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Footnote

WAT: White Adipose Tissue

LW: Large White

MS: Meishan

BFT: BackFat Thickness

Abstract

In pig, backfat deposition is strongly related to the growth and reproductive performance. However, the molecular regulatory mechanisms of adipose tissue are not clearly understood. Adipose tissue is now recognized as an important endocrine organ that secretes a variety of factors including adipokines. However, the regulation of expression pattern of these adipokines in both plasma and visceral white adipose tissue (WAT) in lean and fat pig is unclear. In the present study, we used two representative porcine breeds (Large White, LW; Meishan, MS) with contrasting backfat thickness and sexual maturity age. Using specific ELISA assays, we determined the plasma profile of eight adipokines, leptin, adiponectin, visfatin, apelin, chemerin, resistin, omentin and vaspin in LW and MS sows. By RT-qPCR and western-blot we also investigated the mRNA and protein levels of these adipokines and their cognate receptors (LEPR, ADIPOR1, ADIPOR2, CMKLR1, CCRL2, GPR1, APLNR, TLR4, ROR1, CAP1 and HSPA5) in the peri renal WAT, respectively. At both plasma and peri renal WAT level, we found that the amounts of leptin, chemerin, resistin and vaspin were higher whereas those of adiponectin and omentin were lower in MS than LW sows. Plasma and adipose tissue visfatin and apelin levels were not different between the two breeds. Moreover, we noted that the variations of peri renal WAT adipokines observed between MS and LW were similar at the protein and mRNA level except for chemerin and apelin suggesting post-transcriptional modifications for these two adipokines. Finally, among the eight adipokines studied, we showed that only the plasma concentrations of leptin and chemerin were positively and those of adiponectin, negatively associated with the thickness of fat and opposite correlation was found for the onset of puberty in both LW and MS animals. Taken together, these results support a potential involvement of adipokines in WAT regulation and its link with the onset of the puberty in sows.

Key words: adipokines, pig, puberty, fattening, plasma, white adipose tissue

1. Introduction

In pig, fat deposition plays a key role in the meat quality and the nutritional value of meat (Wood *et al*. 2008). Different adipose traits have been studied in pig breeds. Among them the most crucial include thickness of subcutaneous fat tissue on the back at different locations. In pig, backfat (BF) deposition is strongly related to the growth and reproductive performance. Indeed, the lower the level of deposited fat, the better the growth performance (Wood, et al. 2008). Some evidence shows also positive relationships of BF thickness with the subsequent gilt reproduction performance including the age at the onset of puberty and the littersize (Kummer *et al*. 2006, Tummaruk *et al*. 2001, Tummaruk *et al*. 2007). To investigate potential molecular mechanisms involved in the fattening and the sexual maturity, we used two representative pig breeds : Meishan (MS) and Large White (LW). Meishan gilts reach puberty at an earlier age and they are fatter than LW gilts (Bazer *et al*. 1988, Bidanel *et al*. 1991, Miyano *et al*. 1990). Adipocyte size-increasing is a major contributor to the greater backfat thickness for Meishan pigs (Nakajima *et al*. 2011). Adipose tissue is not only a site of lipid storage, but it is also described as an active endocrine organ (Kershaw & Flier 2004). It is the main tissue involved in the regulation of lipid metabolism and it is the site of *de novo* fatty acid synthesis in pigs (Ohea & Leveille 1969). Moreover, it is able to release hormones named adipokines involved in metabolic actions, but also adipogenesis (Jialal & Devaraj 2018, Luo & Liu 2016). Using pig breeds with diverging backfat thicknesses several studies described the white adipose tissue (WAT) transcriptome (Tao *et al*. 2017, Xing *et al*. 2019). They showed the involvement of pathways and genes of lipid metabolism in the backfat deposition (for review (Poklukar *et al*. 2020)). However, few studies investigate the potential role of adipokines at the plasma and visceral adipose tissue level in the pig fattening.

In mammals, many adipokines are associated with body mass index (BMI), food intake and reproductive functions (Bluher & Mantzoros 2015, Estienne *et al*. 2019, Rak *et al*. 2017b). In pig, the best-known adipokines are leptin (LEP) and adiponectin (ADIPOQ). Fat swine express higher levels of *LEP* mRNA and protein than lean swine at similar body weight (Ramsay *et al*. 1998). Meishan swine with high backfat express lower adiponectin transcripts in subcutaneous adipose tissue than Landrace swine with lower backfat (Nakajima *et al*. 2019). In our knowledge, no similar study was conducted to investigate plasma or WAT expression levels of other adipokines such as visfatin (NAMPT), chemerin (RARRES2), omentin (ITLN1), apelin (APLN), resistin (RETN) and vaspin (SERPINA12) in pig. The nucleotide sequences of porcine *NAMPT*, *RARRES*2 and *RETN* were cloned and they encode for 52, 16 and 12 kDa proteins, respectively (Chen *et al*. 2007, Dai *et al*. 2006, Huang *et al.* 2010). The entire sequence for *APLN, ITLN1* and *SERPINA12* gene is still lacking. Only a partial sequence for porcine APLN is known (Del Ry *et al.* 2009). In mammals, adiponectin, apelin, and chemerin bind seven-transmembrane domain receptors (ADIPOR1 and ADIPOR2 for adiponectin, APLNR for apelin, CMKLR1, GPR1, and CCRL2 for chemerin) whereas leptin acts through LEPR, a member of class I cytokine receptor family. All these receptors have been described in various pig tissues (Huang *et al*. 2010, Lin *et al*. 2000, Lord *et al*. 2005, Rak *et al*. 2017a, Rozycka *et al.* 2018, Smolinska *et al*. 2019). In mammals, the receptor for resistin remains unknown. However, some reports have suggested potential receptors for resistin such as the receptor tyrosine kinase-like orphan receptor 1 (ROR1) (Sanchez-Solana *et al*. 2012), toll-like receptor 4 (TLR4) (Benomar *et al.* 2016) or adenylyl cyclase-associated protein 1 (CAP1) (Lee *et al.* 2014). Also, vaspin could bind to the HSPA5 receptor to regulate the proliferation apoptosis balance (Nakatsuka *et al.* 2013). Until now, no study investigated the expression of these receptors in pig WAT. However, there is some

evidence showing that adipokines may exert autocrine or paracrine effects in adipose tissue (Karastergiou &Mohamed-Ali 2010). Thus, it becomes crucial to deepen our understanding of the potential involvement of all these adipokines as key regulators of fattening and the sexual maturity in sows.

In this present study, we aimed to compare the plasma profile of eight adipokines (leptin, adiponectin, chemerin, visfatin, apelin, resistin, omentin and vaspin) between MS and LW sows. Furthermore, we investigated the expression pattern of these adipokines and their cognate receptors in the peri renal adipose tissue in both breeds. Finally, we determined whether the plasma adipokines could be associated to the fattening and to the age of the onset of puberty in these animals.

2. Materials and Methods

2.1 Ethical issues

Plasma and peri renal WAT tissue have been collected during meat processing as abattoir by-products by highly qualified and experienced laboratory staff. All the zootechnical parameters (body weight, backfat thickness, age at the puberty…) were routinely collected by the UEPAO (doi: 10.15454/1.5573896321728955E12) and Pig phenotyping and Innovative breeding facility (doi:10.15454/1.5572415481185847E12) experimental units for the monitoring of the breeding. Thus, according to the ethical issues for the protection of animals, this project does not require the consent of the competent ethics committee for animal experiments. The UEPAO and Pig phenotyping and Innovative breeding facility experimental units are registered by the Ministry of Agriculture with the license number D-37-175-1 and A17-661 for animal experimentation, respectively. All experiments were performed in accordance with the European Communities Council Directive 2010/63/UE. Once collected, tissues were immediately sampled, snap-frozen and stored at -80°C until use.

2.2 Animals, tissue and blood sampling and plasma preparation

WAT around the kidney and plasma were collected from 16 MS and 16 LW sexually mature at a local abattoir under veterinarian control, less than 20 min after slaughter. Tissues were transported to the laboratory in PBS with antibiotic-antimycotic solution within 30 min of collection. Blood samples and WAT were collected from 16 LW and 16 MS pigs on days 4–6 (early luteal phase) of oestrous cycle by morphological examination of the ovaries (Akins & Morrissette 1968). Plasma samples were prepared by low-speed centrifugation (2,000 ***g*** at

4°C for 10 min) and stored at −20°C to determine adipokines concentration. To determine adipokines and adipokine receptors mRNA and protein expression, WAT were immediately frozen in liquid nitrogen and stored at −70°C. Zootechnical parameters were also studied from animal databases implemented by two differerent experimental units. From the Pig phenotyping and Innovative breeding facility unit, we analysed the backfat thickness in LW and MS sows by using a database including 57 MS and 65 LW animals at 140 days old. Backfat thickness was measured as the average of ultrasonix measurements (Agroscan, E.C.M, Angoulême, France) determined directly at the last rib (P2 site), and last lumbar vertebra locations and taken 5 cm off the midline on each side of the pig. These data were collected from 2017 to 2019. From the UEPAO unit, we analysed the reproductive parameters (age at the puberty, maximum number of litter, size of litter and total number of piglet per female during its entire life) in 54 MS and 61 LW collected from 2008 to 2015. Pubertal signs were checked and recorded twice daily by inspection of the vulva and assessment of the standing reflex and using the back-pressure test (Dorries, et al. 1997).

