British Journal of Nutrition (2020), 124, 668–680
doi:10.1017/S0007114520001701

Comparison between Tibetan and Small-tailed Han sheep in adipocyte phenotype, lipid metabolism and energy homoeostasis regulation of adipose tissues when consuming diets of different energy levels

Xiaoping Jing[1,2], Jianwei Zhou[3]*, Allan Degen[4], Wenji Wang[1], Yamin Guo[3], Jingpeng Kang[3], Peipei Liu[1], Luming Ding[1], Zhanhuan Shang[1], Qiang Qiu[1], Xuezhi Ding[5] and Ruijun Long[1]*

[1]*State Key Laboratory of Grassland and Agro-Ecosystems, International Centre for Tibetan Plateau Ecosystem Management, School of Life Sciences, Lanzhou University, Lanzhou 730000, People's Republic of China*
[2]*Laboratory for Animal Nutrition and Animal Product Quality, Department of Animal Sciences and Aquatic Ecology, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium*
[3]*State Key Laboratory of Grassland and Agro-Ecosystems, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730020, People's Republic of China*
[4]*Desert Animal Adaptations and Husbandry, Wyler Department of Dryland Agriculture, Blaustein Institutes for Desert Research, Ben-Gurion University of Negev, Beer Sheva 8410500, Israel*
[5]*Lanzhou Institute of Animal Husbandry and Veterinary Medicine, Chinese Academy of Agriculture Sciences, Lanzhou 730050, People's Republic of China*

(Submitted 14 January 2020 – Final revision received 4 May 2020 – Accepted 6 May 2020 – First published online 14 May 2020)

Abstract

This study aimed to gain insight into how adipose tissue of Tibetan sheep regulates energy homoeostasis to cope with low energy intake under the harsh environment of the Qinghai-Tibetan Plateau (QTP). We compared Tibetan and Small-tailed Han sheep (*n* 24 of each breed), all wethers and 1·5 years of age, which were each divided randomly into four groups and offered diets of different digestible energy (DE) densities: 8·21, 9·33, 10·45 and 11·57 MJ DE/kg DM. When the sheep lost body mass and were assumed to be in negative energy balance: (1) adipocyte diameter in subcutaneous adipose tissue was smaller and decreased to a greater extent in Tibetan than in Small-tailed Han sheep, but the opposite occurred in the visceral adipose tissue; (2) Tibetan sheep showed higher insulin receptor mRNA expression and lower concentrations of catabolic hormones than Small-tailed Han sheep and (3) Tibetan sheep had lower capacity for glucose and fatty acid uptake than Small-tailed Han sheep. Moreover, Tibetan sheep had lower AMPK*α* mRNA expression but higher mammalian target of rapamycin mRNA expression in the adipocytes than Small-tailed Han sheep. We concluded that Tibetan sheep had lower catabolism but higher anabolism in adipose tissue and reduced the capacity for glucose and fatty acid uptake to a greater extent than Small-tailed Han sheep to maintain energy homoeostasis when in negative energy balance. These responses provide Tibetan sheep with a high ability to cope with low energy intake and with the harsh environment of the QTP.

Key words: Tibetan sheep: Small-tailed Han sheep: Adipocyte phenotypes: Lipid metabolism and energy homoeostasis regulation

The Qinghai-Tibetan Plateau (QTP), known as the world's 'Third Pole', is characterised by an extremely harsh environment and its sensitivity to global climate change(1,2). The QTP covers about 2·5 million km^2, a quarter of China, of which 64 % is alpine grassland, and, therefore, makes it pivotal for ecosystem services and ecological security(2). Tibetan sheep (*Ovis aries*) with the largest number of livestock in this area have been raised on the QTP for thousands of years and even have thrived under the harsh conditions. These sheep play a vital role in the livelihoods of Tibetan pastoralists and the QTP ecosystem, as well as

---

Abbreviations: ACC1, acetyl-CoA carboxylase 1; AMPKα, AMP-activated protein kinase-α; BW, body weight; CPT1, carnitine palmitoyl-transferase-1; FABP4, fatty acid binding protein 4; FAS, fatty acid synthase; GPAT, glycerol-3-phosphate acyltransferase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; mTOR, mammalian target of rapamycin; QTP, Qinghai-Tibetan Plateau.

* **Corresponding authors:** Jianwei Zhou, email zhoujw@lzu.edu.cn; Ruijun Long, email longrj@lzu.edu.cn

in the maintenance of Tibetan culture. Furthermore, Tibetan sheep are important for the development of the economy in the QTP, especially in the western region of Tibet, where they comprise up to 96 % of the livestock(3). Under traditional management, Tibetan sheep graze on rangeland all year round without receiving supplementary feed. However, the biomass and nutrients of the forage vary greatly among seasons and are often well below maintenance requirements for grazing livestock in the winter(4,5). Consequently, during the long, cold season, they face enormous challenges of negative energy balance and can lose 40 % of their body weight (BW)(6). However, Tibetan sheep have evolved adaptive mechanisms for the harsh environment of the QTP(7–9).

Adipose tissue is the largest and most effective energy store in mammals, and adipocytes are able to change in size substantially to conform to changes in energy needs of the body(10). This tissue plays a central role in the regulation of energy metabolism and maintenance of whole-body energy homoeostasis through lipolysis or lipogenesis. In addition, adipocytes maintain energy homoeostasis by secreting adipokines in response to hormonal signals(10–12). The energy intake of Tibetan sheep varies greatly with seasonal forage fluctuations and can be extremely low for extended periods in the cold season. However, limited information is available on how adipose tissue in Tibetan sheep regulates energy homoeostasis when consuming low-energy diets and, consequently, the aim of this study, at least in part, was to fill this gap.

