Journal of Developmental Origins of Health and Disease (2017), **8**(1), 3–7.

doi:10.1017/S2040174416000702

RAPID COMMUNICATION

Maternal undernutrition programs the apelinergic system of adipose tissue in adult male rat offspring

S. Lecoutre[1], L. Marousez[1], A. Drougard[2], C. Knauf[2], C. Guinez[1], D. Eberlé[1], C. Laborie[1], D. Vieau[1], J. Lesage[1] and C. Breton[1]*

[1]*EA4489, Équipe Malnutrition Maternelle et Programmation des Maladies Métaboliques, Univ. Lille, Lille, France*
[2]*European Associated Laboratory NeuroMicrobiota (INSERM/UCL), Team 3, IRSD, INSERM U1220, Toulouse, France*

Based on the Developmental Origin of Health and Disease concept, maternal undernutrition has been shown to sensitize adult offspring to metabolic pathologies such as obesity. Using a model of maternal 70% food restriction in pregnant female rats throughout gestation (called FR30), we previously reported that obesity-prone adult male rat offspring displayed hyperleptinemia with modifications in leptin and leptin receptor messenger RNA (mRNA) levels in white adipose tissue (WAT). Apelin is a member of the adipokine family that regulates various aspects of energy metabolism and WAT functionality. We investigated whether apelin and its receptor APJ could be a target of maternal undernutrition. Adult male rat offspring from FR30 dams showed increased plasma apelin levels and apelin gene expression in WAT. Post-weaning high-fat diet led to marked increase in APJ mRNA and protein levels in offspring's WAT. We demonstrate that maternal undernutrition and post-weaning diet have long-term consequences on the apelinergic system of adult male rat offspring.

Received 24 May 2016; Revised 16 November 2016; Accepted 19 November 2016

Key words: adipogenesis, apelin, APJ, fetal programming, HF diet

Introduction

Intrauterine growth retardation (IUGR) and low birth weight induced by maternal undernutrition are associated with an increased risk of developing metabolic disorders in adulthood. These observations led to the concept of 'Developmental Origin of Health and Disease' also called 'fetal programming.'[1,2] To unravel the underlying mechanisms, we used a model of maternal 70% food restriction in pregnant female rats throughout gestation (called FR30). We showed that maternal FR30 induces IUGR and programs energy metabolism disturbances in adult male rat offspring. They displayed mild hypertension, hypercorticosteronemia and subtle food intake modifications.[3,4] Despite normoglycemia and normoinsulinemia, they exhibited mild glucose intolerance, with a defect in glucose-induced insulin secretion during a glucose tolerance test.[4] Although showing a lean phenotype, adult male rat offspring from FR30 dams were predisposed to adiposity exhibiting adipocyte hypertrophy and hyperleptinemia.[5] Maternal undernutrition programs adult male offspring white adipose tissue (WAT) gene expression profile. In particular, obesity-prone adult offspring from FR30 dams displayed higher expression of leptin messenger RNA (mRNA).[5] Lower expression of leptin receptor mRNA associated with reduced leptin-induced STAT3 phosphorylation suggested that leptin sensitivity was impaired in WAT of adult male offspring from FR30 dams. Post-weaning high-fat diet (HFD) exacerbated this profile.[5]

Thus, WAT may represent a prime target of metabolic programming induced by maternal undernutrition. We hypothesized that other adipokines, such as apelin, might be a target of maternal undernutrition.[6,7] Indeed, apelin is a relatively recent member of the adipokine family identified as an endogenous ligand of the apelin receptor APJ.[8] Apelin and APJ are expressed in many mammalian tissues including WAT.[9] The apelinergic system controls various aspects of energy metabolism and is dysregulated in metabolic syndrome. In humans and mice, plasma apelin levels are increased in obesity,[10] diabetes[11] and with insulin resistance.[12] Apelin expression is increased in adipocytes of obese patients.[13] Apelin is also known for its anti-obesity and anti-diabetic properties.[14] Apelin decreases WAT mass and triglyceride levels in obese mice, whereas apelin knock-out mice exhibit increased adiposity and serum-free fatty acid levels due to enhanced lipolysis.[15,16] In addition, apelin modulates adipogenesis[16,17] and promotes browning of WAT.[18] Apelin also improves insulin secretion[19] and insulin sensitivity by promoting glucose utilization in adipose and muscle tissues.[20] In this context, we decided to investigate whether maternal FR30 would modify plasma apelin levels and apelin/APJ gene expression levels in WAT in adult male offspring. We also assessed whether post-weaning HFD would impact on these parameters.