2.3 RT-qPCR

Peri renal WAT (n=16 for each breed) was homogenized in the TRIzol® reagent using an Ultraturax (Invitrogen ™ by Life Technologies ™, Villebon sur Yvette, France) and total RNA was extracted using the RNeasy Midi kit (Quiagen®, Courtaboeuf, France) according to the manufacturer's recommendations. Concentration and purity of isolated RNA were determined with a NanoDrop Spectrophotometer (Peqlab Biotechnologie GmbH, Erlangen, Germany). The integrity of RNA was checked on 1.25 % agarose-formaldehyde gels. The cDNA was generated by reverse transcription (RT) of total RNA (1µg) in a mixture comprising: 0.5 mM each deoxyribonucleotide triphosphate (dATP, dGTP, dCTP, DTTP) 2 M of RT buffer, 15 µg / µL of oligodT, 0.125 U of ribonuclease inhibitor, and 0.05 U MMLV

(Moloney murine leukemia virus reverse transcriptase) for one hour at 37°C. Real-time PCR was performed using the MyiQ Cycle device (Bio-Rad, Marnes-la-Coquette, France), in a mixture of SYBR Green Supermix 1X reagent (Bio-Rad, Marnes la Coquette, France), 250 nM specific primers (Invitrogen ™ by Life Technologies ™, Villebon sur Yvette, France) (**Table 1**) and 5 µL of cDNA diluted to the fifth for a total volume of 20 µl. The samples were duplicated on the same plate. PCR amplification with water, instead of cDNA, was performed systematically as a negative control. After incubation for 2 minutes at 50°C and a denaturation step of 10 minutes at 95°C, samples were subjected to 40 cycles (30 seconds at 95°C, 30 seconds at 60°C, 30 seconds at 72°C), following by the acquisition of the melting curve. A test of primers' efficiency (E) was performed from serial dilutions of a pool of cDNA obtained from the samples. The efficiency was calculated with the following formula: $E = 10^{-1/\text{slope value}}$ and was ranged from 1.85 to 2.00 (**Table 1**). For each gene, expression was calculated according to primer efficiency (E) and quantification cycle (Cq), where expression $= E^{-Cq}$. Then, relative expression of the target gene to the reference gene was analyzed. We have tested several reference genes and selected a combination of three genes: tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta polypeptide (*YWHAZ*), peptidylprolyl isomerase (*PPIA*), succinate dehydrogenase complex subunit A (*SDHA*) that were not affected by the breed or the time of oestrus cycle. Indeed, the geometric mean of multiple housekeeping genes has been reported as an accurate normalization factor (Vandesompele *et al.* 2002).

2.4 Western-blot

Peri renal WAT and plasma from 8 animals per breed were lysed using an Ultraturax (Invitrogen ™ by Life Technologies ™, Villebon sur Yvette, France) in lysis buffer (Tris 1 M (pH 7.4), NaCl 0.15 M, EDTA 1.3 mM, EGTA 1 mM, VO_4^{3-} 23 mM, NaF 0.1 M, NH_2PO_4

1 %, Triton 0.5 %). The lysates were centrifuged for 20 minutes at 16,000 g at 4 °C and the supernatant containing proteins was collected and kept on ice. The protein concentration was measured using the bicinchoninic acid (BCA) protein assay (Interchim, Montluçon, France). Lysate protein (60 µg) was mixed with Laemmli buffer 5 X and proteins were denatured for 5 minutes by heat shock at 95 °C. Proteins were loaded in an electrophoresis sodium dodecyl sulfate-polyacrylamide gel (12 % for high protein weight (110 – 20 kDa) and 15% for low protein weight (< 20 kDa)). Then, proteins were transferred to a nitrocellulose membrane. Membranes were blocked with Tris-Buffered Saline Tween buffer containing 0.05% of Tween 20 and 5% of milk for 30 minutes at room temperature. Membranes were incubated overnight at 4 °C with the appropriate primary antibody (**Table 2**). The validation of all these antibodies was already reported in pig tissue (cf references in **Table 2**). As shown in **Table 3**, adipokines and adipokines receptors protein studied are relatively conserved between pig and human species. Membranes were then incubated 90 minutes at room temperature with a HorseRadish Peroxidase-conjugated anti-rabbit or anti-mouse IgG (**Table 2**). Proteins of interest were detected by enhanced chemiluminescence (Western Lightning Plus-ECL, Perkin Elmer, Villebon-sur-Yvette, France) with a G-box SynGene (Ozyme, St Quentin en Yvelines, France) and GeneSnap software ((release 7.09.17), Ozyme, St Quentin en Yvelines, France). Then, proteins were quantified with GeneTools software (release 4.01.02).

2.5 Adipokine assays

Commercially available porcine leptin (SEA084PO), total adiponectin (RD591023200R), chemerin (all forms) (EP0169), visfatin (MBS 7230081), resistin (EK-028-36), apelin (RAB0018), omentin (MBS750058) and vaspin(MBS 267502) ELISA kits as described in **Table 4**, were used to quantify leptin, adiponectin, chemerin, visfatin, resistin, apelin, omentin and vaspin concentrations in LW and MS plasma. For all the assays, the inter-

and intra-experimental coefficients of variation were <12 and <10%, respectively. Samples were run in duplicate within the same assay. The sensitivity of each ELISA kit used is indicated in the **Table 4**.

2.6 Statistical analysis

The Statview® software (version 9.3) was used for all analyses. One-way analysis of variance (ANOVA) was performed on data from age, body weight, backfat thickness, age at the puberty, litter size, number of alive piglets per sow, plasma adipokines as well as the expression levels of adipokines and their receptors in peri renal WAT. All experimental data are presented as means ± SEM. The level of statistical significance was set at $P < 0.05$. The relationships between quantitative parameters (plasma adipokines vs backfat thickness and plasma adipokines vsonset of puberty) were investigated by Pearson's correlation analyses using the Z-test of Statview software.

3. Results

3.1 Backfat thickness and reproductive performance in LW and MS

As showed in **Table 5**, we observed that MS animals had a higher backfat thickness (BFT) than LW animals (24.3 ± 0.4 vs 16.6 ± 0.3 mm, $P<0.001$, n=16 animals in each breed). This result was confirmed from a more important database provided by the Pig phenotyping and Innovative breeding facility experimental unit (INRAE, Magneraud, France) including 57 MS and 65 LW animals at 140 days old (19.5 ± 0.5 vs 11.1 ± 0.1 mm, $P<0.001$). Concerning the reproductive performance, we noted that MS animals have an earlier sexual maturity than LW animals (86.1 ± 1.30 days vs 171.9 6 ± 2.8, $P<0.001$, n=16 animals in each breed). Again, we confirmed these data from another larger database implemented by our porcine experimental unit (UEPAO, INRAE, Nouzilly, France) including 61 LW and 54 MS animals studied until their culling. With this later database, we showed that the maximum number of litter was higher in MS than LW sows (MS: 18 vs LW: 12, **Figure 1A**). Moreover, even if the litter size at the first parturition was similar between the two breeds (LW: 13.7 ± 0.2 vs MS : 12.5 ± 0.7, $P=0.8$, **Figure 1B**), the total amount of piglets produced during the entire life was significantly greater in MS than LW sows (122.9 ± 8.5 vs 81.2 ± 2.50, $P<0.001$, **Figure 1C**).

3.2 Plasma adipokines in LW and MS

We investigated the plasma adipokines in overnight fasted MS and LW sows. As showed in **Table 5** and **Figure 2**, we observed by using specific ELISA assays that plasma leptin, chemerin, resistin and vaspin concentrations were significantly higher in MS than LW sows ($P = 0.0001$) whereas plasma total adiponectin and omentin concentrations were lower in MS than LW ($P = 0.0001$). Furthermore, plasma visfatin and apelin levels were similar between the two breeds. We confirmed the ELISA assay data by immunoblot for adiponectin,

chemerin and omentin that were the three most plasma concentrated adipokines in our study (**Figure 3**).