For this purpose, we compared Tibetan sheep with Small-tailed Han sheep, which were introduced to the QTP due to their high lamb production and are raised in feedlots and graze natural pasture only in summer when the forage is of good quality(13). Earlier studies have reported that Tibetan sheep have lower maintenance energy requirements, higher nutrient digestibility and greater average daily gain than Small-tailed Han sheep when receiving the same diet(9). Based on their different backgrounds and energy requirements, we hypothesised that Tibetan and Small-tailed Han sheep would differ in their lipid metabolism in adipose tissues to maintain energy homoeostasis and predicted that the differences would allow Tibetan sheep to cope better with low energy intake than Small-tailed Han sheep. The morphology of adipocytes, the pre-prandial serum concentrations of hormones and adipokines, the activity of the key enzymes of lipid metabolism and the mRNA expression of regulatory genes of energy homoeostasis in adipose tissues were examined and compared between Tibetan sheep and Small-tailed Han sheep when offered diets of different energy levels.

Materials and methods

The protocol in this study was approved by the Institutional Animal Care and Use Committee of Lanzhou University. The study was done at the Yak and Tibetan Sheep Research Station of Lanzhou University, Tianzhu Tibetan Autonomous County, Gansu Province, north-eastern QTP, China, during October and December 2016. Average air temperature and relative humidity during the study were 6°C and 76 %, respectively.

Animals and diets

The experimental design was described previously(9). Briefly, twenty-four Tibetan sheep (BW = 48·5 (SD 1·89) kg) and twenty-four Small-tailed Han sheep (BW = 49·2 (SD 2·21) kg) were used, all wethers aged 1·5 years. Sheep of each breed were divided randomly into one of four groups (six sheep/group per breed) and received a diet at 4·5 % of BW$^{0.75}$ per d DM yielding a digestible energy density of: 8·21, 9·33, 10·45 or 11·57 MJ/kg DM (online Supplementary Table S1), which were 0·8, 0·9, 1·0 and 1·1 times maintenance digestible energy requirements, respectively, according to the Feeding Standard of Meat-producing Sheep and Goats of China, NY/T 816-2004(14). The number of sheep was based on effect size index (*d*-value) which was calculated using estimated standard deviations of the means of measured variables from previous similar studies. A sample size of six per group per breed resulted in a *d*-value close to 0·5, which is a medium and acceptable effect size(15). The diets all contained approximately 70 g/kg crude protein, which was similar to the average crude protein content in forage of the QTP during the cold season(16). The sheep were maintained under a three-sided roofed shelter and penned individually in a 1·5 × 2·5 m pen that had a sand floor and that was equipped with a water tank and a feed trough. They were allowed 14 d to adapt to the conditions, which was followed by a 42-d feeding period in which diets were offered in two equal portions at 08.00 and at 18.00 hours, and feed intake was measured. Sheep were weighed every 2 weeks before morning feeding, and average daily gain was calculated. By design, a large range of energy intakes were offered so that the sheep lost, maintained or gained body mass (online Supplementary Fig. S1). We assumed that a change in body mass inferred a change in energy balance in the same direction. This allowed comparisons between sheep breeds and also within sheep breeds at different stages of energy balance, that is, the response of sheep losing energy compared with sheep in energy balance.

Sampling procedures

Jugular vein blood samples (15 ml) were collected before feeding on the morning of day 42, and serum was removed after centrifuging at 2000 ***g*** for 15 min and stored at –80°C for determination of concentrations of hormones and adipokines. After the feeding period, the sheep were slaughtered humanely 2–3 h after morning feeding(17). Subsequently, subcutaneous (abdomen) and visceral (omentum) adipose tissue samples were collected within 20 min, cut into small pieces, placed into 1·5 ml tubes (Eppendorf, GCS), snap-frozen in liquid N_2 and stored at –80°C for determination of enzymes activity and mRNA expression. Additional three samples from each of the subcutaneous and visceral adipose tissues were collected from each sheep and fixed in 4 % (v/v) paraformaldehyde solution for adipocyte morphology determination.

Serum hormones and adipokines determination

Serum concentrations of insulin, glucocorticoid, adrenaline and noradrenaline were measured with ELISA kits (Ovis, Shanghai

Bangyi Bioscience Co. Ltd), as were the serum concentrations of leptin, IL-6, TNF-α, adiponectin and resistin.

Adipocytes morphology measurements

The fixed subcutaneous and visceral adipose tissue samples were embedded in paraffin, blocks were cut into 5 µm sections using a rotary microtome (RM2235, Leica) and the sections (four slices of each sample) were stained by haematoxylin–eosin. Slides were viewed using a digital microscope (Olympus DP2-BSW) with a 20× objective lens. Adipocyte diameter was determined by Image-Pro Plus 6.0 software (Media Cybernetics Inc.).

Enzyme activity determination

Both subcutaneous and visceral adipose tissues were homogenised with 9 ml tissue homogenisation buffer (pH 7·4) per g tissue, then centrifuged at 1000 ***g*** for 10 min at 4°C to remove debris. The activities of hormone-sensitive lipase (HSL), carnitine palmitoyl-transferase-1 (CPT1), acetyl-CoA carboxylase 1 (ACC1), fatty acid synthase (FAS) and glycerol-3-phosphate acyltransferase (GPAT) were determined in the supernatant of tissue homogenates using commercial ELISA kits (Ovis, Shanghai Bangyi Bioscience Co. Ltd).

RNA extraction and mRNA expression determination

Both subcutaneous and visceral adipose tissues were ground using a sterilised mortar with liquid N_2. Total RNA was then isolated and used to generate complementary DNA (cDNA) by a Prime Script® RT Reagent Kit (Takara), and the cDNA was amplified by real-time PCR using an SYBR Green real-time PCR master mix kit (Takara) with the Agilent StrataGene Mx3000P (Agilent Technologies Inc.), as previously described(18). Primers are presented in online Supplementary Table S2. β-Actin was selected as a housekeeping gene. The oligonucleotides were synthesised by Takara Biotechnology Co. Ltd. Relative gene mRNA expression levels are presented as $2^{-\Delta\Delta Ct}$(19).