Materials and methods

Animal use accreditation was granted by the French Ministry of Agriculture (no. 04860). Experiments were conducted in

*Address for correspondence: C. Breton, EA4489, Équipe Malnutrition Maternelle et Programmation des Maladies Métaboliques, Univ. Lille, F59000 Lille, France.
(Email christophe.breton@univ-lille1.fr)

accordance with the principles of laboratory animal care (European Communities Council Directive of 1986, 86/609/EEC). Wistar rats were purchased from Charles River Laboratories (L'Arbresle, France). After mating, pregnant females were transferred into individual cages with free access to water and to standard chow diet (SAFE 04, 2900 cal/g, containing 16% protein, 3% fat, 60% carbohydrates; UAR, Augy, France). Control pregnant dams were fed *ad libitum* with a standard diet (NCD), whereas pregnant dams from the food-restricted group were fed 30% (FR30) of the daily intake of NCD pregnant dams, from day 1 (E1) of pregnancy until delivery (E21). At parturition, litter size was adjusted to eight pups per dam. To obviate any litter effects, animals used for further experiments were randomly chosen in different litters and only a limited number of animals ($n = 1$–2) was used from each litter. Food-restricted pups were nursed by FR30 dams fed *ad libitum* during lactation. After weaning, male offspring from both groups (NCD and FR30) were housed individually. They were fed either a standard (NCD) or an HFD (SAFE, D12451, 4720 cal/g, containing 23% protein, 23% fat, 40% carbohydrates; UAR) composing four groups (NCD/NCD, NCD/HFD, FR30/NCD, FR30/HFD; $n = 16$/group). Offspring's body weight were measured weekly until adulthood. At 4 months of age, male rat offsprings were killed between 9 and 10 am after 16-h overnight fasting. Trunk blood samples were collected and plasma was stored at −20°C. Gonadal (GWAT) and perirenal (PWAT) WAT fat pads were rapidly removed, weighed, frozen in liquid nitrogen and stored at −80°C. A commercially available enzyme-linked immunosorbent assay kit was used to measure plasma apelin levels (Phoenix Pharmaceuticals, Burlingame, CA, USA) that assays all isoforms of apelin from apelin-12 to apelin-36. Methods for quantitative reverse transcription polymerase chain reaction (RT-qPCR) analysis have been previously described.[5] RT-qPCR was performed with a LightCycler480 SYBR Green I Master and a LightCycler480 (Roche). Primers for APLN (forward: GCATGAATCT GAGTTTCTGCGTGCA, reverse: GCTTTAGAAAGGCAT GGGTCCCTTA), APLNR (forward: CCTTCATCATCAT GCTGACCTGTT, reverse: TGCATCGGTTCTCCTCCCT TGCACA) genes were designed using the Primer Premier software (Premier Biosoft International). The level of gene expression was normalized to three reference genes (cyclophilin A, β2 microglobulin, lactate dehydrogenase A).[5] Methods for Western blot analysis have been previously described.[5] Frozen GWAT and PWAT were homogenized in lysis buffer: 250 mM sucrose,