3.3 Adipokines and adipokine receptors mRNA expression profile in visceral WAT

In the visceral WAT (peri-renal fat) of LW and MS animals, we examined the expression profiles of adipokines (*LEP*, *RARRES2*, *RETN*, *SERPINA12*, *ADIPOQ*, *ITLN1*, *NAMPT* and *APLN*) and those of receptors for adiponectin (*ADIPOR1* and *ADIPOR2*), chemerin (*CMKLR1*, *GPR1*, *CCRL2*), leptin (*LEPR LF (Long Form)*), resistin (potential receptors: *TLR4*, *ROR1*, *CAP1*), vaspin (potential receptor: *HSPA5*) and apelin (*APLNR*) at the mRNA levels by RT-qPCR. As showed in the **Table 6**, except for *NAMPT* and *APLNR* all adipokines tested and their receptor expression profiles were significantly different between the two breeds of sows ($P<0.05$). *LEP*, *APLN*, *RETN*, *SERPINA12*, *ADIPOR1*, *CCRL2*, *GPR1*, *ROR1*, *CAP1* and *HSPA5* mRNA expression were significantly higher in MS than LW animals.

3.4 Adipokines and adipokine receptors protein expression profile in visceral WAT

We next determined the protein expression profile of all adipokines and adipokines receptors studied at the mRNA levels in the peri renal adipose tissue except for leptin receptor (long form), ROR1 and CAP1 due to the lack of specific porcine antibodies. Concerning the adipokines, as shown in **Figure 4**, leptin, chemerin, resistin and vaspin protein levels were significantly higher in MS than in LW animals whereas the amount of visfatin and apelin protein was similar in both breeds. Moreover, the adiponectin and omentin protein amounts were significantly lower in MS than LW animals (**Figure 4**). For the adipokines receptors, as shown in **Figure 5**, except for apelin receptor and TLR4, all adipokines receptors expression profiles were significantly different between the two breeds of sows. ADIPOR1, GPR1, CCRL2 and HSPA5 protein levels were significantly higher in MS than in LW sows (**Figure**

5). Thus, similar regulations at the mRNA and protein levels in peri renal adipose tissue from LW and MS sows were observed for all adipokines and adipokines receptors studied except for chemerin and apelin.

3.5 Correlation between the plasma adipokines, and the backfat thickness and the age at the puberty in both LW and MS sows

To link the concentration of plasma adipokines studied (leptin, chemerin, resistin, vaspin, adiponectin, omentin, visfatin and apelin) to the backfat thickness or to the age of sexual maturity we performed Pearson correlations. As shown in **Tables 7 and 8**, we found that only plasma leptin and chemerin were positively associated with the backfat thickness (**Table 7**, leptin: r = 0.78, P = 0.002 and chemerin: r = 0.91, P = 0.0001 for LW sows; and leptin: r = 0.90, P = 0.0001 and chemerin: r = 0.92, P = 0.0001 for MS sows) and negatively with the onset of the puberty (**Table 8**, leptin: r = -0.80, P = 0.0001 and chemerin: r = -0.91, P = 0.0001 for LW sows; and leptin: r = -0.97, P = 0.0001 and chemerin: r = -0.83, P = 0.0001 for MS sows) in both LW and MS. At the opposite, plasma adiponectin was negatively associated with these parameters in both LW and MS (**Table 7** for backfat thickness, adiponectin : r = -0.95, P = 0.0001 for LW sows and r = -0.98, P = 0.0001 for MS sows; **Table 8** and positively for the onset of the puberty, adiponectin : r = 0.90, P = 0.0001 for LW sows and r = 0.91, P = 0.0001 for MS sows).

Discussion

The efficiency and quality of pig meat production is strongly dependent on the metabolic processes involved in the lipid synthesis and deposition. Consequently, in order to optimize production traits in pigs, it is crucial to better understand the biological processes involved in the regulation of the adipogenesis and lipid metabolism in WAT of pigs. Moreover, the

knowledge of these processes in pig is relevant for the study of obesity and metabolic disorders in human. In mammals, the adipokines, leptin, adiponectin, visfatin, chemerin, apelin, resistin, omentin and vaspin are involved in the regulation of lipid metabolism in human and/or rodents. In the present study, we showed that these eight adipokines are differently regulated between MS and LW animals in both plasma and peri-renal WAT. Furthermore, we showed that only plasma leptin and chemerin were positively correlated to both BFT thickness and negatively with onset of the puberty whereas the opposite was observed for adiponectin.

Our data concerning the higher plasma leptin in MS with a greater BF thickness are in a good agreement with the literature. Indeed, as observed in various studies in humans and rodents (for review (GuerreMillo 1997)), greater adipose tissue increases circulating plasma leptin in pig (Lee *et al*. 2019). In swine, the positive correlation between circulating leptin in plasma and adipose tissue mass is also recognized as a potent regulator of reproductive neuroendocrine axis in gilts (Barb *et al*. 2005). In our study, we found that plasma leptin was negatively associated with the age at the beginning of the puberty in the sow (MS and LW). So, it means that animals with higher plasma leptin concentrations are earlier sexually mature. These data are in a good agreement with some bibliography showing that leptin could participate in the promotion of reproductive maturity (Elias 2012). In the present study we observed that MS animals had higher plasma leptin levels and were earlier sexually mature as compared to LW animals. Our results are in good agreement with human data showing that leptin levels are higher in girls with central precocious puberty (Verrotti *et al.* 2003, Zurita-Cruz *et al*. 2017). One of the major mechanisms whereby leptin influences the timing of puberty is via its ability to regulate the hypothalamic Kiss1 system. Indeed, a close association between higher leptin levels, increased Kiss1/kisspeptin expression and earlier

puberty onset (or vice versa) has been demonstrated in the female rat (Castellano *et al*. 2011). Even if this association remains to demonstrate in pig, it will be very interesting to compare kisspeptin system expression in LW and MS. Various studies in humans and rodents with leptin deficiency showed that, while leptin is indispensable for puberty to proceed, leptin alone cannot trigger early puberty, therefore supporting a major permissive role of this hormone in the metabolic gating of pubertal maturation. Although serum leptin increases during the puberty in the gilt, other factors such serum estradiol in addition to leptin may regulate the onset of puberty (Barb *et al*. 2004). Moreover, leptin directly affects ovarian function in prepubertal pigs by stimulating estradiol secretion and inhibiting cell apoptosis (Gregoraszczuk *et al*. 2006).

Concerning adiponectin, Daniele et al., 2008, analysed mRNA *ADIPOQ* expression in two genetically different breeds of pigs, lean type, Large White and fat type, Casertana and they observed higher *ADIPOQ* expression in LW showing for the first time that *ADIPOQ* expression was negatively correlated with the "fat" phenotype in pig (Daniele *et al.* 2008). Moreover, a recent study of Nakajima et al., 2019 reported a significant difference in *ADIPOQ* and *ADIPOR1* mRNA levels in lean and fat pigs (Nakajima *et al.* 2019). Lean-type pigs exhibited higher *ADIPOQ* mRNA expression, fat-type pigs had slightly high expression in its receptor, *ADIPOR1*. In the present study, the *ADIPOQ* expression pattern confirms previous data indicating that fatter genotype of pigs can be characterized as decreased expression of adiponectin in subcutaneous fat and low adiponectin circulation as well (Jacobi *et al*. 2004, Lord *et al*. 2005). In our study, we showed that MS had lower plasma adiponectin and peri renal WAT adiponectin and ADIPOR2 expression but higher peri renal WAT ADIPOR1. However, Lord et al found that *ADIPOQ* and *ADIPOR2* mRNA levels, but not *ADIPOR1*, are modulated in pig visceral fat tissues (Lord *et al.* 2005) suggesting not only

different regulation between ADIPOR1 and ADIPOR2 but also according to the location of WAT. In human subcutaneous adipose tissue, during adipocyte differentiation the expression of *ADIPOR2* is increased, whereas *ADIPOR1* mRNA levels are relatively stable (Rasmussen *et al*. 2006). In our study, we did not compare the adipocyte differentiation between LW and MS animals, but we could speculate that the opposite profile observed between ADIPOR1 and ADIPOR2 is associated with a different structure or functioning of the peri-renal adipose tissue between LW and MS animals. Concerning the location of WAT, Depreester et al investigated the expression of adipokines in five different WAT in dairy cow and they showed a higher mRNA expression of *ADIPOQ* in intra pelvic and peri-renal as compared to subcutaneous, omental or mesenteric WAT (Depreester *et al*. 2018). Until now, even if no comparable data are available in pig species, we can hypothesize variation of adipokine and adipokine receptors expression according to the location of WAT. In our study, we showed a positive correlation between plasma adiponectin and the onset of the puberty. In mice, adiponectin deficiency altered the peak concentrations of LH surge (Cheng *et al.* 2016) and adiponectin can modulate *in vitro* LH and FSH secretion by pig gonadotrophs (Kiezun *et al*. 2014). The role of adiponectin *in vivo* in the regulation of the sexual maturity in pig remains to determine.

In the present study, we did not observe any difference between LW and MS in term of plasma and peri-renal WAT visfatin suggesting that this adipokine is not involved in the fattening in swine. Similar conclusions have been observed by Palin et al., 2008 reporting that visfatin cannot be considered as a marker of fat accumulation in pig since the highest *NAMPT* expression levels (mRNA and protein) were associated with the leaner animals (Palin *et al.* 2008). In adult humans, plasma concentrations of visfatin were shown to be positively associated with obesity, although negative associations were also reported (Sommer *et al*.