Statistical analyses

Data were analysed using the mixed model with Tukey-adjusted P values of the SAS statistical package (SAS version 9.4, SAS Institute Inc.) as the following model: $Y = \mu + E + B + (E \times B) + e$, where Y is the dependent variable, μ is the overall mean, E is the effect of dietary energy level, B is the effect of sheep breed, $E \times B$ is the interaction between sheep breed and dietary energy level and e is the residual error. Sheep breed and dietary energy level were fixed effects, and the experimental animals were random effects. The effect of dietary energy level was determined using polynomial contrasts to determine whether the effect was linear, quadratic or cubic with an increase in dietary intake. When there was a significant interaction between dietary energy level and breed, differences between breeds at the same dietary energy level were separated using t tests(8). Differences were considered significant at $P < 0{\cdot}05$.

Results

Daily DM intake and body weight change

Daily DM intakes and average daily gain of the sheep were reported previously(9). The digestible energy intake, by design, increased linearly ($P < 0{\cdot}001$) with an increase in dietary energy level, but was similar between breeds (online Supplementary Table S3; $P > 0{\cdot}05$). Average daily gain was significantly greater in Tibetan than Small-tailed Han sheep across treatments (linear dietary energy level × breed, $P = 0{\cdot}003$) and increased linearly ($P < 0{\cdot}001$) in both breeds with an increase in dietary energy level. Small-tailed Han sheep lost BW at dietary energy intakes of 8·21 and 9·33 MJ/kg, but Tibetan sheep lost BW only at dietary energy intake of 8·21 MJ/kg (online Supplementary Fig. S1).

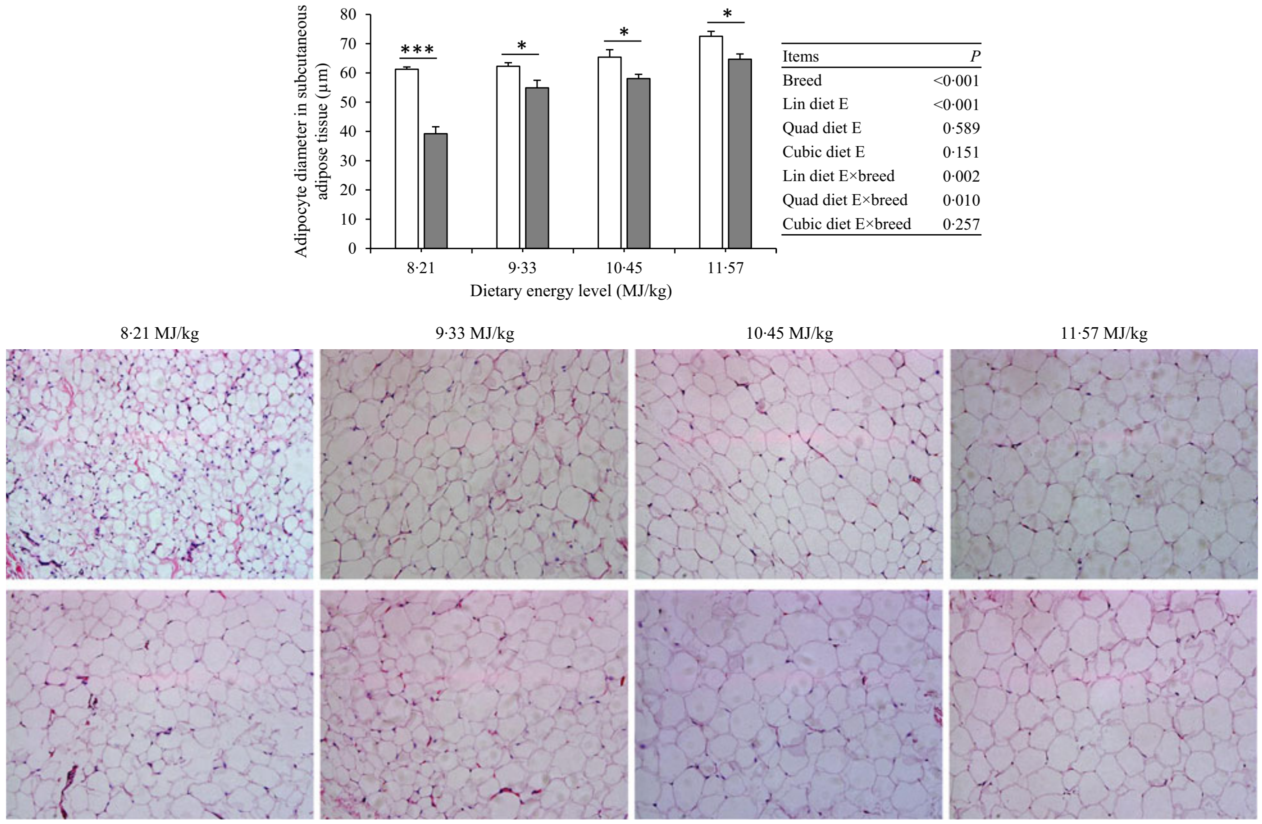
Morphology of adipocytes

In the subcutaneous adipose tissue, the adipocyte diameter increased linearly with an increase in dietary energy level ($P < 0{\cdot}001$; Fig. 1) and was greater in Small-tailed Han than in Tibetan sheep (linear dietary energy level × breed, $P = 0{\cdot}002$). In the visceral adipose tissue, the adipocyte diameter also increased linearly with an increase in dietary energy level ($P < 0{\cdot}001$; Fig. 2), however, was greater in Tibetan than in Small-tailed Han sheep ($P < 0{\cdot}001$).