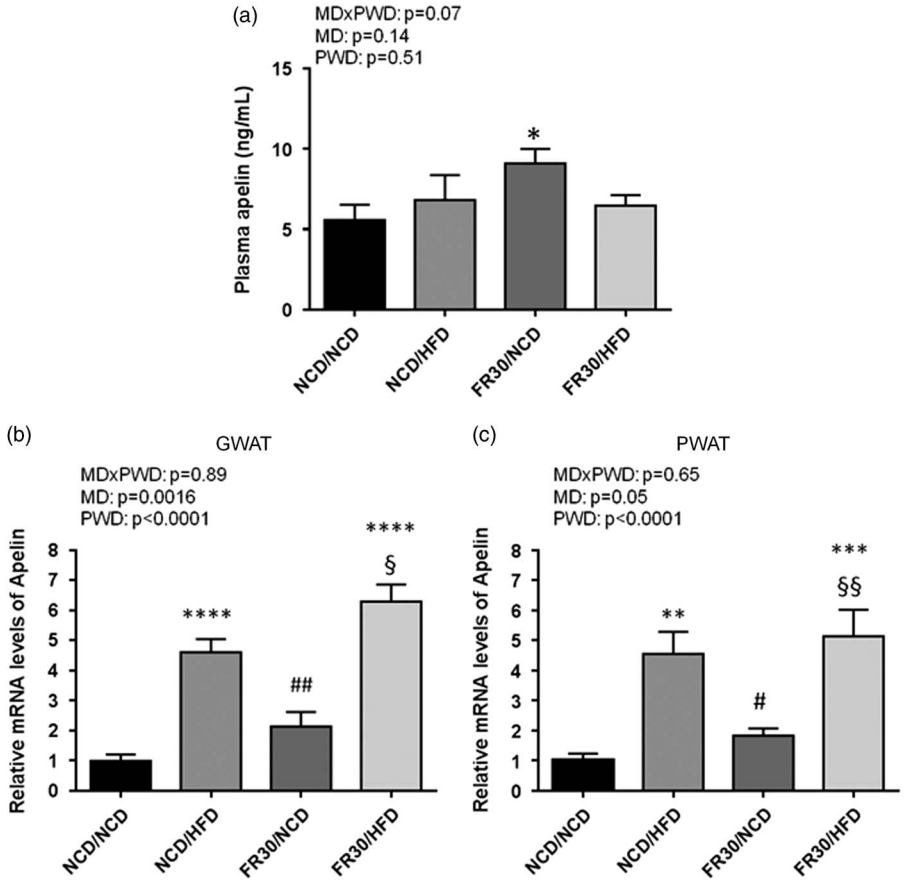

Fig. 1. Plasma apelin levels (*a*) and messenger RNA (mRNA) expression levels of apelin in gonadal white adipose tissue (GWAT) (*b*) and perirenal white adipose tissue (PWAT) (*c*) in adult NCD and FR30 rats under standard (NCD) and high-fat diet (HFD). Values are means ± S.E.M. ($n = 10$/group). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ *v.* NCD/NCD; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *v.* NCD/HFD; $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ *v.* FR30/NCD. MD, maternal diet; PWD, post-weaning diet; FR30, 70% food restriction.

https://doi.org/10.1017/S2040174416000702 Published online by Cambridge University Press

10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-Tris, 1 mM ethylenediaminetetraacetic acid, 1% Triton X-100, protease inhibitor cocktail 4-(2-aminoethyl)benzenesulfonyl fluoride, pepstatin A, E-64, bestatin, leupeptin and aprotinin, and phosphatase inhibitor cocktail (10 mM sodium fluoride, 1 mM sodium orthovanadate, 20 mM sodium β-glycerophosphate and 10 mM benzamidine). Protein concentrations were determined using a protein assay kit (Bio-Rad, France). Proteins were subjected to 8% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels and transferred onto nitrocellulose membranes. Blots were blocked with 5% bovine serum albumin (Sigma, France) and then incubated in the presence of appropriate primary antibodies (rabbit anti-rat APJ; Santa Cruz Biotechnology, Heidelberg, Germany) and secondary antibodies. Mouse anti-rat actin antibody (Sigma-Aldrich, Saint-Quentin-Fallavier, France) was used as a loading control. Following nitrocellulose membrane washing, targeted proteins (50 kDa for APJ, 42 kDa for actin) were revealed using enhanced chemiluminescence reagents (Amersham Life Science, Les Ulis, France) according to the manufacturer's recommendations. The intensity of bands was quantified by using Quantity One Bio-Rad and the APJ/actin ratios were calculated. All data are presented as means ± S.E.M. Statistical analysis was performed by two-way analysis of variance [maternal diet (MD) and post-weaning diet (PWD) as co-factors] followed by Tukey's *post-hoc* analysis. Mann–Whitney test was used for Western blot analysis. A P level <0.05 was considered as statistically significant.

