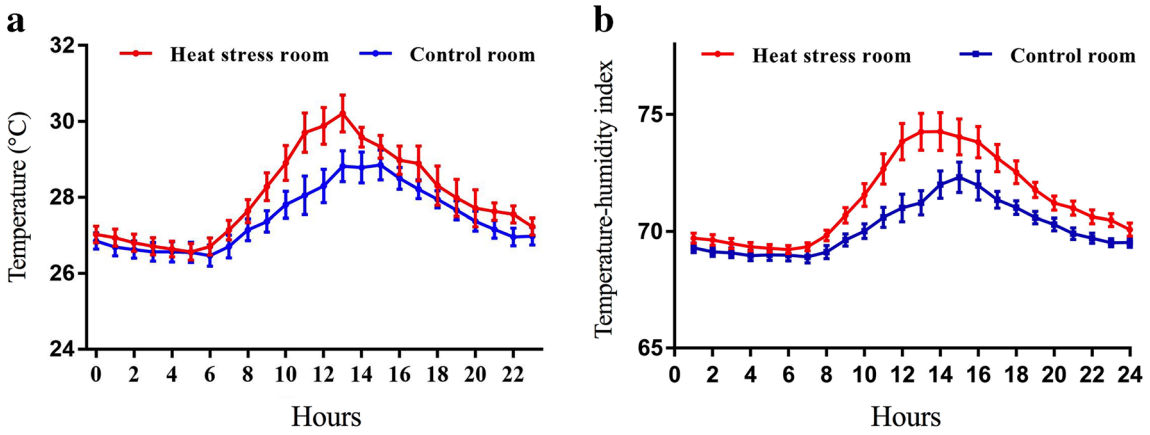

Fig. 1 Environmental temperature (**a**) and average hourly temperature-humidity index (THI) (**b**) in gestating room and lactating room

genes associated with fat synthesis, such as FASN, DGAT1, PPAR-γ, and sterol regulatory element-binding protein 1 (SREBP-1c) ($P < 0.01$, Fig. 3a) as well as ACACA ($P < 0.05$, Fig. 3a), while no changes were observed in genes involved in fat hydrolysis (HSL, ATGL, and CPT-1A). In addition, the expression of FABP4 ($P < 0.01$, Fig. 3c) was higher in abdominal adipose tissue after heat shock stress, whereas no such change was observed for CD36 expression. In the liver tissue, heat stress treatment significantly increased the expression of SREBP-1c ($P < 0.05$, Fig. 3d) and DGAT1 ($P < 0.01$, Fig. 3d). However, mRNA levels of ATGL and CPT-1A were lower in the heat stress group than the control group ($P < 0.05$, Fig. 3e). Compared with the control group, heat stress treatment significantly increased CD36 expression in liver tissue ($P < 0.01$, Fig. 3f).

Effect of heat stress on m^{6}A RNA methylation

The level of m^{6}A was significantly elevated in both abdominal fat and liver of piglets challenged by heat stress ($P < 0.01$, Fig. 4).

Effect of heat stress on mRNA expression of m^{6}A-related enzymes

Changes in gene expression of m^{6}A-related enzymes in both abdominal fat and liver were listed in Fig. 5. Compared with the control group, heat stress significantly upregulated the expression of WTAP, METTL14, and FTO in abdominal adipose tissue ($P < 0.01$, Fig. 5a), and similar results were observed in the expression of METTL14, FTO, and YTHDF2 in liver samples ($P < 0.01$, Fig. 5b). In addition, we found heat stress increased mRNA expression of YTHDF2 in abdominal adipose tissue ($P < 0.05$, Fig. 5a) and increased METTL3 and WTAP in liver tissue ($P < 0.05$, Fig. 5b).

Effect of heat stress on protein abundance of m^{6}A-related enzymes

The protein abundances of METTL3, METTL14, FTO, and YTHDF2 in both abdominal fat and liver were shown in Fig. 6. METTL14 and FTO protein levels in abdominal adipose tissue were significantly higher than those in the control group ($P < 0.01$, Fig. 6a), which were consistent with the real-

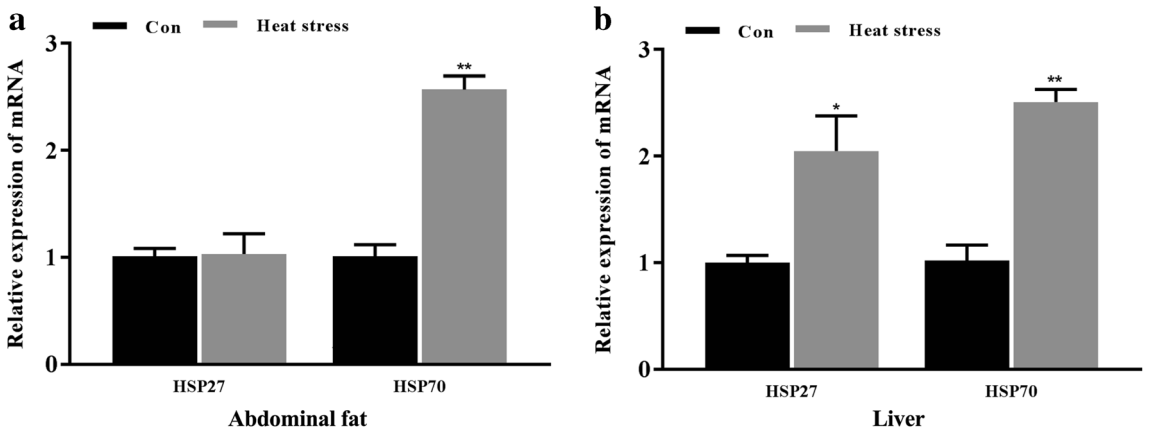

Fig. 2 Effect of heat shock on the expression of HSP-related genes. mRNA expression of HSP27 (HSPB1) and HSP70 (HSP1B) in abdominal fat (**a**) and liver (**b**). Data were shown as mean ± SEM ($n = 4$). *$P \leq 0.05$, **$P \leq 0.01$