Activities of key enzymes of lipid metabolism in adipose tissues

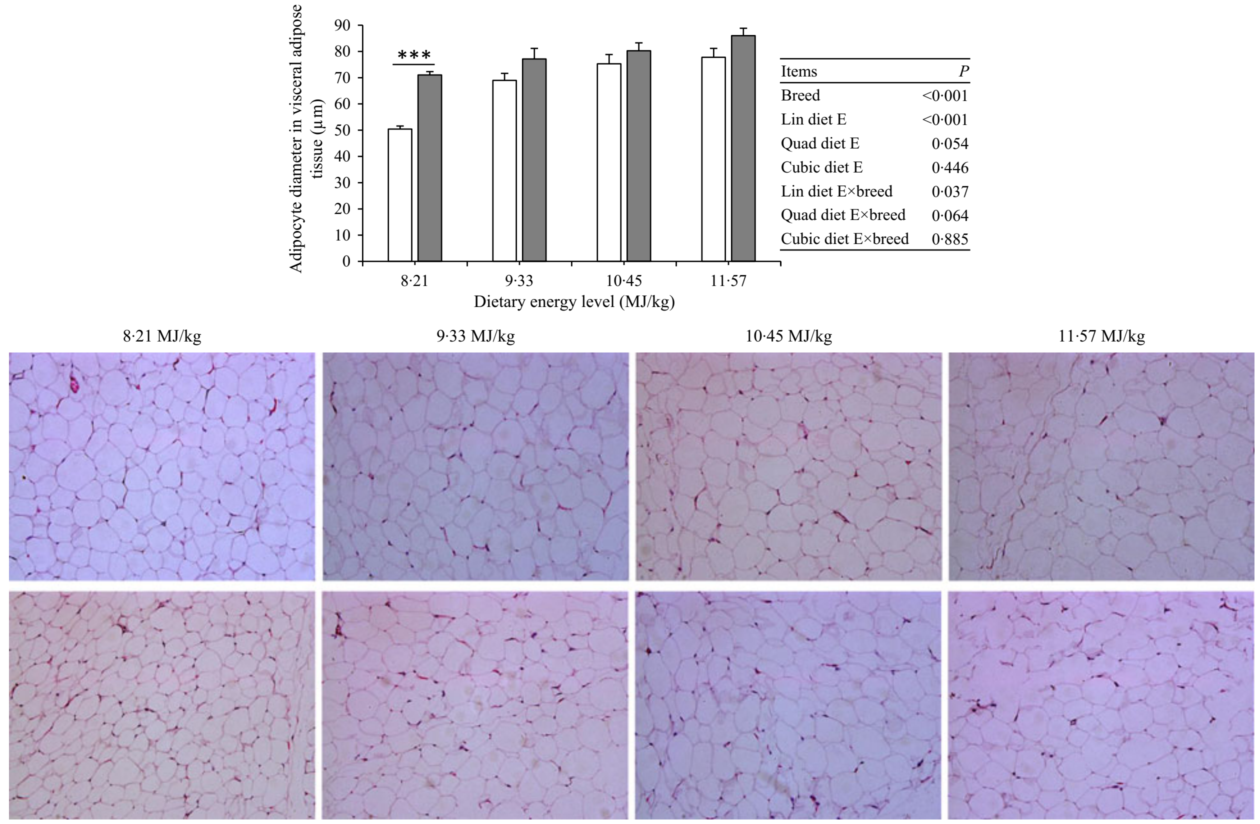
The activities of key enzymes of fatty acid metabolism are presented in Tables 1 and 2. In comparing the key enzymes of lipolysis in subcutaneous adipose tissue, HSL activity was higher in Tibetan than in Small-tailed Han sheep at the three lowest dietary energy levels (8·21, 9·33 and 10·45 MJ/kg; linear dietary energy level × breed, $P = 0{\cdot}002$; Table 1) and CPT1 activity decreased linearly with an increase in dietary energy level ($P < 0{\cdot}001$) and was higher in Tibetan than in Small-tailed Han sheep ($P = 0{\cdot}007$). The activities of ACC1 and FAS, the key enzymes of fatty acid synthesis, decreased quadratically with an increase in dietary energy level ($P < 0{\cdot}001$) and were higher in Tibetan than in Small-tailed Han sheep ($P < 0{\cdot}001$). The activity of GPAT, the key enzyme of esterification, did not differ between breeds ($P > 0{\cdot}05$).

In the visceral adipose tissue, HSL activity was higher in Small-tailed Han than in Tibetan sheep ($P = 0{\cdot}002$; Table 2), while the CPT1 activity was also higher in Small-tailed Han than in Tibetan sheep but only at the three lowest dietary energy levels (quadratic dietary energy level × breed, $P = 0{\cdot}003$). The activity of ACC1 decreased quadratically with an increase in dietary energy level ($P < 0{\cdot}001$) and was higher in Small-tailed Han than in Tibetan sheep at the two lowest dietary energy levels (linear dietary energy level × breed, $P = 0{\cdot}003$), while the activity of FAS also decreased quadratically with an increase in dietary energy level ($P = 0{\cdot}001$) and was higher in Small-tailed Han than in Tibetan sheep ($P < 0{\cdot}001$). The activity of GPAT was higher in Small-tailed Han than in Tibetan sheep at the two highest dietary energy levels (linear dietary energy level × breed, $P = 0{\cdot}012$).