Results

FR30/NCD rats had 1.6-fold higher plasma apelin levels compared with NCD/NCD animals. By contrast, no difference

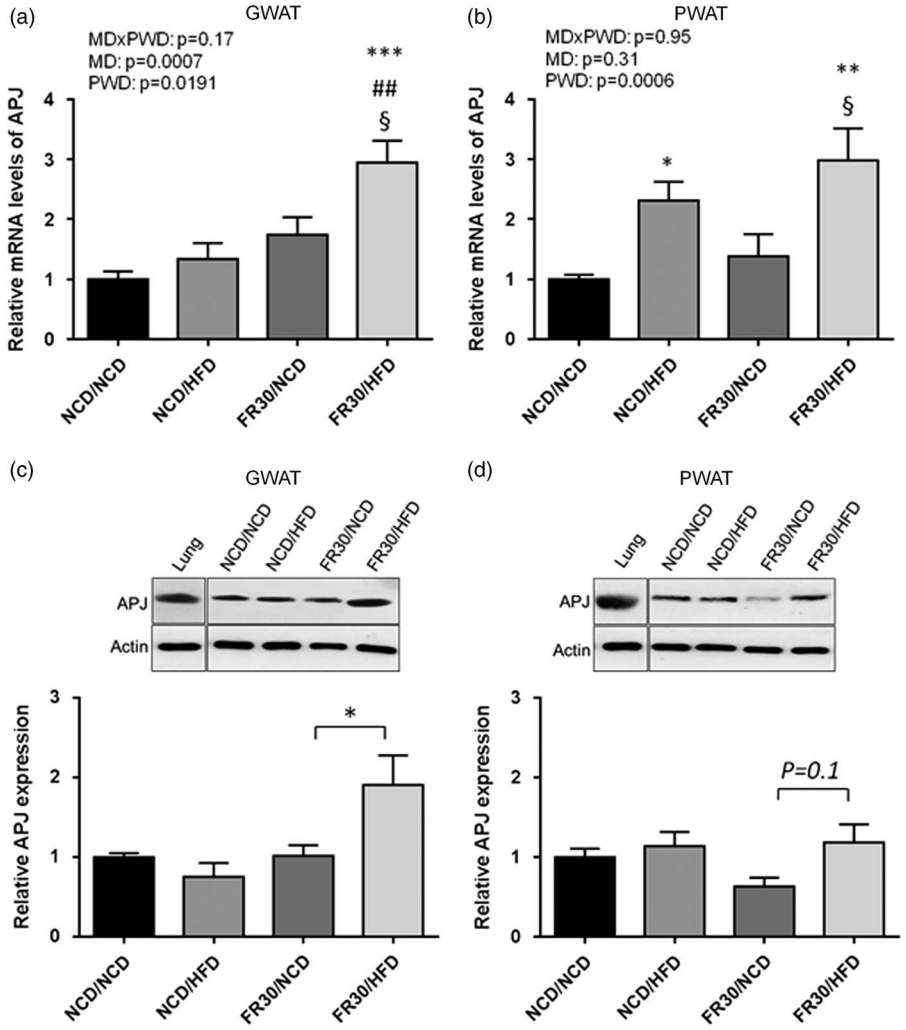

Fig. 2. Messenger RNA (mRNA) expression levels of APJ in gonadal white adipose tissue (GWAT) (*a*) and perirenal white adipose tissue (PWAT) (*b*) in adult NCD and FR30 rats under standard (NCD) and high-fat diet (HFD). Values are means ± S.E.M. (n = 10/group). $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ *v.* NCD/NCD; $^{\#\#}P<0.01$ *v.* NCD/HFD; $^{\$}P<0.05$ *v.* FR30/NCD. Western blot analysis of APJ in GWAT (*c*) and PWAT (*d*) protein extracts from adult NCD and FR30 rats under NCD or HFD. Representative Western blot of three similar analyses are shown. Lung was used as a positive control. Values are means ± S.E.M. (n = 4/group). $^{*}P<0.05$, NCD *v.* HFD in the same condition. MD, maternal diet; PWD, post-weaning diet; FR30, 70% food restriction.