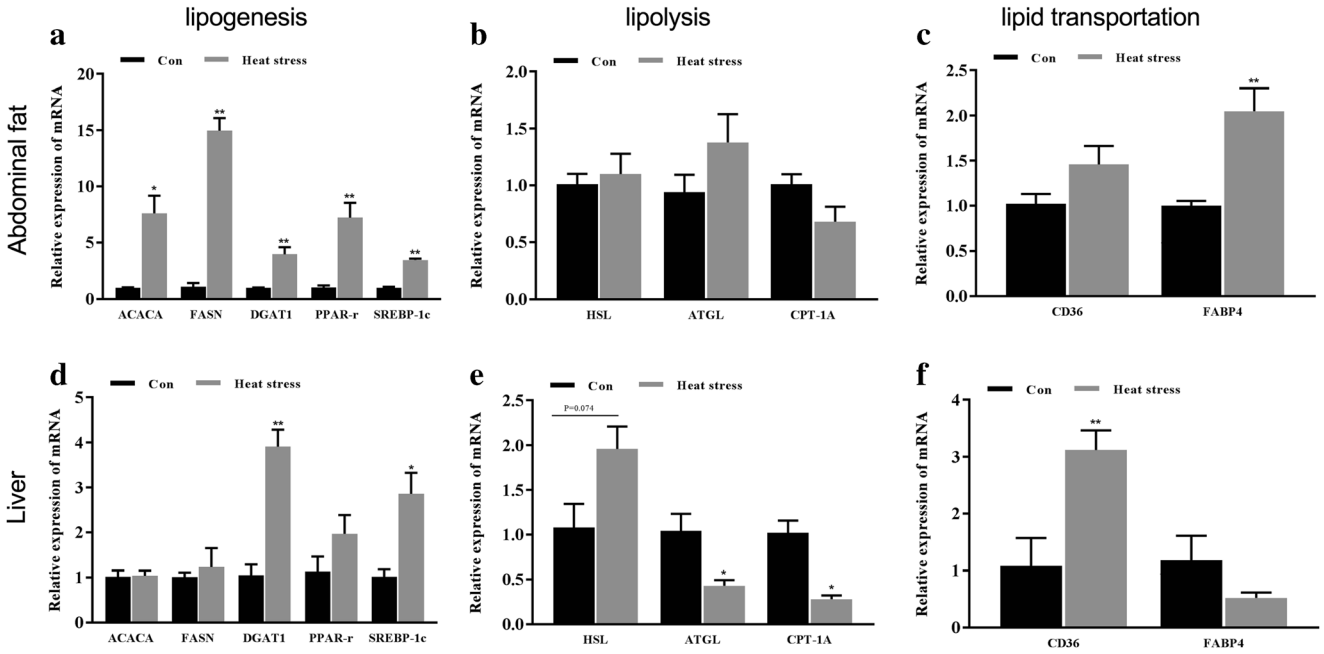

Fig. 3 Effect of heat shock on the expression of genes involved in fat metabolism in abdominal fat and liver. mRNA expression of ACACA, FASN, DGAT1, PPAR-γ, and SREBP-1c associated with lipogenesis in abdominal fat (**a**) and liver (**d**). mRNA expression of HSL, ATGL, and CPT-1A associated with fat hydrolysis in abdominal fat (**b**) and liver tissues (**e**). mRNA expression of CD36 and FABP4 associated with fat transportation in abdominal fat (**c**) and liver tissues (**f**). Data were shown as mean ± SEM ($n = 4$). *$P \leq 0.05$, **$P \leq 0.01$

time PCR results. Although heat stress did not induce a significant change in mRNA expression of METTL3 in abdominal fat ($P = 0.075$, Fig. 5a), its protein abundance was significantly increased by heat stress ($P < 0.05$, Fig. 6a). In liver tissue, heat stress significantly increased the protein level of YTHDF2 ($P < 0.05$, Fig. 6b).

Discussion

Heat shock proteins (HSPs) are a group of proteins which can be triggered by heat stress and commonly named according to their molecular weight. As one of the major inducible HSPs, HSP70 is widely used as a heat stress marker in various studies (Morimoto 1993). Heat exposure can upregulate HSP70 in embryos (Skjærven et al. 2011; Sakatani et al. 2012) and increase HSP27 in mammary gland (Li et al. 2019), liver (Zhang et al. 2016), and cardiac cells (Parida et al. 2019). Similarly, our current study found that HSP70 was increased in both abdominal fat and liver and HSP27 was increased in the liver, indicating the adaptive response of these piglets in response to heat stress even when they were in utero of sows and during lactation period.