Items	*P*
Breed	<0·001
Lin diet E	<0·001
Quad diet E	0·589
Cubic diet E	0·151
Lin diet E×breed	0·002
Quad diet E×breed	0·010
Cubic diet E×breed	0·257

Fig. 1. Morphology of the subcutaneous adipose tissue in Tibetan (T, ■) and Small-tailed Han (H, □) sheep offered diets of different energy densities. The dietary energy levels are digestible energy on a DM basis. Values are means with their standard errors. Magnification 200×. Lin, linear; Quad, quadratic. * $P < 0{\cdot}05$; *** $P < 0{\cdot}001$.



Items	*P*
Breed	<0·001
Lin diet E	<0·001
Quad diet E	0·054
Cubic diet E	0·446
Lin diet E×breed	0·037
Quad diet E×breed	0·064
Cubic diet E×breed	0·885

Fig. 2. Morphology of the visceral adipose tissue in Tibetan (T, ■) and Small-tailed Han (H, □) sheep offered diets of different energy densities. The dietary energy levels are digestible energy on a DM basis. Values are means with their standard errors. Magnification 200×. Lin, linear; Quad, quadratic. *** $P < 0{\cdot}001$.

Table 1. Key enzyme activities of fatty acid metabolism in the subcutaneous adipose tissue of Tibetan (T) and Small-tailed Han (H) sheep offered diets of different energy densities
(Mean values with their pooled standard errors)

		Dietary energy level (MJ/kg)*					*P*			
Items	Breed	8·21	9·33	10·45	11·57	SE	Breed	E-L	E-Q	E-C
HSL (U/l)	T	417^{a}	386^{a}	387^{a}	422	10·4	<0·001	<0·001	<0·001	0·239
	H	358^{b}	335^{b}	337^{b}	439			0·002†	0·056†	0·253†
CPT1 (U/ml)	T	131	127	107	98·3	5·97	0·007	<0·001	0·992	0·314
	H	113	107	99·4	98·5			0·062†	0·565†	0·617†
ACC1 (U/ml)	T	194^{a}	164	196^{a}	236^{a}	6·9	<0·001	0·018	<0·001	0·526
	H	168^{b}	158	146^{b}	156^{b}			<0·001†	0·014†	0·076†
FAS (×10 U/ml)	T	260^{a}	251	259	295	6·9	<0·001	<0·001	<0·001	0·102
	H	225^{b}	238	236	279			0·318†	0·443†	0·290†
GPAT (U/l)	T	768	823	732	795	40·2	0·128	0·943	0·317	0·035
	H	750	748	667	774			0·990†	0·380†	0·900†

E-L, linear effect of dietary energy level; E-Q, quadratic effect of dietary energy level; E-C, cubic effect of dietary energy level; HSL, hormone-sensitive lipase; CPT1, carnitine palmitoyl-transferase-1; ACC1, acetyl-CoA carboxylase; FAS, fatty acid synthase; GPAT, glycerol-3-phosphate acyltransferase.
^{a,b} Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
* Digestible energy on a DM basis.
† *P* value for the interaction of dietary energy level effect with breeds.

Table 2. Key enzyme activities of fatty acid metabolism in the visceral adipose tissue of Tibetan (T) and Small-tailed Han (H) sheep offered diets of different energy densities
(Mean values with their pooled standard errors)

		Dietary energy level (MJ/kg)*					*P*			
Items	Breed	8·21	9·33	10·45	11·57	SE	Breed	E-L	E-Q	E-C
HSL (U/l)	T	357	323	364	417	9·1	0·002	<0·001	<0·001	0·028
	H	390	338	378	442			0·691†	0·272†	0·933†
CPT1 (U/ml)	T	112^{b}	117^{b}	123^{b}	126	4·2	<0·001	0·046	0·003	0·030
	H	123^{a}	133^{a}	150^{a}	121			0·192†	0·003†	0·073†
ACC1 (U/ml)	T	155^{b}	137^{b}	170	189	5·3	<0·001	<0·001	<0·001	0·007
	H	190^{a}	156^{a}	169	193			0·003†	0·148†	0·430†
FAS (×10 U/ml)	T	255	237	248	252	6·1	<0·001	0·064	0·001	0·057
	H	289	252	257	262			0·057†	0·270†	0·860†
GPAT (U/l)	T	674	800	738^{b}	725^{b}	35·8	<0·001	0·002	0·011	0·508
	H	722	845	941^{a}	924^{a}			0·012†	0·986†	0·166†

E-L, linear effect of dietary energy level; E-Q, quadratic effect of dietary energy level; E-C, cubic effect of dietary energy level; HSL, hormone-sensitive lipase; CPT1, carnitine palmitoyl-transferase-1; ACC1, acetyl-CoA carboxylase; FAS, fatty acid synthase; GPAT, glycerol-3-phosphate acyltransferase.
^{a,b} Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).
* Digestible energy on a DM basis.
† *P* value for the interaction of dietary energy level effect with breeds.

Serum concentrations of hormones and adipokines

The pre-prandial serum concentration of insulin decreased quadratically with an increase in dietary energy level ($P = 0{\cdot}003$; Table 3) and was higher in Small-tailed Han than in Tibetan sheep at the three lowest dietary energy levels (quadratic dietary energy level × breed, $P = 0{\cdot}015$), whereas the concentrations of adrenaline and noradrenaline increased linearly with an increase in dietary energy level ($P < 0{\cdot}01$) and was higher in Small-tailed Han than in Tibetan sheep at the two lowest dietary energy levels (8·21 and 9·33 MJ/kg; linear dietary energy level × breed, $P < 0{\cdot}01$). The glucocorticoid concentration was also higher in Small-tailed Han than in Tibetan sheep ($P < 0{\cdot}001$).