2008). However, associations between polymorphisms of human NAMPT, insulin levels and obesity were reported (Bailey *et al*. 2006, Blakemore *et al.* 2009). Thus, this discrepancy between pig and human about the involvement of visfatin in fattening remains to be investigated. In human, serum chemerin levels are increased in obesity as a result of enhanced synthesis in fat tissues (Rourke *et al.* 2013). Indeed, *RARRES2* mRNA expression in subcutaneous and omental adipose tissues of patients was higher in obesity, and it decreased upon bariatric surgery evoked weight loss (Chakaroun *et al.* 2012). In good agreement with these data, we observed a higher plasma levels and peri renal WAT protein amount of chemerin in « fat » MS as compared to LW. However, it is well known that several C-terminally truncated chemerin isoforms are present in plasma (Buechler *et al*. 2019) and these isoforms are not distinguished by most of ELISA assays. So it will be interesting to quantify these different isoforms in fat and lean pig to better understand the role of chemerin in the pig fattening. In our study, we also observed that the protein level of the three chemerin receptors was differently regulated in MS and LW. Indeed, GPR1 and CCRL2 were higher whereas CMKLR1 was lower expressed in MS as compared to LW. In pig, Huang et al found that GPR1 was more expressed in epididymal and subcutaneous than CMKLR1 suggesting that GPR1 could be involved in the lipid metabolism regulation in pig (Huang *et al*. 2010).

Concerning resistin, we showed that this adipokine was higher expressed in plasma and peri renal WAT of MS as compared to LW animals suggesting that resistin could be involved in the fattening in pig. These data are in good agreement with those of Chen et al., 2004 showing that resistin is expressed in adipose tissue and increased during the development of obesity in pig (Chen *et al*. 2004). In humans, mutations in the *RETN* locus have been associated with obesity and insulin resistance (Engert *et al.* 2002). Moreover, Ortega et al. found a positive association between resistin levels and body fat percentage, especially in

girls (Ortega *et al.* 2013). In pig associations between the polymorphisms in *RETN* gene with several phenotypic measurements, including fat traits have been suggested (Otieno *et al.* 2005). However, in our study plasma resistin was not significantly correlated with the backfat thickness that is a good indicator for fat deposition (Gray *et al.* 1968). Thus, further studies are necessary to determine whether resistin is associated to fattening in pig. Morash et al., 2002 showed an increase in *RETN* expression in pituitary of prepubertal mice (Moras *et al.* 2004) and Rak-Mardyła et al., 2013 documented local-ovarian resistin action in prepubertal pig (Rak-Mardyla *et al.* 2013) suggesting that resistin could be involved during prepubertal time.

Here, we found that apelin levels in plasma and in peri renal WAT were similar in MS and LW suggesting that this adipokine is not involved in the adipose tissue development in pig. In the literature, no data about a potential role of apelin in the regulation of lipid metabolism or in the sexual maturity has been yet described in pig. In human, an absence of correlation between plasma apelin concentrations and body mass index has often been described *(Castan-Laurell et al.* 2011). Concerning omentin and vaspin, like apelin, no role in the control of energy metabolism or the puberty in female has been yet described in pig. Interestingly, we observed that omentin and vaspin levels (plasma and peri renal WAT) were decreased and increased in MS as compared to LW, respectively. These data are in good agreement with human data showing that plasma omentin and vaspin were lower and higher in obese as compared to lean human (Feng *et al.* 2014, Watanabe *et al.* 2017). However, we found no significant association between the plasma levels of these two adipokines and the backfat thickness and the sexual maturity.

4. Conclusions

Taken together, our results report for the first time the pattern of expression of eight adipokines in both plasma and peri renal WAT in two pig breeds with different fattening and fertility parameters. We found that these eight adipokines except for visfatin and apelin are differently regulated between MS and LW animals in both plasma and peri-renal WAT. Furthermore, we showed that only plasma leptin and chemerin were positively correlated to both BFT thickness and negatively with onset of the puberty whereas the opposite was observed for adiponectin. Future research needs to assess the profile of the different forms of blood circulating adipokines in order to better understand their role in the interactions between energy metabolism and fertility in sow.

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Figures legends

Figure 1: Reproductive parameters in Large White (LW) and Meishan (MS). **A.** Percentage of sow in each number of litter in each breed. Fifty-four MS and sixty-one LW sows were followed in term of number of litter until their culling. **B.** Litter size at the first parturition (litter number 1). MS (n=54) and LW (n=61). Data are shown as the mean ± SEM. **C**. Total amount of piglets produced during the entire life in MS (n=54) and LW (n=61). Data

are shown as the mean ± SEM. Groups showing different letters are significantly different ($P < 0.05$).

Figure 2: Plasma concentration of leptin (A), adiponectin (B), visfatin (C), chemerin (D), omentin (E), apelin (F), resistin (G) and vaspin (H) determined by ELISA assays in Large White (LW) and Meishan (MS) sows. Data are shown as the mean ± SEM; n = 16 animals per breed. Groups showing different letters are significantly different ($P < 0.05$).

Figure 3: Protein level of adiponectin (A), chemerin (B) and omentin (C) determined by immunoblot in plasma in Large White (LW) and Meishan (MS) sows. All serum samples contained equal amounts of proteins, as confirmed by staining the nitrocellulose membrane with Ponceau. Blots were quantitated and the results are expressed as intensity signal in arbitrary units after normalization, allowed by the use of reversible ponceau staining, as an internal standard. Data are shown as the mean ± SEM; n = 8 animals per breed. Groups showing different letters are significantly different ($P < 0.05$).

Figure 4: Protein expression of leptin (A), adiponectin (B), visfatin (C), chemerin (D), omentin (E), apelin (F), resistin (G) and vaspin (H) determined by immunoblot in peri renal WAT in Large White (LW) and Meishan (MS) sows. Vinculin is used as a loading control. Blots were quantitated, and the ratios of adipokines to vinculin were represented. Data are shown as the mean ± SEM; n = 8 animals per breed. Groups showing different letters are significantly different ($P < 0.05$).

Figure 5: Protein expression of ADIPOR1 (A), ADIPOR2 (B), CMKLR1 (C), GPR1 (D), CCRL2 (E), APLNR (F), TLR4 (G) and HSPA5 (H) determined by immunoblot in peri

renal WAT in Large White (LW) and Meishan (MS) sows. Vinculin is used as a loading control. Blots were quantitated, and the ratios of adipokines receptors to vinculin were represented. Data are shown as the mean ± SEM; n = 8 animals per breed. Groups showing different letters are significantly different ($P < 0.05$).

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Table 1: Oligonucleotide primer sequences

Gene	Product size (bp)	Forward	Reverse	Number of accession	Efficiency
LEP	101	5'- AGGGTCACCGGTTTGGACTT -3'	5'- AGACTGGTGAGGATCTGTTGGTAGA -3'	**NM_213840.1**	1.95
LEPR LF (Long Form)	71	5'- AAGAGTGCGGTGCCCATTC -3'	5'- GTGAACAGCTCTCCTGGAGGAT -3'	**NM_001024587.1**	1.90
ADIPOQ	64	5'- GCTGTACTCTTCACCTACGACCAGTA -3'	5'- CCAGACTTGGTCCCCCTTCT -3'	**NM_214370.1**	2.00
ADIPOR1	74	5'- GCCATGGAGAAGATGGAGGA -3'	5'- AGCACGTCGTACGGGATGA -3'	**NM_001007193.1**	1.90
ADIPOR2	71	5'- TGTTCGCCACCCCTCAGTAT -3'	5'- AATGATTCCACTCAGGCCCA -3'	**NM_001007192.1**	1.85
NAMPT	71	5'- TGCTGATCCCAACAAAAGGT -3'	5'- AAATTCCCTCCTGGTGTCCT-3'	**DQ001974**	2.00
RARRES2	147	5'- AGTTCCACAAGCACCCACCC -3'	5'- GCTTTCTTCCAGTCCCTCTT -3'	**EU660865.1**	1.90
CMKLR1	137	5'- CTAACCATCCCTGCCTCCCT -3'	5'- ATCCTGACCACCCTGCCTTC -3'	**EU660866.2**	1.90
GPR1	168	5'- CCACCCTGTCTTATCTCATCGG -3'	5'- AGGATCTTGCTCGTGGAAGTTGT -3'	**NM001190244.1**	2.00
CCRL2	149	5'- AGCACCAGAGGATGACTATG -3'	5'- TTGTCCAGGAGGCCAACCAA -3'	**NM001001617.1**	1.85
APLN	117	5'- AAGGCAACGTCCGCTATTTG -3'	5'- ATGGGGCCCTTGTGGGAGA -3'	**XM_003360446.4**	2.00
APLNR	188	5'- ACCTTGGTGCCGTTCTCGG -3'	5'- CTCAGCTTCGACCGCTACCT -3'	**XM_021082015.1**	1.95
RETN	149	5'- ATGAAGGCTCTCTCCCTCCT -3'	5'- TTCCAGGCCAATGTTCCTAA -3'	**NM_213783.2**	1.85
TLR4	165	5'- TCAGTTCTCACCTTCCTCCTG -3'	5'- GTTCATTCCTCACCCAGTCTTC -3'	**KF460453.1**	2.00
ROR1	149	5'- GGATCCAATCAGGAAGCAAA- 3'	5'- GTTGGCGTCTCCTAGCAAAG -3'	**XM_021097759.1**	1.85
CAP1	100	5'- ATTATGGCTGACATGCAAAA -3'	5'- CTCCATATCCACAGTGTGTG -3'	**XM_003127824**	1.95
SERPINA12	149	5'-GCTGTGAGTCGTGACCAAGT -3'	5'- CACAGAGATGCTCCAAGGG -3'	**XM_013978403.2**	2.00
HSPA5	344	5'- CATCGAGTTGGCTTTCCGTG -3'	5'- GTCAGCAGGCAGGGCTTCGC -3'	**X92446.1**	1.95
YWHAZ	178	5'- ATGCAACCAACACATCCTATC -3'	5'- GCATTATTAGCGTGCTGTCTT -3'	**DQ178130]**	2.00
PPIA	171	5'- CACAAACGGTTCCCAGTTTT -3'	5'- TGTCCACAGTCAGCAATGGT -3'	**NM_214353.1**	1.85
SDHA	100	5'- TCCGACTGGCTGGGGGACCA -3'	5'- GTTCTGCTGAACGGCATGCC -3'	**DQ402993**	2.00