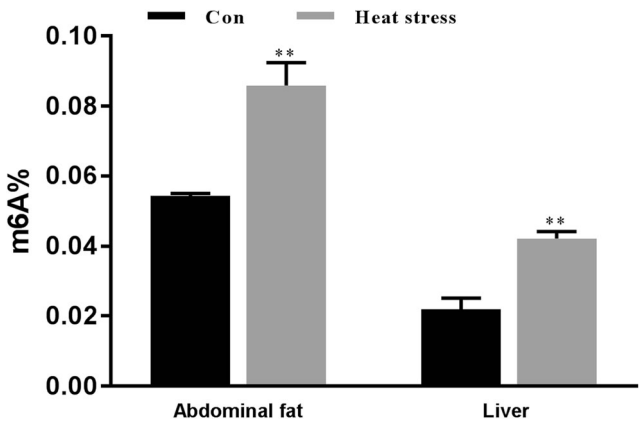

Fig. 4 Effect of heat shock on m^{6}A RNA methylation in abdominal fat and liver. Data were shown as mean ± SEM ($n = 3$). *$P \leq 0.05$, **$P \leq 0.01$

It has also been reported that heat stress elevated glyceroneogenesis and increased fat storage in pigs (Qu et al. 2016; Qu and Ajuwon 2018). Liver and lipid tissue are two major organs regulating lipogenesis in animals. Although fat synthesis for humans and rats is primarily in the liver, de novo lipogenesis of pigs largely exists in fat tissue (O'Hea and Leveille 1969). Despite the fact that liver is not the major organ for lipogenesis in pigs, it still participates in lipid metabolism through fatty acid transportation and oxidation, and synthesis of cholesterol, phospholipids, and ketogenesis (Odle et al. 1995). To investigate the effect of heat stress on lipid metabolism, the expression of rate-limiting enzymes involved in lipogenesis and lipolysis was measured in the current study. A number of key enzymes and transcription factors are involved in lipogenesis (Kersten 2001). Briefly, in liver and adipose tissue, acetyl-CoA is firstly carboxylated into malonyl-CoA with ACCα. Subsequently, malonyl-CoA is further converted to palmitic acid by fatty acid synthase (FAS). Our research found that the expression of acetyl-CoA

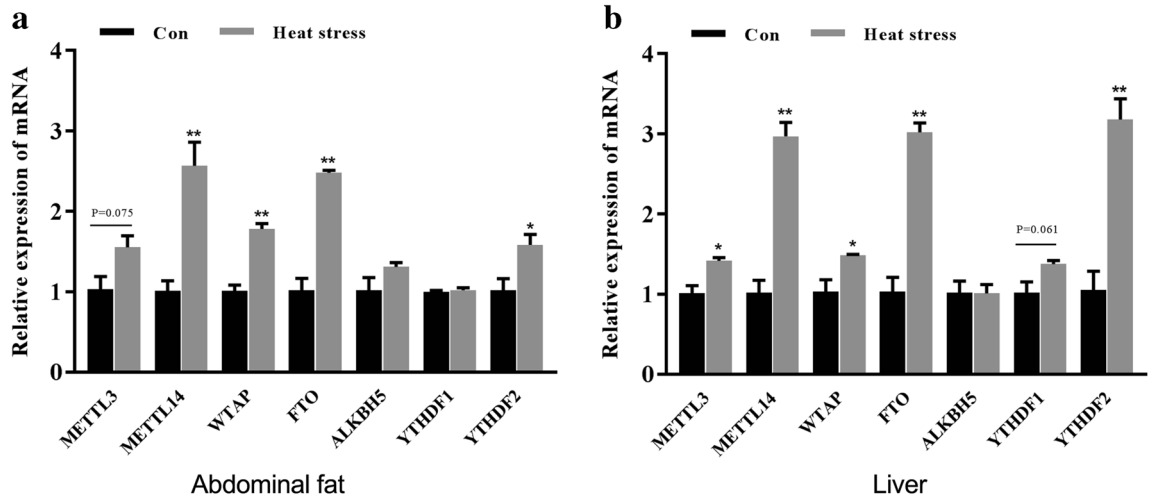

Fig. 5 Effect of heat shock on the expression of genes related to m^{6}A RNA methylation. mRNA expression of METTL3, METTL14, FTO, ALKBH5, YTHDF1, and YTHDF2 in abdominal fat (**a**) and liver (**b**). Data were shown as mean ± SEM ($n = 4$). *$P \leq 0.05$, **$P \leq 0.01$

carboxylase 1 (ACACA) and fatty acid synthase (FASN) was significantly increased in abdominal adipose tissue in piglets under heat stress. Diglyceride acyltransferase (DGAT1), which is mainly expressed in liver and adipose tissue, participates in the final step of triacylglycerol synthesis (Cui et al. 2011). In the current study, we also demonstrated that expression of DGAT1 was significantly upregulated in the liver and adipose tissue under heat stress. It is possible that certain transcription factors may be involved in the regulation of genes involved in lipolysis and lipogenesis (Lodhi et al. 2011). For example, Kakehashi et al. (2013) reported that oxidative stress increased activation of PPAR-γ, which is a