https://doi.org/10.1017/S2040174416000702 Published online by Cambridge University Press

of plasma apelin levels was observed in FR30/HFD *v.* NCD/HFD rats (Fig. 1a). MD and PWD affected apelin gene expression in both fat depots of adult rat offspring. In GWAT (Fig. 1b) and PWAT (Fig. 1c), a tendency toward increased apelin mRNA levels was observed in FR30/NCD (1.9-fold and 1.8-fold, respectively) compared with NCD/NCD rats. This is consistent with increased levels of serum apelin in FR30/NCD animals (Fig. 1a). In GWAT and PWAT, apelin mRNA levels were increased to a similar extent in NCD/HFD *v.* NCD/NCD rats (4.6-fold and 4.5-fold, respectively) and FR30/HFD *v.* FR30/NCD rats (3.3-fold and 2.8-fold, respectively). MD and PWD affect APJ gene expression in GWAT (Fig. 2a), whereas only PWD affects APJ gene expression in PWAT (Fig. 2b) of adult rat offspring. In GWAT and PWAT, APJ mRNA levels were markedly increased in FR30/HFD *v.* FR30/NCD rats (1.7-fold and 2.2-fold, respectively). The increase of AJP protein expression in FR30/HFD *v.* FR30/NCD rats (1.9-fold) was confirmed by Western blot analysis in both fat depots (Fig. 2c and 2d).

Discussion

We have previously shown that maternal FR30 programs metabolic disorders in adult male rat offspring. In particular, they exhibited impaired glucose tolerance with a defect in glucose-induced insulin secretion.[4] Obesity-prone adult male rat offspring from FR30 dams displayed hyperleptinemia, adipocyte hypertrophy with changes in adipogenic gene expression levels (i.e. leptin/leptin receptor) in WAT, especially after post-weaning HFD.[5] Here, we demonstrate that offspring's apelinergic system is also altered as a consequence of maternal undernutrition. Indeed, adult male rat offspring exhibited hyperapelinemia as well as a tendency toward increased apelin mRNA levels in two different fat pads. Post-weaning HFD resulted in additional gene expression increases of genes in the apelinergic system in WAT, regardless of MD. However, a marked difference in the level of AJP protein was only observable in rat offspring from FR30 dams under HFD. Elevated plasma apelin levels may result, at least in part, from elevated WAT apelin release as has been reported in obese patients.[19] However, elevated plasma apelin may also originate from other tissues.[9] As observed in different HFD-fed rodent models of obesity and in type 2 diabetic and obese patients, the expression of apelin/APJ in WAT was positively correlated with fat mass and glucose intolerance.[11,12,14,21] Increased plasma apelin levels may participate in impairing glucose-stimulated insulin secretion observed in adult male rat offspring from undernourished dams.[4,22] In line with our previous observations,[5] it is tempting to speculate that the up-regulation of AJP in WAT of rat offspring from FR30 dams under HFD might represent an adaptive mechanism for limiting the development of WAT and fat accumulation. Indeed, apelin inhibits adipogenesis,[16,17] decreases lipid storage,[23] promotes browning in WAT and activates energy expenditure.[18] However, further experiments are needed to determine whether apelin sensitivity is modified in WAT of rat offspring from FR30 dams. Elevated leptin expression in adult mice from undernourished dams was shown to be correlated with hypomethylation of the leptin promoter in WAT.[24] Although the underlying programming mechanisms remain elusive, the up-regulation of apelin in WAT of rat offspring from FR30 dams might also result from altered epigenetic control as described in pulmonary edema.[25] Together, our data show that, in addition to leptin, apelin is another adipokine target of fetal programming in WAT of adult rat offspring from undernourished dams.

Acknowledgments

The authors thank Anne Dickes-Coopman and Charlène Pourpe for their excellent technical assistance.

Financial Support

This study was supported by grant from the French Ministry of Education.

Conflicts of Interest

None.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national guids on the care and use of laboratory animals (European Communities Council Directive of 1986, 86/609/EEC) and has been approved by the institutional committee (French Ministry of Agriculture No. 04860).

References

1. Barker DJP. Developmental origins of adult health and disease. J Epidemiol Community *Health*. 2004; 58, 114–115.
2. Ravelli AC, van Der Meulen JH, Osmond C, Barker DJ, Bleker OP. Obesity at the age of 50 y in men and women exposed to famine prenatally. *Am J Clin Nutr*. 1999; 70, 811–816.
3. Rivière G, Michaud A, Breton C, *et al*. Angiotensin-converting enzyme 2 (ACE2) and ACE activities display tissue-specific sensitivity to undernutrition-programmed hypertension in the adult rat. *Hypertension*. 2005; 46, 1169–1174.
4. Breton C, Lukaszewski M-A, Risold P-Y, *et al*. Maternal prenatal undernutrition alters the response of POMC neurons to energy status variation in adult male rat offspring. *Am J Physiol Endocrinol Metab*. 2009; 296, E462–E472.
5. Lukaszewski M-A, Mayeur S, Fajardy I, *et al*. Maternal prenatal undernutrition programs adipose tissue gene expression in adult male rat offspring under high-fat diet. *Am J Physiol Endocrinol Metab*. 2011; 301, E548–E559.
6. Breton C. The hypothalamus–adipose axis is a key target of developmental programming by maternal nutritional manipulation. *J Endocrinol*. 2013; 216, R19–R31.
7. Lukaszewski M-A, Eberlé D, Vieau D, Breton C. Nutritional manipulations in the perinatal period program adipose tissue in offspring. *Am J Physiol Endocrinol Metab*. 2013; 305, E1195–E1207.