Table 2: **List of primary and secondary antibodies used for protein detection by western blot.**

Primary antibodies	Dilution	Supplier	Host	Molecular weight (kDa)	References
Vinculin	1/1000	Sigma (hVIN-1)	Mouse	110	Reverchon, et al. 2014
Chemerin	1/1000	Abcam (ab203040)	Rabbit	16	Smolinska *et al*., 2019
CMKLR1	1/1000	Abcam (ab230442)	Rabbit	42	Smolinska *et al*., 2019
GPR1	1/1000	Abcam (ab169331)	Mouse	43	Smolinska *et al*., 2019
CCRL2	1/1000	Abcam (ab85224)	Rabbit	56	Smolinska *et al*., 2019
Apelin	1/1000	Santa Cruz (sc-293441)	Mouse	8	Różycka *et al*., 2018
APLNR	1/1000	Santa Cruz (sc-33823)	Rabbit	42	Różycka *et al*., 2018
Visfatin	1/1000	Phoenix (G-003-81)	Rabbit	52	Palin *et al*., 2008
Omentin	1/1000	R&D system	sheep	40	Cloix, et al. 2014
Resistin	1/1000	Santa Cruz (sc-17575)	Rabbit	12	Rak *et al*., 2015
TLR4	1/1000	Abcam (ab13556)	Rabbit	90	Dai, et al. 2016
Vaspin	1/1000	ThermoFisher (PA5-30989)	Rabbit	47	Kurowska, et al. 2019
HSPA5	1/1000	Santa Cruz (sc-13968)	Rabbit	78	Ferenc, et al. 2017
Leptin	1/1000	Santa Cruz (A-20)	Rabbit	16	Aquila, et al. 2008
Adiponectin	1/1000	Santa Cruz (sc-26496)	Rabbit	28	Rak *et al.*, 2017
ADIPOR1	1/1000	Santa Cruz (sc-46749)	Rabbit	42	Rak *et al*., 2017
ADIPOR2	1/1000	Santa Cruz (sc-46751)	Rabbit	43	Rak *et al*., 2017
Secondary antibodies					
Rabbit	1/50000	Biorad	Goat		
Sheep	1/10000	Biorad	Goat		
Mouse	1/50000	Interchim	Goat		

Table 3: **Percentage of amino acid identity between pig and human for adipokines and adipokine receptors studied.**

Adipokines or adipokines receptors	%	Accession number (human and pig) or references
Chemerin	83.4	**EU660865** and **NM_002889.4**, Huang *et al*., 2010
CMKLR1	86.2	**EU660866.2** and **AY497547.1** , Huang *et al*., 2010
GPR1	86.8	**FJ234899** and **EF577403.1** , Huang *et al*., 2010
CCRL2	65.4	**NM_001001617.1** and **AY337001.1**
Apelin	83.1	**XM_003360446.4** (predicted) and **NM_017413.5**
APLNR	92.7	**XM_021082015** (predicted) and **NM_005161.4**
Visfatin	97.1	Chen *et al*., 2007
Omentin	81.7	**EU652941** and **AY549722**
Resistin	75.2	Dai *et al*., 2006
TLR4	73.1	**AJ628065** and **AH009665**, Alvarez, et al. 2006
ROR1	98.2	**XM 021097759.1** (predicted) and **XM 017001376.1**
CAP1	100	**XM 011504510.1** (predicted) and **XM021095996.1**
Vaspin	61.2	**XM_013978403** (predicted) and **AY326420**
HSPA5	nd	nd and **M19645**
Leptin	86.0	Dai, et al. 2000
LEPR	83.9	**NM_001024587** and **U43168.1**
Adiponectin	83.0	**EF601160.1** and **NM_001177800**, Ding *et al*., 2004
ADIPOR1	97.0	Ding *et al*., 2004
ADIPOR2	94.0	Ding, et al. 2004

Table 4: List of ELISA assays used for measurement of porcine plasma adipokine concentration.

Adipokine	Supplier	Host	Sensitivity of the used ELISA kit	References
Leptin	Cloud-Clone Corps distributed by Euromedex (SEA084PO)	Pig	less than 12.7pg/mL	Fan, et al. 2020
Total Adiponectin	BioVendor Research and Diagnostic Products (cat. # RD591023200R)	Pig	0.026 ng/ml	Rak *et al*., 2017
Chemerin	FineTest, Wuhan Fine Biotech Co distributed by Euromedex (cat # EP0169)	Pig	0.1ng/ml	Smolinska *et al*., 2019
Visfatin	MyBioSource (cat # MBS 7230081)	Pig	0.26ng/ml	
Resistin	Phoenix Pharmaceuticals Inc. (cat # EK-028-36)	Human	0.016–1 ng/ml	Rak, et al. 2015
Apelin	Sigma-Aldrich (cat. # RAB0018)	Human	5.84 pg/ml	Rak *et al*., 2017
Omentin	Hölzel Diagnostika (cat # MBS750058)	Pig	0.05 ng/ml	
Vaspin	MyBioSource (cat # MBS 267502)	Pig	0.05 ng/ml	Kurowska *et al*., 2019

Table 5: **Age, body weight (BW), backfat thickness (BFT), ratio BFT/BW, age at the puberty and plasma leptin, adiponectin, visfatin, chemerin, omentin, apelin, resistin and vaspin concentrations in Large White (LW, n=16) and Meishan (MS, n=16) sows.**

	LW	MS	*P*-value
Age (day)	214.7 ± 8.2	179.0 ± 6.1	**0.001**
Body weight (BW, kg)	126.5 ± 1.6	68.5 ± 1.6	**0.0001**
Backfat thickness (BFT, mm)	16.6 ± 0.3	24.3 ± 0.4	**0.0001**
Ratio BFT/BW	0.13 ± 0.002	0.35 ± 0.005	**0.0001**
Age at puberty	171.9 6 ± 2.8	86.1 ± 1.30	**0.0001**
Leptin (ng/ml)	0.81 ± 0.005	1.56 ± 0.07	**0.0001**
Adiponectin (µg/ml)	5.67 ± 0.15	2.55 ± 0.09	**0.0001**
Visfatin (ng/ml)	2.40 ± 0.15	2.66 ± 0.15	0.23
Chemerin (ng/ml)	168.24 ± 1.99	232.97 ± 3.51	**0.0001**
Omentin (ng/ml)	225.69 ± 4.80	129.44 ± 3.18	**0.0001**
Apelin (ng/ml)	1.02 ± 0.06	1.01 ± 0.05	0.87
Resistin (ng/ml)	3.15 ± 0.21	8.20 ± 0.24	**0.0001**
Vaspin (ng/ml)	0.45 ± 0.03	0.86 ± 0.04	**0.0001**

Results are represented as mean ± SEM. *P*-value was considerate significant when $P < 0.05$ (bold).