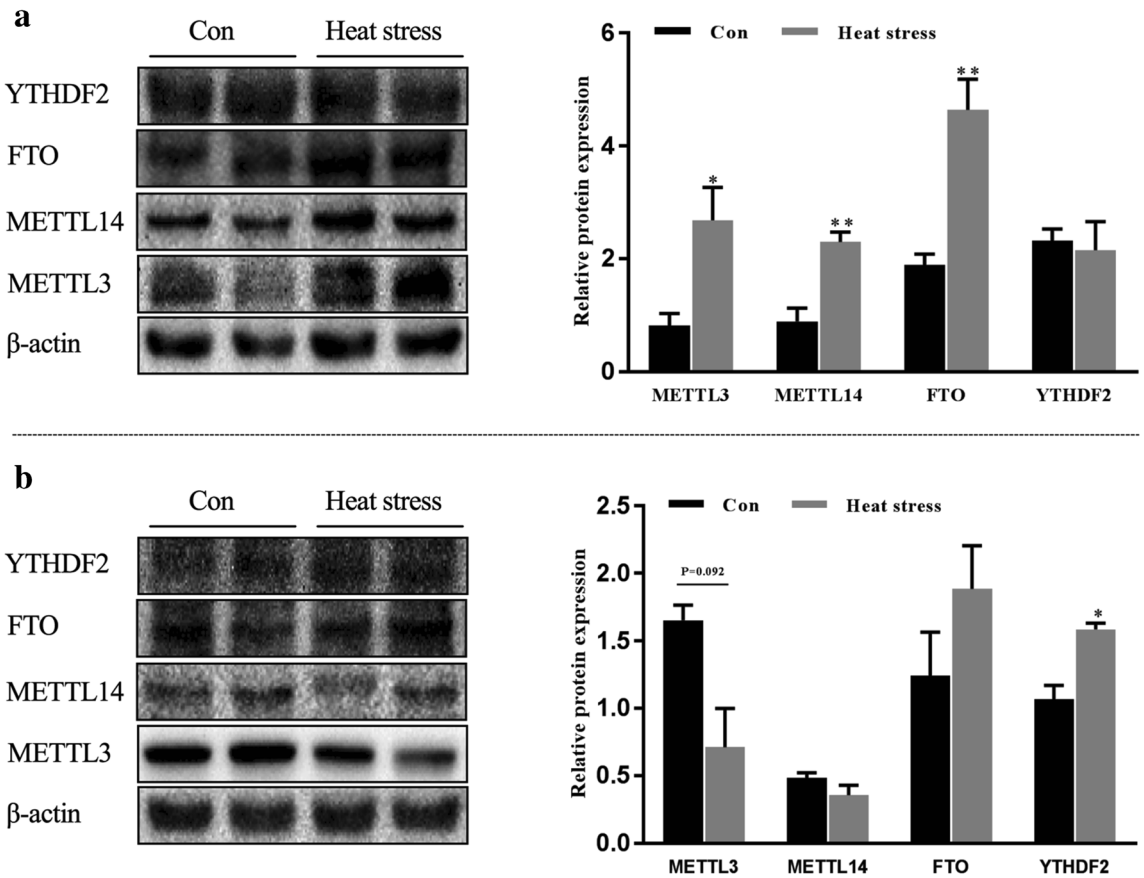

Fig. 6 Effect of heat shock on METTL3, METTL14, FTO, and YTHDF2 protein level in abdominal fat (**a**) and liver (**b**). Data were shown as mean ± SEM ($n = 3$). *$P \leq 0.05$, **$P \leq 0$

master regulator of fat metabolism. In our study, we also found that PPAR-γ was upregulated in abdominal adipose tissue, which was consistent with previous studies. In addition, PPAR is important to the induction of a number of HSPs (Vallanat et al. 2010; Xu et al. 2012). PPAR-γ activation increases lipogenic genes, including genes involved in lipid uptake (FAT/CD36) and transport (FABP4) (Ravacci et al. 2015; Blanchard et al. 2016). In this trial, we found heat stress significantly increased the expression of FABP4 in adipose tissue, while expression of FAT/CD36 was increased in the liver. Differently from PPARs, SREBP1-c is a transcription factor that functions to activate lipogenic gene expression in liver (Ferre and Foufelle 2010; Hagiwara et al. 2012). Our study also showed that SREBP-1c was significantly increased in the liver under heat stress.

Activation of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) mobilizes the stored triglycerides and increases free fatty acid secretion, which is the first step in the oxidation of fatty acids (Morak et al. 2012). In this study, no difference in the expression of HSL and ATGL was observed when piglets were challenged by heat stress. After long-chain fatty acid is activated by acyl-CoA synthetase on the outer mitochondrial membrane, they are transported into the mitochondrial matrix via carnitine palmitoyltransferase (CPT1) (Calderon-Dominguez et al. 2016). CPT1 is also known as carnitine acyltransferase I, which contains three isoforms including CPT1A (widely existing in liver and adipose tissue), CPT1B (predominately expressed in muscle), and CPT1C (highly expressed in the brain) (Houten and Wanders 2010). In our research, ATGL and CPT-1A were decreased in the liver under heat stress, indicating lipogenesis was inhibited under high temperatures. However, genes involved in lipolysis did not change in the experimental group challenged with heat stress. It is reasonable to conclude here that increased fatness in pigs upon heat stress may be due to increased lipogenesis while lipolysis is not changed.