The serum concentration of leptin decreased quadratically with an increase in dietary energy level ($P = 0{\cdot}002$; Table 4) and was higher in Small-tailed Han than in Tibetan sheep ($P < 0{\cdot}001$). The concentration of IL-6 was also higher in Small-tailed Han than in Tibetan sheep (breed, $P < 0{\cdot}001$), whereas the TNF-α concentration was higher in Tibetan than in Small-tailed Han sheep at the lowest dietary energy level but was lower in Tibetan than in Small-tailed Han sheep at dietary energy levels of 9·33 and 11·57 MJ/kg (linear dietary energy level × breed, $P = 0{\cdot}001$). Resistin concentration increased linearly with an increase in dietary energy level ($P < 0{\cdot}001$) and was higher in Tibetan than in Small-tailed Han sheep at the lowest dietary energy level, but was lower in Tibetan than in Small-tailed Han sheep at the two highest energy levels (linear dietary energy level × breed, $P < 0{\cdot}001$). The concentration of adiponectin decreased quadratically with an increase in dietary energy level ($P < 0{\cdot}001$) and was higher in Tibetan sheep than in Small-tailed Han sheep ($P < 0{\cdot}001$).

Table 3. Concentrations of pre-prandial serum hormone in Tibetan (T) and Small-tailed Han (H) sheep offered diets of different energy densities (Mean values with their pooled standard errors)

		Dietary energy level (MJ/kg DM)*					*P*			
Items	Breed	8·21	9·33	10·45	11·57	SE	Breed	E-L	E-Q	E-C
Insulin (µIU/ml)	T	23·9^{b}	20·2^{b}	24·2^{b}	27·1	1·09	<0·001	0·218	0·003	0·230
	H	30·9^{a}	28·6^{a}	28·1^{a}	29·5			0·015†	0·355†	0·213†
Glucocorticoid (ng/ml)	T	8·26	9·86	9·43	6·72^{b}	0·727	<0·001	0·881	0·026	0·946
	H	9·51	10·5	10·9	11·3^{a}			0·027†	0·082†	0·859†
Adrenaline (ng/ml)	T	2·16^{b}	2·92^{b}	3·90^{b}	6·36	0·454	<0·001	<0·001	0·323	0·985
	H	5·98^{a}	6·11^{a}	6·70^{a}	6·42			<0·001†	0·112†	0·371†
Noradrenaline (ng/ml)	T	3·33^{b}	3·32^{b}	2·85	3·43	0·198	<0·001	0·003	0·012	0·018
	H	4·75^{a}	4·22^{a}	3·30	3·70			0·005†	0·553†	0·861†

E-L, linear effect of dietary energy level; E-Q, quadratic effect of dietary energy level; E-C, cubic effect of dietary energy level.

[a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

* Digestible energy on a DM basis.

† *P* value for the interaction of dietary energy level × breed.

Table 4. Concentrations of pre-prandial serum adipokines in Tibetan (T) and Small-tailed Han (H) sheep offered diets of different energy densities (Mean values with their pooled standard errors)

		Dietary energy level (MJ/kg DM)*					*P*			
Items	Breed	8·21	9·33	10·45	11·57	SE	Breed	E-L	E-Q	E-C
Leptin (ng/ml)	T	10·4	9·89	10·4	10·9	0·360	<0·001	0·022	0·002	0·038
	H	11·9	10·3	11·8	12·6			0·509†	0·183†	0·220†
IL-6 (pg/ml)	T	38·4^{b}	49·8^{b}	59·3	71·2^{b}	3·53	<0·001	<0·001	0·003	0·886
	H	74·5^{a}	61·0^{a}	64·5	84·0^{a}			0·002†	0·003†	0·814†
TNF-α (pg/ml)	T	351^{a}	296^{b}	324	319^{b}	6·9	0·070	0·566	0·028	0·008
	H	316^{b}	327^{a}	339	344^{a}			0·001†	0·008†	0·023†
Resistin (µg/l)	T	9·90^{a}	9·43	9·52^{b}	11·1^{b}	0·380	0·010	<0·001	0·215	0·769
	H	7·97^{b}	9·76	12·0^{a}	13·2^{a}			<0·001†	0·020†	0·304†
Adiponectin (µg/ml)	T	4·36	4·34	3·80	4·52	0·131	<0·001	0·400	<0·001	0·009
	H	4·18	3·60	3·37	4·04			0·480†	0·178†	0·160†

E-L, linear effect of dietary energy level; E-Q, quadratic effect of dietary energy level; E-C, cubic effect of dietary energy level.

[a,b] Mean values within a column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

* Digestible energy on a DM basis.

† *P* value for the interaction of dietary energy level effect with breeds.

Expression of regulatory genes of lipid metabolism and energy homoeostasis in adipose tissues

Insulin receptor mRNA expression was higher in Tibetan than in Small-tailed Han sheep in both subcutaneous and visceral adipose tissues ($P < 0{\cdot}001$; Fig. 3). The GLUT4 mRNA expression was higher in Small-tailed Han than in Tibetan sheep in both subcutaneous and visceral adipose tissues at the lowest dietary energy level (linear dietary energy level × breed, $P < 0{\cdot}001$; Fig. 4); and fatty acid binding protein 4 (FABP4) mRNA expression was higher in Small-tailed Han than in Tibetan sheep in both subcutaneous and visceral adipose tissues ($P < 0{\cdot}001$; Fig. 5(a) and (c)). However, the lipoprotein lipase (LPL) mRNA expression in Tibetan sheep was higher than in Small-tailed Han sheep at the three highest dietary energy levels (9·33, 10·45 and 11·57 MJ/kg) but was lower than in Small-tailed Han sheep at the lowest dietary energy level (8·21 MJ/kg) in both subcutaneous and visceral adipose tissues (linear dietary energy level × breed, $P < 0{\cdot}001$; Fig. 5(b) and (d)).