Table 6: **Relative mRNA expression of adipokines (*LEP*, *ADIPOQ*, *NAMPT*, *RARRES2*, *ITLN1*, *APLN*, *RETN* and *SERPINA12*) and adipokine receptors (*LEPR*, *ADIPOR1*, *ADIPOR2*, *CMKLR1*, *CCRL2*, *GPR1*, *APLNR*, *TLR4*, *ROR1*, *CAP1* and *HSPA5*) in peri renal adipose tissue of LW and MS sows.**

		LW	MS	*P*-value
Adipokines	*LEP*	0.02 ± 0.002	1.96 ± 0.5	**0.001**
	ADIPOQ	0.16 ± 0.02	0.02 ± 0.003	**0.0002**
	NAMPT	0.18 ± 0.05	0.20 ± 0.06	0.1
	RARRES2	2.37 ± 0.30	0.14 ± 0.03	**0.0001**
	ITLN1	2.61 ± 0.75	0.02 ± 0.08	**0.002**
	APLN	0.15 ± 0.03	2.19 ± 0.84	**0.0005**
	RETN	0.07 ± 0.01	2.47 ± 0.56	**0.0001**
	SERPINA12	0.43 ± 0.03	2.16 ± 0.17	**0.00001**
Adipokine receptors				
	LEPR	1.85 ± 0.28	0.16 ± 0.02	**0.0001**
	ADIPOR1	0.14 ± 0.02	0.62 ± 0.13	**0.001**
	ADIPOR2	0.04 ± 0.02	0.02 ± 0.004	**0.01**
	CMKLR1	0.11 ± 0.02	0.03 ± 0.006	**0.0001**
	CCRL2	0.07 ± 0.02	0.12 ± 0.02	**0.002**
	GPR1	0.13 ± 0.009	0.22 ± 0.01	**0.05**
	APLNR	0.08 ± 0.02	0.08 ± 0.007	0.77
	TLR4	0.08 ± 0.002	0.06 ± 0.009	**0.01**
	ROR1	0.03 ± 0.007	0.05 ± 0.001	**0.001**
	CAP1	0.02 ± 0.004	0.08± 0.03	**0.001**
	HSPA5	0.76 ± 0.17	5.62 ± 0.98	**0.0007**

Results are represented as mean ± SEM. *P*-value was considerate significant when $P < 0.05$ (bold).

Table 7: Pearson correlation coefficient (r) calculated between the backfat thickness and plasma adipokines concentration (leptin, adiponectin, visfatin, chemerin, omentin, apelin, resistin and vaspin) in both LW and MS sows.

		LW	MS
Leptin	r	**0.78**	**0.90**
	P	**0.002**	**0.0001**
Adiponectin	r	**-0.95**	**-0.98**
	P	**0.0001**	**0.0001**
Visfatin	r	0.15	0.07
	P	0.59	0.79
Chemerin	r	**0.91**	**0.92**
	P	**0.0001**	**0.0001**
Omentin	r	0.06	-0,38
	P	0.84	0.15
Apelin	r	-0.06	-0.22
	P	0.82	0.42
Resistin	r	0.14	-0.06
	P	0.62	0.83
Vaspin	r	0.28	-0.006
	P	0.31	0.98

The correlation was noted "r" and *P*-value (*P*) was considerate significant if $P < 0.05$ (bold).

Table 8: Pearson correlation coefficient (r) calculated between the age of puberty and plasma adipokines concentration (leptin, adiponectin, visfatin, chemerin, omentin, apelin, resistinand vaspin) in both LW and MS sows.

		LW	MS
Leptin	r	**-0.80**	**-0.97**
	P	**0.0001**	**0.0001**
Adiponectin	r	**0.90**	**0.91**
	P	**0.0001**	**0.0001**
Visfatin	r	0.06	-0.02
	P	0.80	0.93
Chemerin	r	**-0.91**	**-0.83**
	P	**0.0001**	**0.0001**
Omentin	r	0.15	-0.35
	P	0.59	0.20
Apelin	r	-0.22	-0.20
	P	0.40	0.45
Resistin	r	0.18	-0.18
	P	0.51	0.50
Vaspin	r	0.29	0.04
	P	0.28	0.90

The correlation was noted "r" and *P*-value (*P*) was considerate significant if $P < 0.05$ (bold).

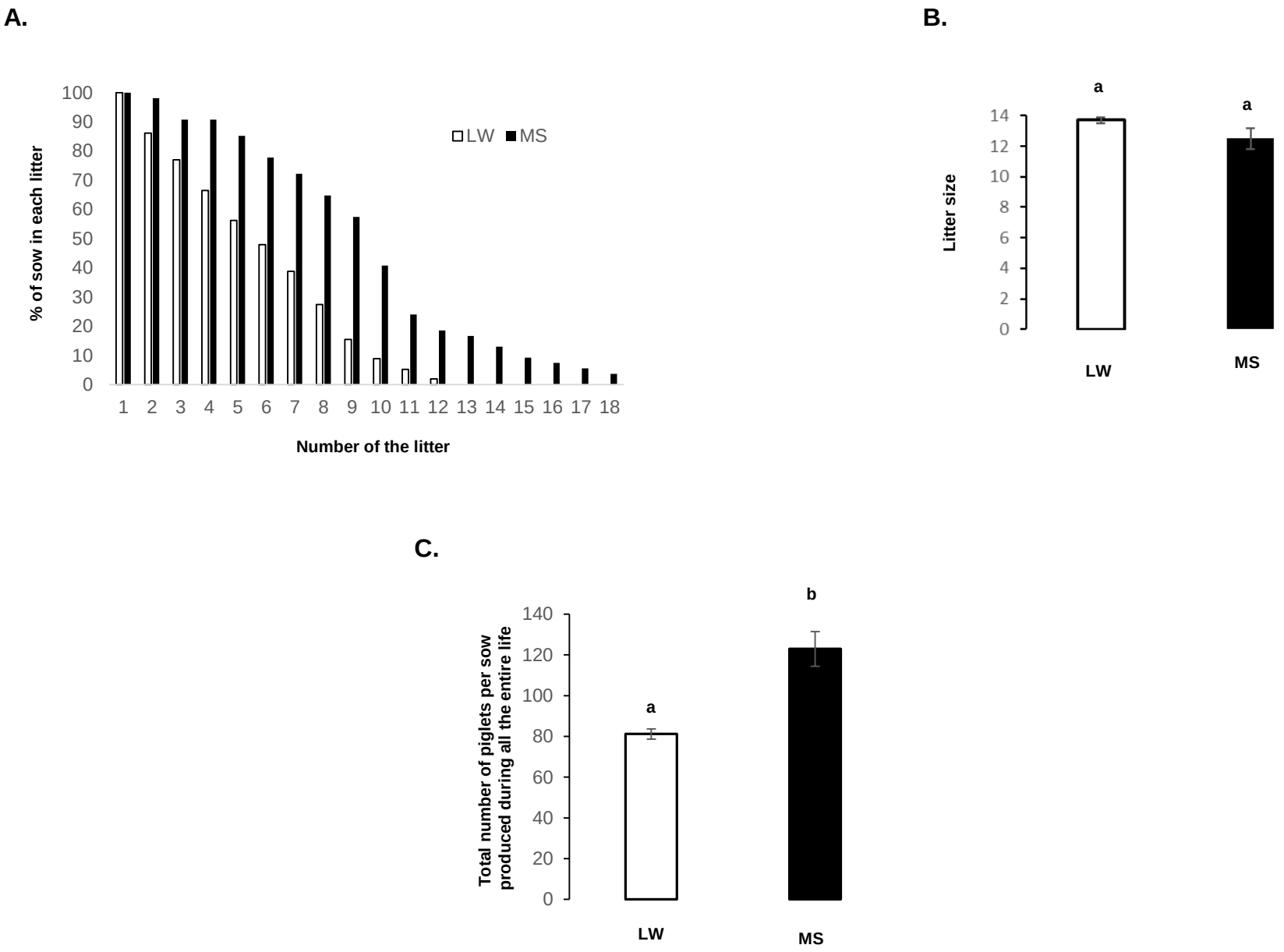
A.
% of sow in each litter
100
90
80
70
60
50
40
30
20
10
0
LW
MS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
Number of the litter
B.
Litter size
14
12
10
8
6
4
2
0
a
a
LW
MS
C.
Total number of piglets per sow produced during all the entire life
140
120
100
80
60
40
20
0
a
b
LW
MS