Recent study demonstrated that m^6A RNA methylation played a vital role in fat metabolism. Around 3000 m^6A peaks were observed in adipose tissue transcriptomes, which were mainly enriched in stop codons, 3′-untranslated regions, and coding regions (Tao et al. 2017). Demethylation modification of m^6A by FTO plays a part in preadipocyte differentiation and hepatic fat accumulation (Tao et al. 2017; Kang et al. 2018). In detail, FTO regulates adipogenesis through changing cell cycle in preadipocytes (Wu et al. 2018), and modification of m^6A RNA methylation in PPARα transcript is important for liver lipid metabolism (Zhong et al. 2018). As expected, we found that expression of FTO, which has a strong correlation to fat deposition, was significantly increased in abdominal adipose tissue and the liver under heat stress. Our results also showed that methyltransferases such as METTL3 and METTL14 were significantly increased in abdominal adipose tissue. In the liver, however, only YTHDF2 was upregulated under heat stress, which indicated the effect of heat stress on m^6A RNA methylation may vary among tissues. It has been reported that m^6A demethylation is active during adipogenesis. For instance, Wang et al. (2015) found that FTO decreased m^6A levels and induced adipogenesis in porcine adipocytes, while METTL3 played an opposite role. Furthermore, Cai et al. (2018) reported loss of m^6A increased adipogenesis via a m^6A-YTHDF2-dependent way in porcine adipocytes. However, the results of m^6A RNA methylation quantification in our study showed that heat stress enhanced m^6A RNA methylation, indicating the function as "writer" (Mettle3, Mettle14, and WTAP) was greater than "eraser" (FTO). Recently, WTAP, METTL3, and METTL14 were reported to promote adipogenesis by enhancing cell cycle transition (Kobayashi et al. 2018). Furthermore, knockdown of any of these three proteins could induce cell cycle arrest and be detrimental to adipogenesis (Kobayashi et al. 2018). In agreement with our study, Lu et al. (2019) reported that sheep under heat stress significantly increased the m^6A level and upregulated mRNA levels of HSPs (HSP70, HSP90, and HSP110) and m^6A-related enzymes (METTL3, METTL14, WTAP, FTO, ALKBH5, YTHDF1-3, and YTHDC1-2). This novel finding indicated methyltransferase also plays a vital role in adipogenesis, which still requires further study. However, one study found heat stress largely decreased expression of METTL3 while increasing the expression of YTHDF2 in HepG2 cells (Yu et al. 2018). The inconsistent results among various studies might be caused by different experimental models (animals, organs, and cell lines) and different levels of heat stress. Therefore, more researches are still needed to decipher the effects of heat stress on m^6A RNA methylation.

Recently, it was reported that m^6A RNA methylation even played an essential role in fetal developmental programming (Kaspi et al. 2018). Maternal diet can regulate fetal programming through m^6A RNA methylation. The effect of maternal high-fat intake on expression of FTO and METTL3 in offspring varied at different times (Li et al. 2016). m^6A levels in visceral fat were increased at 3 weeks old, with downregulated FTO and upregulated METTL3, while changes were reversed at 8 weeks old (Li et al. 2016). Furthermore, METTL3 was involved in the regulation of fetal hematopoiesis (Gao et al. 2018). Our study was the first to report that maternal heat stress might also regulate fetal programming through m^6A RNA methylation, which played a vital role in thermal stabilization during heat stress (Baldridge and Contreras 2014). Uncovering the role of m^6A RNA methylation during fetal developmental programming is of great importance for us to understand the intimate and complex relationship between maternal and fetal interaction and will shed new light to our understanding of obesity and diabetes in the future. In addition, it is worth mentioning that heat stress might also indirectly regulate the fetal lipid metabolism

through pregnancy-related factors (such as gestational age at delivery, the length of labor, neonatal birthweight, and nursing behavior), which needs further investigation.

Conclusion

In conclusion, our study found m^{6}A RNA methylation played an important role in fat deposition of piglets from sows challenged by heat stress from day 85 of gestation to day 21 of lactation. This novel finding indicated that heat stress may be involved in the regulation of lipid metabolism during late gestation and early weaning period, which may lead to the increased fatness in finishing pigs.

Author contributions JH Heng, SH Zhang, and WT Guan designed the experiment and supervised the project. JH Heng, M Tian, and WF Zhang performed the experiments and conducted the lab work. JH Heng, M Tian, and F Chen conducted the statistical analysis. JH Heng and SH Zhang wrote the paper. All authors read and approved the final manuscript.

Funding This work was supported by National Natural Science Foundation of the P. R. of China (nos. 31802067 and 31872364) and the Natural Science Foundation of Guangdong Province (No. 2018A030310201) and the National Key R&D Program of China (No. 2018YFD0500600 and No. 2018YFD0501000).

Compliance with ethical standards

Ethics approval All animal use and care protocols were approved by the Committee of the South China Agricultural University Animal Care and Use (20110107–1).

References

Ameer F, Scandiuzzi L, Hasnain S, Kalbacher H, Zaidi N (2014) De novo lipogenesis in health and disease. Metabolism 63(7):895–902

Baldridge KC, Contreras LM (2014) Functional implications of ribosomal RNA methylation in response to environmental stress. Crit Rev Biochem Mol Biol 49(1):69–89

Baziz HA, Geraert P, Padilha J, Guillaumin S (1996) Chronic heat exposure enhances fat deposition and modifies muscle and fat partition in broiler carcasses. Poult Sci 75(4):505–513

Blanchard PG, Turcotte V, Cote M, Gelinas Y, Nilsson S, Olivecrona G, Deshaies Y, Festuccia WT (2016) Peroxisome proliferator-activated receptor γ activation favours selective subcutaneous lipid deposition by coordinately regulating lipoprotein lipase modulators, fatty acid transporters and lipogenic enzymes. Acta Physiol 217(3):227–239