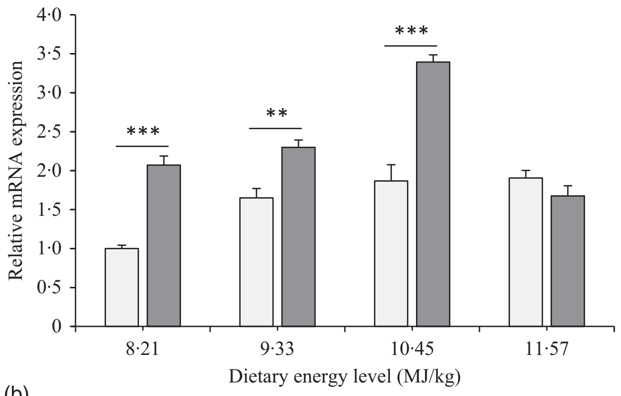
The PPARγ mRNA expression was higher in Tibetan than in Small-tailed Han sheep in the subcutaneous adipose tissue; however, it was higher in Small-tailed Han than in Tibetan sheep in the visceral adipose tissue ($P < 0{\cdot}05$; Fig. 6(a) and (b)). The AMP-activated protein kinase-α (AMPKα) mRNA expression was higher in Small-tailed Han than in Tibetan sheep in both subcutaneous and visceral adipose tissues ($P < 0{\cdot}001$; Fig. 6(c) and (d)); however, the mammalian target of rapamycin (mTOR) mRNA expression was higher in Tibetan than in Small-tailed Han sheep in both subcutaneous and visceral adipose tissues ($P < 0{\cdot}001$; Fig. 6(e) and (f)).

Discussion

Understanding how Tibetan sheep thrive under harsh conditions is important for improvement in production and management strategy decisions. The energy intake of Tibetan sheep varies greatly among seasons and is often well below maintenance requirements in the winter. In the present study, the diets of different energy densities included below and above energy requirement levels, as often occurs on the QTP. Soyabean oil and maize starch were used to increase dietary energy density by adding them together. Soyabean oil was chosen as a source of lipids because of its relatively high unsaturated fatty acids, as is generally the case of the pasture consumed by Tibetan sheep on the QTP(20). Additionally, the contents of both unsaturated fatty acids and carbohydrates of the pasture on the QTP increase and decrease synchronously in the warm and cold seasons,

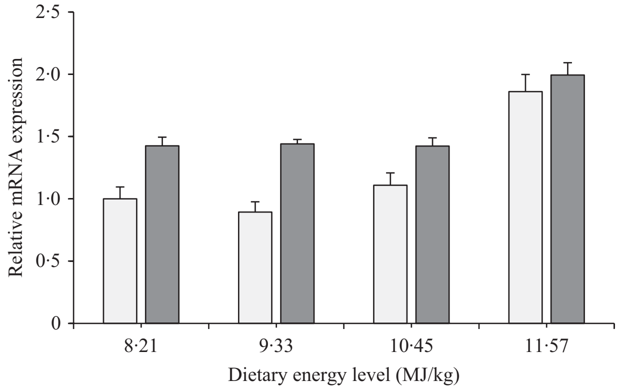
(a) Insulin Receptor

Subcutaneous adipose



Items	*P*
Breed	<0·001
Lin diet E	0·001
Quad diet E	<0·001
Cubic diet E	<0·001
Lin diet E×breed	<0·001
Quad diet E×breed	<0·001
Cubic diet E×breed	<0·001

(b) Visceral adipose



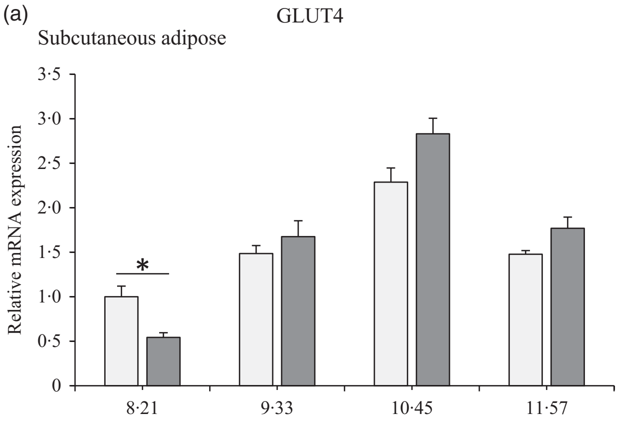
Items	*P*
Breed	<0·001
Lin diet E	<0·001
Quad diet E	<0·001
Cubic diet E	0·156
Lin diet E×breed	0·064
Quad diet E×breed	0·246
Cubic diet E×breed	0·485

Fig. 3. Expression of insulin receptor mRNA in the subcutaneous and visceral adipose tissues of Tibetan (T, ▨) and Small-tailed Han (H, ☐) sheep offered diets of different energy densities. The dietary energy levels are digestible energy on a DM basis. Values are means with their standard errors. Lin, linear; Quad, quadratic. ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$.

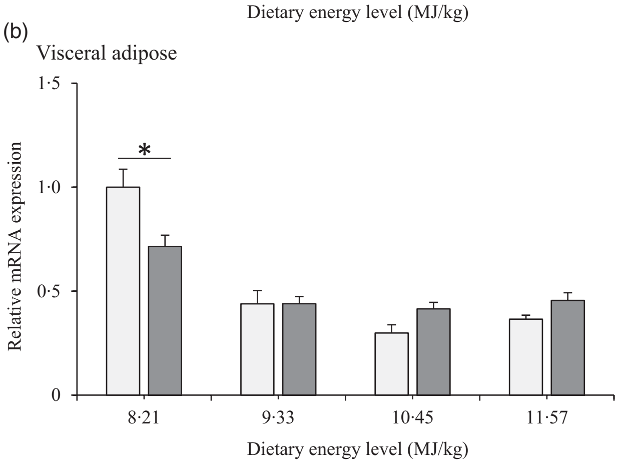
respectively(20,21). In the present study, lipid metabolism and energy homoeostasis regulation in adipose tissues were examined and compared between Tibetan sheep and Small-tailed Han sheep when offered diets of different energy levels in order to investigate how the adipose tissue in Tibetan sheep regulates energy homoeostasis when consuming low-energy diets.