Figure 1

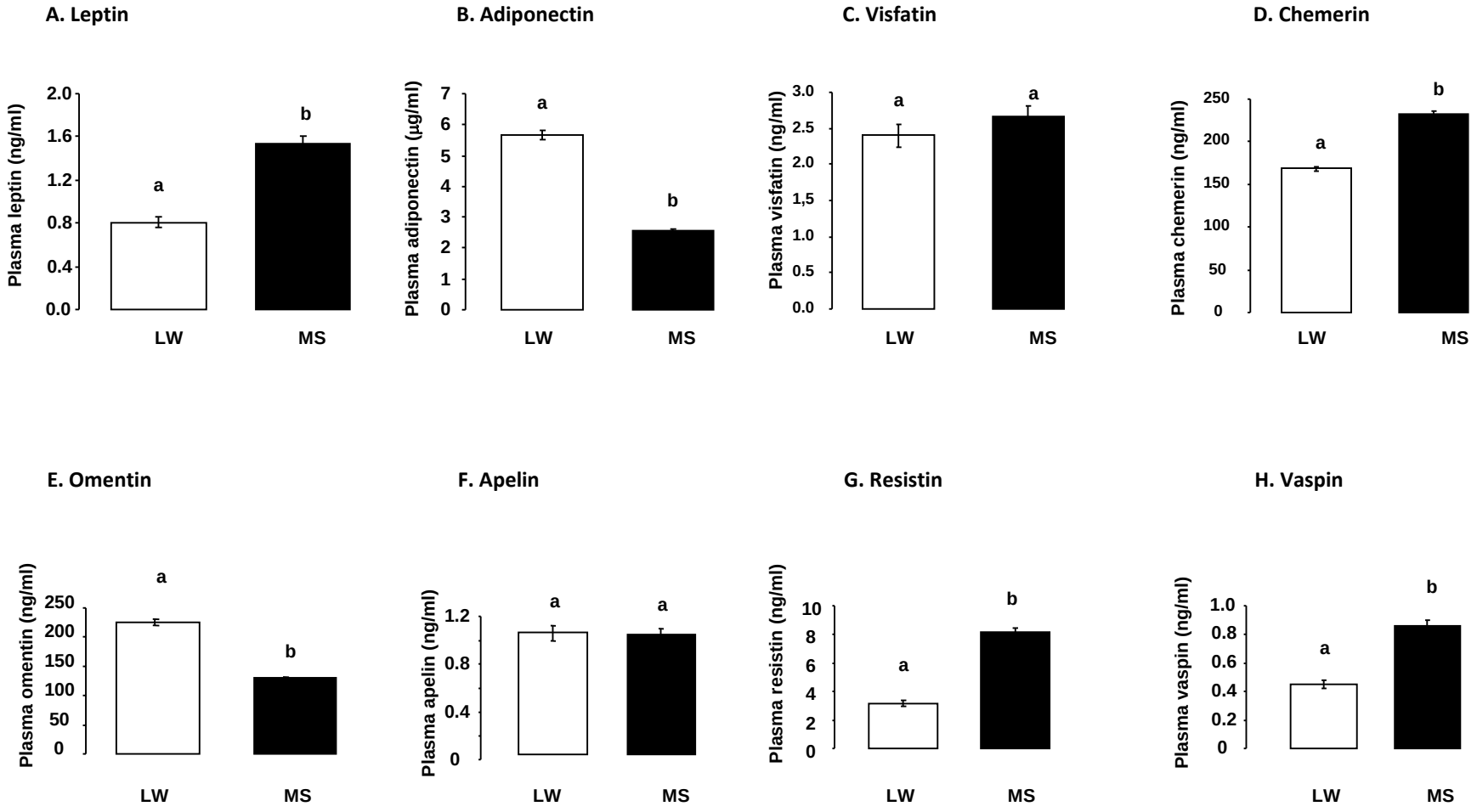
A. Leptin
Plasma leptin (ng/ml)
2.0
1.6
1.2
0.8
0.4
0.0
a
b
LW
MS
B. Adiponectin
Plasma adiponectin (µg/ml)
7
6
5
4
3
2
1
0
a
b
LW
MS
C. Visfatin
Plasma visfatin (ng/ml)
3.0
2.5
2.0
1,5
1.0
0.5
0.0
a
a
LW
MS
D. Chemerin
Plasma chemerin (ng/ml)
250
200
150
100
50
0
a
b
LW
MS
E. Omentin
Plasma omentin (ng/ml)
250
200
150
100
50
0
a
b
LW
MS
F. Apelin
Plasma apelin (ng/ml)
1.2
0.8
0.4
0
a
a
LW
MS
G. Resistin
Plasma resistin (ng/ml)
10
8
6
4
2
0
a
b
LW
MS
H. Vaspin
Plasma vaspin (ng/ml)
1.0
0.8
0.6
0.4
0.2
0
a
b
LW
MS

Figure 2

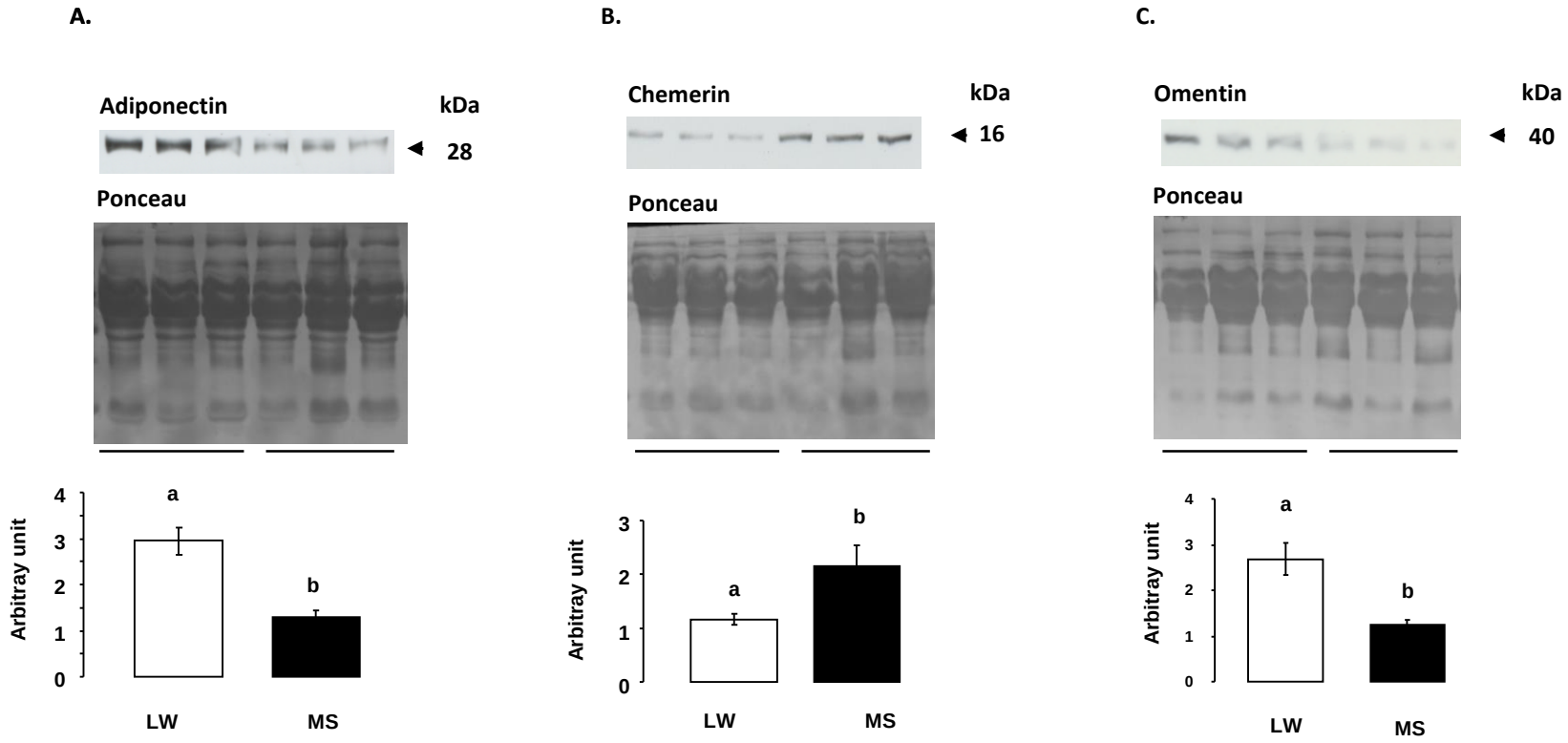
A.
Adiponectin
kDa
28
Ponceau
Arbitray unit
4
3
2
1
0
a
b
LW
MS
B.
Chemerin
kDa
16
Ponceau
Arbitray unit
3
2
1
0
a
b
LW
MS
C.
Omentin
kDa
40
Ponceau
Arbitray unit
4
3
2
1
0
a
b
LW
MS