Boddicker RL, Seibert JT, Johnson JS, Pearce SC, Selsby JT, Gabler NK, Lucy MC, Safranski TJ, Rhoads RP, Baumgard LH, Ross JW (2014) Gestational heat stress alters postnatal offspring body composition indices and metabolic parameters in pigs. PLoS One 9(11):e110859

Cai M, Liu Q, Jiang Q, Wu R, Wang XX, Wang YZ (2018) Loss of m6A on FAM134B promotes adipogenesis in porcine adipocytes through m6A-YTHDF2-dependent way. IUBMB Life 00(00):1–7

Calderon-Dominguez M, Sebastián D, Fucho R, Weber M, Mir JF, García-Casarrubios E, Obregón MJ, Zorzano A, Valverde ÁM, Serra D, Herrero L (2016) Carnitine palmitoyltransferase 1 increases lipolysis, UCP1 protein expression and mitochondrial activity in brown adipocytes. PLoS One 11(7):e0159399

Chen X, Zhou B, Luo Y, Huang Z, Jia G, Liu G, Zhao H (2016) Tissue distribution of porcine FTO and its effect on porcine intramuscular preadipocytes proliferation and differentiation. PLoS One 11(3): e0151056

Chinetti G, Fruchart JC, Staels B (2000) Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation. Inflamm Res 49(10):497–505

Collin A, van Milgen J, Dubois S, Noblet J (2001) Effect of high temperature on feeding behaviour and heat production in group-housed young pigs. Br J Nutr 86(1):63–70

Cui JX, Zeng YQ, Wang H, Chen W, Du JF, Chen QM, Hu YX, Yang L (2011) The effects of DGAT1 and DGAT2 mRNA expression on fat deposition in fatty and lean breeds of pig. Livest Sci 140(1–3):292–296

Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobson P, Carlsson LM, Kiess W, Vatin V, Lecoeur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le Stunff C, Bougnères P, Kovacs P, Marre M, Balkau B, Cauchi S, Chèvre JC, Froguel P (2007) Variation in FTO contributes to childhood obesity and severe adult obesity. Nat Genet 39(6):724–726

Fan B, Du Z-Q, Rothschild MF (2009) The fat mass and obesity-associated (FTO) gene is associated with intramuscular fat content and growth rate in the pig. Anim Biotechnol 20(2):58–70

Ferre P, Foufelle F (2010) Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c. Diabetes Obes Metab 12(Suppl 2):83–92

Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI (2007) A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316(5826):889–894

Fu Y, Dominissini D, Rechavi G, He C (2014) Gene expression regulation mediated through reversible m 6 A RNA methylation. Nat Rev Genet 15(5):293–306

Gan F, Chen X, Liao SF, Lv C, Ren F, Ye G, Pan C, Huang D, Shi J, Shi X, Zhou H, Huang K (2014) Selenium-enriched probiotics improve antioxidant status, immune function, and selenoprotein gene expression of piglets raised under high ambient temperature. J Agric Food Chem 62(20):4502–4508

Gao Y, Vasic R, Tebaldi T, Song Y, Teng R, Joshi P, Viero G, Xiao A, Batista P, Li H, Flavell R, Halene S (2018) Mettl3 mediated m6A modification is essential in fetal hematopoiesis. Am Soc Hematol 132(Suppl 1):3825

Geraert PA, Padilha JC, Guillaumin S (1996) Metabolic and endocrine changes induced by chronic heatexposure in broiler chickens: growth performance, body composition and energy retention. Br J Nutr 75(2):195–204

Grunnet LG, Nilsson E, Ling C, Hansen T, Pedersen O, Groop L, Vaag A, Poulsen P (2009) Regulation and function of FTO mRNA expression in human skeletal muscle and subcutaneous adipose tissue. Diabetes 58(10):2402–2408

Hagiwara A, Cornu M, Cybulski N, Polak P, Betz C, Trapani F, Terracciano L, Heim MH, Rüegg MA, Hall MN (2012) Hepatic mTORC2 activates glycolysis and lipogenesis through Akt, glucokinase, and SREBP1c. Cell Metab 15(5):725–738

Holmes C (1971) Growth and backfat depth of pigs kept at a high temperature. Anim Sci 13(3):521–527

Houten SM, Wanders RJ (2010) A general introduction to the biochemistry of mitochondrial fatty acid β-oxidation. J Inherit Metab Dis 33(5):469–477

Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG, He C (2011) N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 7(12):885–887

Johnson JS, Sanz Fernandez MV, Patience JF, Ross JW, Gabler NK, Lucy MC, Safranski TJ, Rhoads RP, Baumgard LH (2015) Effects of in utero heat stress on postnatal body composition in pigs: II. Finishing phase. J Anim Sci 93(1):82–92

Kakehashi A, Hagiwara A, Imai N, Nagano K, Nishimaki F, Banton M, Wei M, Fukushima S, Wanibuchi H (2013) Mode of action of ethyl tertiary-butyl ether hepatotumorigenicity in the rat: evidence for a role of oxidative stress via activation of CAR, PXR and PPAR signaling pathways. Toxicol Appl Pharmacol 273(2):390–400

Kang H, Zhang Z, Yu L, Li Y, Liang M, Zhou L (2018) FTO reduces mitochondria and promotes hepatic fat accumulation through RNA demethylation. J Cell Biochem 119(7):5676–5685