8. Tatemoto K, Hosoya M, Habata Y, *et al*. Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun*. 1998; 251, 471–476.
9. Hosoya M, Kawamata Y, Fukusumi S, *et al*. Molecular and functional characteristics of APJ. Tissue distribution of mRNA and interaction with the endogenous ligand apelin. *J Biol Chem*. 2000; 275, 21061–21067.
10. Heinonen MV, Purhonen AK, Miettinen P, *et al*. Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. *Regul Pept*. 2005; 130, 7–13.
11. Soriguer F, Garrido-Sanchez L, Garcia-Serrano S, *et al*. Apelin levels are increased in morbidly obese subjects with type 2 diabetes mellitus. *Obes Surg*. 2009; 19, 1574–1580.
12. Dray C, Debard C, Jager J, *et al*. Apelin and APJ regulation in adipose tissue and skeletal muscle of type 2 diabetic mice and humans. *Am J Physiol Endocrinol Metab*. 2010; 298, E1161–E1169.
13. Boucher J, Masri B, Daviaud D, *et al*. Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology*. 2005; 146, 1764–1771.
14. Castan-Laurell I, Dray C, Knauf C, Kunduzova O, Valet P. Apelin, a promising target for type 2 diabetes treatment? *Trends Endocrinol Metab*. 2012; 23, 234–241.
15. Yue P, Jin H, Xu S, *et al*. Apelin decreases lipolysis via G(q), G(i), and AMPK-dependent mechanisms. *Endocrinology*. 2011; 152, 59–68.
16. Than A, Cheng Y, Foh LC, *et al*. Apelin inhibits adipogenesis and lipolysis through distinct molecular pathways. *Mol Cell Endocrinol*. 2012; 362, 227–241.
17. Zhu S, Cheng G, Zhu H, Guan G. A study of genes involved in adipocyte differentiation. *J Pediatr Endocrinol Metab*. 2015; 28, 93–99.
18. Than A, He HL, Chua SH, *et al*. Apelin enhances brown adipogenesis and browning of white adipocytes. *J Biol Chem*. 2015; 290, 14679–14691.
19. Chen H, Zheng C, Zhang X, *et al*. Apelin alleviates diabetes-associated endoplasmic reticulum stress in the pancreas of Akita mice. *Peptides*. 2011; 32, 1634–1639.
20. Dray C, Knauf C, Daviaud D, *et al*. Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. *Cell Metab*. 2008; 8, 437–445.
21. Butruille L, Drougard A, Knauf C, *et al*. The apelinergic system: sexual dimorphism and tissue-specific modulations by obesity and insulin resistance in female mice. *Peptides*. 2013; 46, 94–101.
22. Sörhede Winzell M, Magnusson C, Ahrén B. The apj receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice. *Regul Pept*. 2005; 131, 12–17.
23. Higuchi K, Masaki T, Gotoh K, *et al*. Apelin, an APJ receptor ligand, regulates body adiposity and favors the messenger ribonucleic acid expression of uncoupling proteins in mice. *Endocrinology*. 2007; 148, 2690–2697.
24. Jousse C, Parry L, Lambert-Langlais S, *et al*. Perinatal undernutrition affects the methylation and expression of the leptin gene in adults: implication for the understanding of metabolic syndrome. *FASEB J*. 2011; 25, 3271–3278.
25. Mishra A, Kohli S, Dua S, *et al*. Genetic differences and aberrant methylation in the apelin system predict the risk of high-altitude pulmonary edema. *Proc Natl Acad Sci U S A*. 2015; 112, 6134–6139.

https://doi.org/10.1017/S2040174416000702 Published online by Cambridge University Press