Figure 3

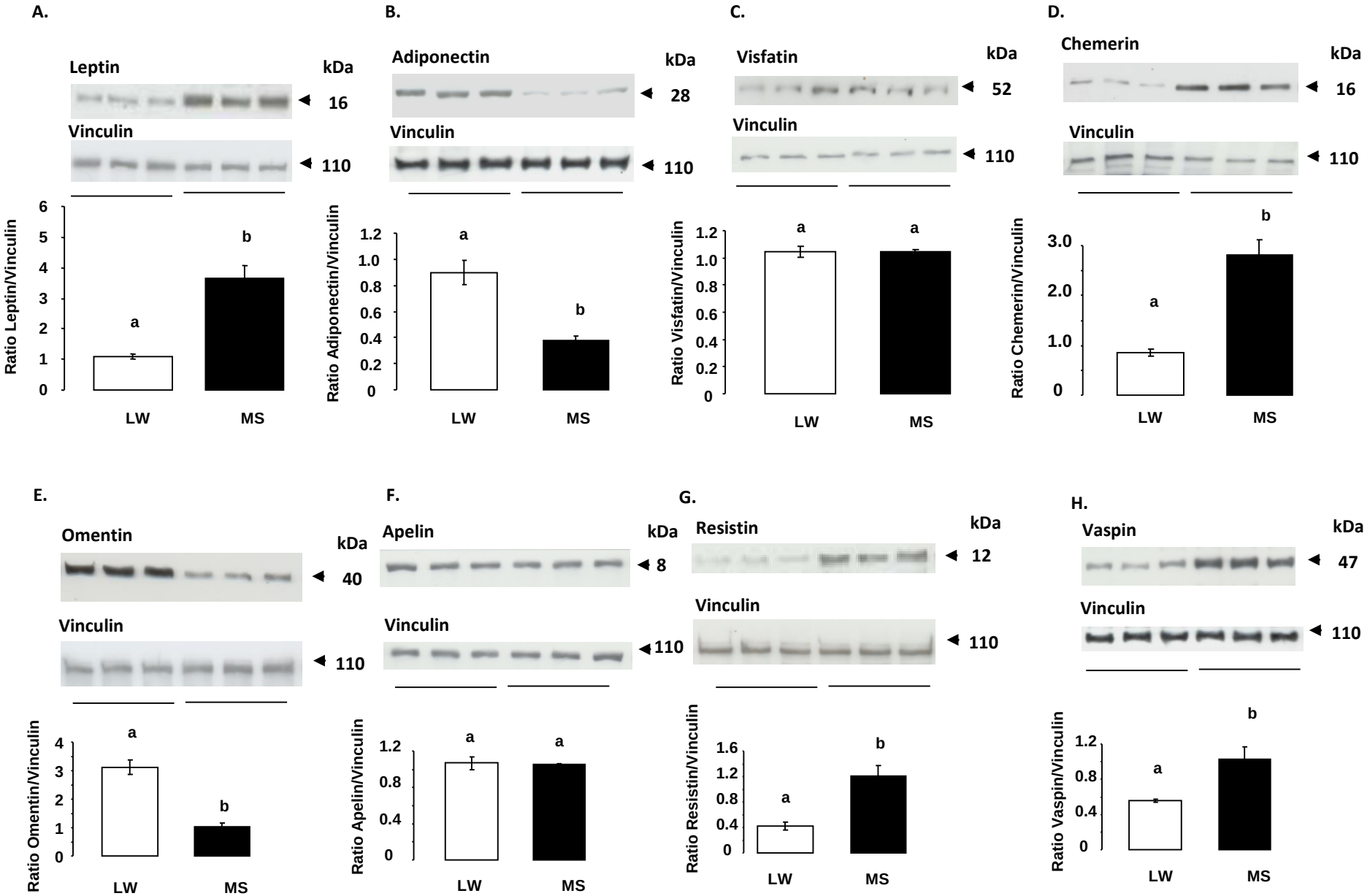
A.
Leptin
kDa
16
Vinculin
110
Ratio Leptin/Vinculin
a
b
LW
MS
B.
Adiponectin
kDa
28
Vinculin
110
Ratio Adiponectin/Vinculin
a
b
LW
MS
C.
Visfatin
kDa
52
Vinculin
110
Ratio Visfatin/Vinculin
a
a
LW
MS
D.
Chemerin
kDa
16
Vinculin
110
Ratio Chemerin/Vinculin
a
b
LW
MS
E.
Omentin
kDa
40
Vinculin
110
Ratio Omentin/Vinculin
a
b
LW
MS
F.
Apelin
kDa
8
Vinculin
110
Ratio Apelin/Vinculin
a
a
LW
MS
G.
Resistin
kDa
12
Vinculin
110
Ratio Resistin/Vinculin
a
b
LW
MS
H.
Vaspin
kDa
47
Vinculin
110
Ratio Vaspin/Vinculin
a
b
LW
MS

Figure 4

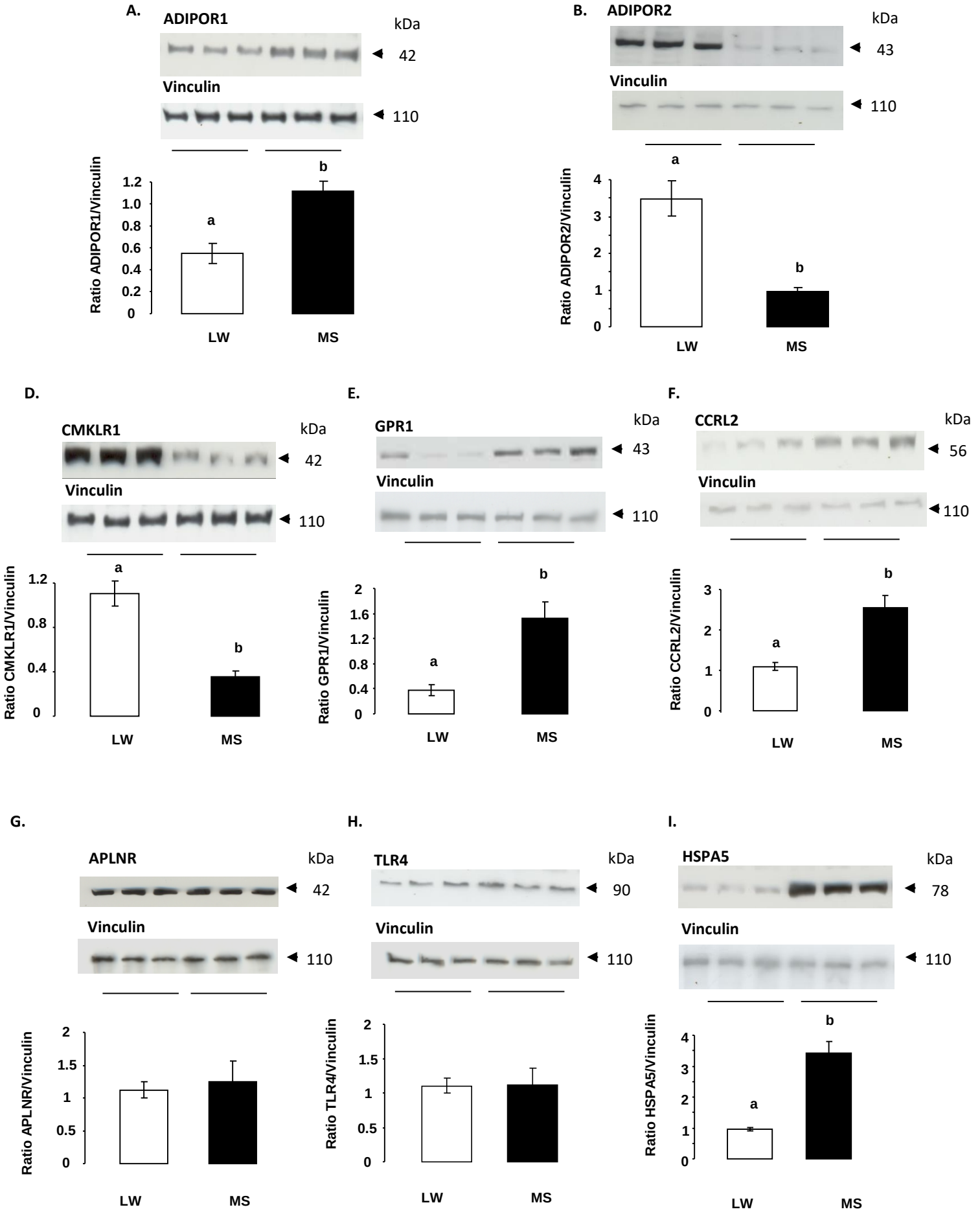
A.
ADIPOR1
kDa
42
Vinculin
110
Ratio ADIPOR1/Vinculin
1.2
1.0
0.8
0.6
0.4
0.2
0
a
b
LW
MS
B.
ADIPOR2
kDa
43
Vinculin
110
Ratio ADIPOR2/Vinculin
4
3
2
1
0
a
b
LW
MS
D.
CMKLR1
kDa
42
Vinculin
110
Ratio CMKLR1/Vinculin
1.2
0.8
0.4
0
a
b
LW
MS
E.
GPR1
kDa
43
Vinculin
110
Ratio GPR1/Vinculin
2
1.6
1.2
0.8
0.4
0
a
b
LW
MS
F.
CCRL2
kDa
56
Vinculin
110
Ratio CCRL2/Vinculin
3
2
1
0
a
b
LW
MS
G.
APLNR
kDa
42
Vinculin
110
Ratio APLNR/Vinculin
2
1.5
1
0.5
0
LW
MS
H.
TLR4
kDa
90
Vinculin
110
Ratio TLR4/Vinculin
2
1.5
1
0.5
0
LW
MS
I.
HSPA5
kDa
78
Vinculin
110
Ratio HSPA5/Vinculin
4
3
2
1
0
a
b
LW
MS

Figure 5