Kaspi A, Khurana I, Ziemann M, Connor T, Spolding B, Zimmet P, Walder K, El-Osta A (2018) Diet during pregnancy is implicated in the regulation of hypothalamic RNA methylation and risk of obesity in offspring. Mol Nutr Food Res 7:e1800134

Katsumata M, Yano H, Ishida N, Miyazaki A (1990) Influence of a high ambient temperature and administration of clenbuterol on body composition in rats. J Nutr Sci Vitaminol (Tokyo) 36(6):569–578

Kersten S (2001) Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Rep 2(4):282–286

Kobayashi M, Ohsugi M, Sasako T, Awazawa M, Umehara AIT, Kobayashi N, Okazaki Y, Kubota N, Suzuki R, Waki H, Horiuchi K, Hamakubo T, Kodama T, Aoe S, Tobe K, Kadowaki T, Uekia K (2018) The RNA methyltransferase complex of WTAP, METTL3, and METTL14 regulates mitotic clonal expansion in adipogenesis. Mol Cell Biol 38(16):00116–00118

Kouba M, Hermier D, Le Dividich J (1999) Influence of a high ambient temperature on stearoyl-CoA-desaturase activity in the growing pig. Comp Biochem Physiol B Biochem Mol Biol 124(1):7–13

Kouba M, Hermier D, Le Dividich J (2001) Influence of a high ambient temperature on lipid metabolism in the growing pig. J Anim Sci 79(1):81–87

Li X, Yang J, Zhu YB, Liu Y, Shi X, Yang GS (2016) Mouse maternal high-fat intake dynamically programmed mRNA m6A modifications in adipose and skeletal muscle tissues in offspring. Int J Mol Sci 17(8):1336

Li CM, Wang YR, Li L, Han ZY, Mao SY, Wang GL (2019) Betaine protects against heat exposure–induced oxidative stress and apoptosis in bovine mammary epithelial cells via regulation of ROS production. Cell Stress Chaperones 24:453–460

Liu J, Yue Y, Han D, Wang X, Fu Y, Zhang L, Jia G, Yu M, Lu Z, Deng X, Dai Q, Chen W, He C (2014) A METTL3–METTL14 complex mediates mammalian nuclear RNA N 6-adenosine methylation. Nat Chem Biol 10(2):93–95

Lodhi IJ, Wei X, Semenkovich CF (2011) Lipoexpediency: de novo lipogenesis as a metabolic signal transmitter. Trends Endocrinol Metab 22(1):1–8

Lossec G, Herpin P, Le Dividich J (1998) Thermoregulatory responses of the newborn pig during experimentally induced hypothermia and rewarming. Experimental Physiology:Translation and Integration 83(5):667–678

Lu Q, Wen J, Zhang H (2007) Effect of chronic heat exposure on fat deposition and meat quality in two genetic types of chicken. Poult Sci 86(6):1059–1064

Lu Z, Ma Y, Li Q, Liu E, Jin M, Zhang L, Wei C (2019) The role of N 6-methyladenosine RNA methylation in the heat stress response of sheep (Ovis aries). Cell Stress Chaperones:1–10

Lucy MC, Safranski TJ (2017) Heat stress in pregnant sows: thermal responses and subsequent performance of sows and their offspring. Mol Reprod Dev 84(9):946–956

Meyer KD, Saletore Y, Zumbo P, Elemento O, Mason CE, Jaffrey SR (2012) Comprehensive analysis of mRNA methylation reveals enrichment in 3′ UTRs and near stop codons. Cell 149(7):1635–1646

Morak M, Schmidinger H, Riesenhuber G, Rechberger GN, Kollroser M, Haemmerle G, Zechner R, Kronenberg F, Hermetter A (2012) Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) deficiencies affect expression of lipolytic activities in mouse adipose tissues. Mol Cell Proteomics 11(12):1777–1789

Morimoto R (1993) Cells in stress: transcriptional activation of heat shock genes. Science 259(5100):1409–1411

Odle J, Lin X, van Kempen TA, Drackley JK, Adams SH (1995) Carnitine palmitoyltransferase modulation of hepatic fatty acid metabolism and radio-HPLC evidence for low ketogenesis in neonatal pigs. J Nutr 125(10):2541–2549

O'Hea EK, Leveille GA (1969) Significance of adipose tissue and liver as sites of fatty acid synthesis in the pig and the efficiency of utilization of various substrates for lipogenesis. J Nutr 99(3):338–344

Parida S, Mishra SR, Mishra C, Mohapatra S, Dalai N, Mahapatra APK, Kundu AK (2019) Impact of heat stress on transcriptional abundance of HSP70 in cardiac cells of goat. Anim Biotechnol 12:1–6

Prunier A, de Braganca MM, Le Dividich J (1997) Influence of high ambient temperature on performance of reproductive sows. Livest Prod Sci 52(2):123–133

Qu H, Ajuwon K (2018) Adipose tissue-specific responses reveal an important role of lipogenesis during heat stress adaptation in pigs. J Anim Sci 96(3):975–989

Qu H, Yan H, Lu H, Donkin S, Ajuwon K (2016) Heat stress in pigs is accompanied by adipose tissue–specific responses that favor increased triglyceride storage. J Anim Sci 94(5):1884–1896

Quiniou N, Noblet J (1999) Influence of high ambient temperatures on performance of multiparous lactating sows. J Anim Sci 77(8):2124–2134

Ravacci GR, Brentani MM, Tortelli TC, Torrinhas RS, Santos JR, Logullo AF, Waitzberg DL (2015) Docosahexaenoic acid modulates a HER2-associated lipogenic phenotype, induces apoptosis, and increases trastuzumab action in HER2-overexpressing breast carcinoma cells. Biomed Res Int 2015:838652

Sakatani M, Alvarez N, Takahashi M, Hansen P (2012) Consequences of physiological heat shock beginning at the zygote stage on embryonic development and expression of stress response genes in cattle. J Dairy Sci 95(6):3080–3091

Skjærven KH, Olsvik PA, Finn RN, Holen E, Hamre K (2011) Ontogenetic expression of maternal and zygotic genes in Atlantic cod embryos under ambient and thermally stressed conditions. Comp Biochem Physiol A Mol Integr Physiol 159(2):196–205

Tao X, Chen J, Jiang Y, Wei Y, Chen Y, Xu H, Zhu L, Tang G, Li M, Jiang A, Shuai S, Bai L, Liu H, Ma J, Jin L, Wen A, Wang Q, Zhu G, Xie M, Wu J, He T, Huang C, Gao X, Li X (2017) Transcriptome-wide N 6-methyladenosine methylome profiling of porcine muscle and adipose tissues reveals a potential mechanism for transcriptional regulation and differential methylation pattern. BMC Genomics 18(1):336

Vallanat B, Anderson SP, Brown-Borg HM, Ren H, Kersten S, Jonnalagadda S, Srinivasan R, Corton JC (2010) Analysis of the heat shock response in mouse liver reveals transcriptional dependence on the nuclear receptor peroxisome proliferator-activated receptor α (PPARα). BMC Genomics 11:16

Wang X, Zhu L, Chen J, Wang Y (2015) mRNA m6A methylation downregulates adipogenesis in porcine adipocytes. Biochem Biophys Res Commun 459(2):201–207

Wegner K, Lambertz C, Daş G, Reiner G, Gauly M (2014) Climatic effects on sow fertility and piglet survival under influence of a moderate climate. Animal 8(9):1526–1533

Wu R, Jiang D, Wang Y, Wang X (2016) N 6-methyladenosine (m 6 A) methylation in mRNA with a dynamic and reversible epigenetic modification. Mol Biotechnol 58(7):450–459

Wu R, Liu Y, Yao Y, Zhao Y, Bi Z, Jiang Q, Liu Q, Cai M, Wang F, Wang Y, Wang X (2018) FTO regulates adipogenesis by controlling cell cycle progression via m6A-YTHDF2 dependent mechanism. Biochim Biophys Acta Mol Cell Biol Lipids 1863(10):1323–1330

Xu D, Murakoshi N, Igarashi M, Hirayama A, Ito Y, Seo Y, Tada H, Aonuma K (2012) PPAR-γ activator pioglitazone prevents age-related atrial fibrillation susceptibility by improving antioxidant capacity and reducing apoptosis in a rat model. J Cardiovasc Electrophysiol 23(2):209–217

Yu J, Li Y, Wang T, Zhong X (2018) Modification of N6-methyladenosine RNA methylation on heat shock protein expression. PLoS One 13(6):e0198604

Yue YN, Liu J, Cui XL, Cao J, Luo GZ, Zhang ZZ, Cheng T, Gao MS, Shu X, Ma HH, Wang FQ, Wang XX, Shen B, Wang YZ, Feng XH, He C, Liu JZ (2018) VIRMA mediates preferential m 6 A mRNA methylation in 3′ UTR and near stop codon and associates with alternative polyadenylation. Cell Discov 4:10

Zhang KX, Zhao PP, Guo GG, Guo Y, Li SW, He Y, Sun X, Chai HL, Zhang W, Xing MW (2016) Arsenic trioxide exposure induces heat shock protein responses in cock livers. Biol Trace Elem Res 170(2):459–465

Zhao BS, Roundtree IA, He C (2017) Post-transcriptional gene regulation by mRNA modifications. Nat Rev Mol Cell Biol 18(1):31–42

Zheng G, Dahl JA, Niu Y, Fedorcsak P, Huang CM, Li CJ, Vågbø CB, Shi Y, Wang WL, Song SH, Lu Z, Bosmans RP, Dai Q, Hao YJ, Yang X, Zhao WM, Tong WM, Wang XJ, Bogdan F, Furu K, Fu Y, Jia G, Zhao X, Liu J, Krokan HE, Klungland A, Yang YG, He C (2013) ALKBH5 is a mammalian RNA demethylase that impacts RNA metabolism and mouse fertility. Mol Cell 49(1):18–29

Zhong X, Yu J, Frazier K, Weng X, Li Y, Cham CM, Dolan K, Zhu X, Hubert N, Tao Y, Lin F, Martinez-Guryn K, Huang Y, Wang T, Liu J, He C, Chang EB, Leone V (2018) Circadian clock regulation of hepatic lipid metabolism by modulation of m6A mRNA methylation. Cell Rep 25(7):1816–1828

Zhou J, Wan J, Gao X, Zhang X, Jaffrey SR, Qian SB (2015) Dynamic m 6 A mRNA methylation directs translational control of heat shock response. Nature 526(7574):591–594

Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M, Lass A, Neuberger G, Eisenhaber F, Hermetter A, Zechner R (2004) Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science (New York, NY) 306(5700):1383–1386