Body weight and adipocyte phenotype of Tibetan and Small-tailed Han sheep

BW change is a strong indicator of body energy balance in animals. In the present study, at the two highest dietary energy levels, both Tibetan and Small-tailed Han sheep gained BW which inferred that they were in positive energy balance, but both lost BW at the lowest dietary energy level which inferred that they were in negative energy balance. Adipocytes store excess energy in the form of TAG, which can hydrolyse into NEFA and glycerol to supply energy when needed, and in this way maintain energy homoeostasis(12). Thus, adipocytes change in size according to energy status of the animal(10,12). In the present study, the size of the adipocytes increased linearly with an increase in dietary energy level in both subcutaneous and visceral adipose tissues in both Tibetan and Small-tailed Han sheep, which was consistent with their BW changes. The adipocyte diameter in the subcutaneous adipose tissue was larger in Small-tailed Han than in Tibetan sheep, but the visceral adipose tissue was smaller in Small-tailed Han than in Tibetan sheep. When in negative energy balance, the diameter of adipocytes in subcutaneous adipose tissue decreased to a greater extent in Tibetan than in Small-tailed Han sheep but the reverse response occurred in the visceral adipose tissue. This suggested that during negative energy balance, Tibetan sheep mobilised subcutaneous adipose tissue preferentially, but Small-tailed Han sheep mobilised visceral adipose tissue preferentially. This premise was supported by the greater activity of the key enzymes of lipolysis (HSL and CPT1) in Tibetan than in Small-tailed Han sheep in subcutaneous adipose tissue and by the greater activity of these enzymes in Small-tailed Han than in Tibetan sheep in visceral adipose tissue. Moreover, greater activities of key enzymes of fatty acid synthesis, ACC1 and FAS, occurred in the subcutaneous adipose tissue in Tibetan sheep, but, in the visceral adipose tissue in Small-tailed Han sheep. This suggested a higher metabolic activity of fatty acid synthesis in subcutaneous adipose tissue in Tibetan sheep, but in visceral adipose tissue in Small-tailed Han sheep.

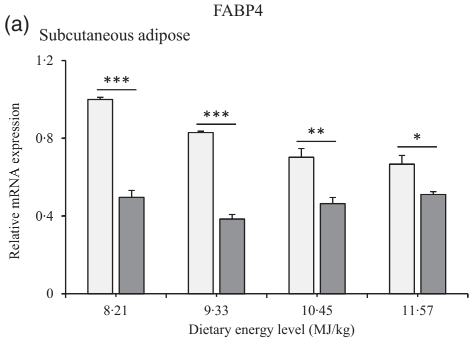


Items	*P*
Breed	0·128
Lin diet E	<0·001
Quad diet E	<0·001
Cubic diet E	<0·001
Lin diet E×breed	0·004
Quad diet E×breed	0·020
Cubic diet E×breed	0·703

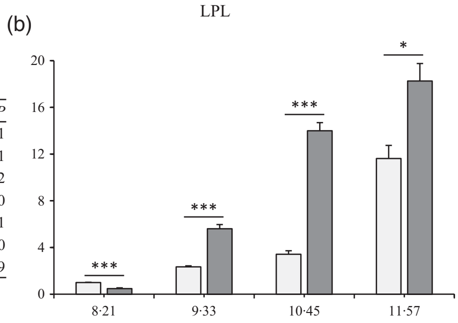


Items	*P*
Breed	0·584
Lin diet E	<0·001
Quad diet E	<0·001
Cubic diet E	0·218
Lin diet E×breed	<0·001
Quad diet E×breed	0·037
Cubic diet E×breed	0·927

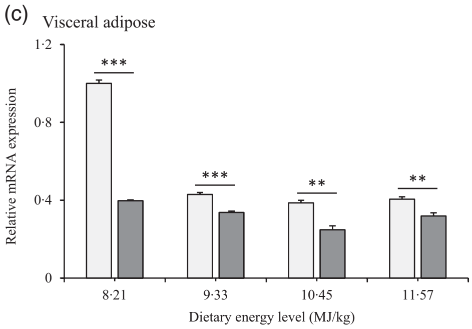
Fig. 4. Expression of GLUT4 mRNA in the subcutaneous and visceral adipose tissues of Tibetan (T, ■) and Small-tailed Han (H, □) sheep offered diets of different energy densities. The dietary energy levels are digestible energy on a DM basis. Values are means with their standard errors. Lin, linear; Quad, quadratic. * $P < 0·05$.



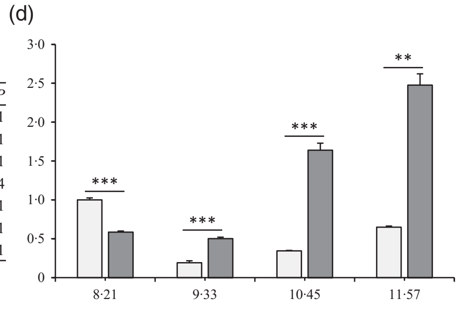
Items	*P*
Breed	<0·001
Lin diet E	<0·001
Quad diet E	0·002
Cubic diet E	0·360
Lin diet E×breed	<0·001
Quad diet E×breed	0·780
Cubic diet E×breed	0·169



Items	*P*
Breed	<0·001
Lin diet E	<0·001
Quad diet E	0·007
Cubic diet E	0·994
Lin diet E×breed	<0·001
Quad diet E×breed	<0·001
Cubic diet E×breed	0·004

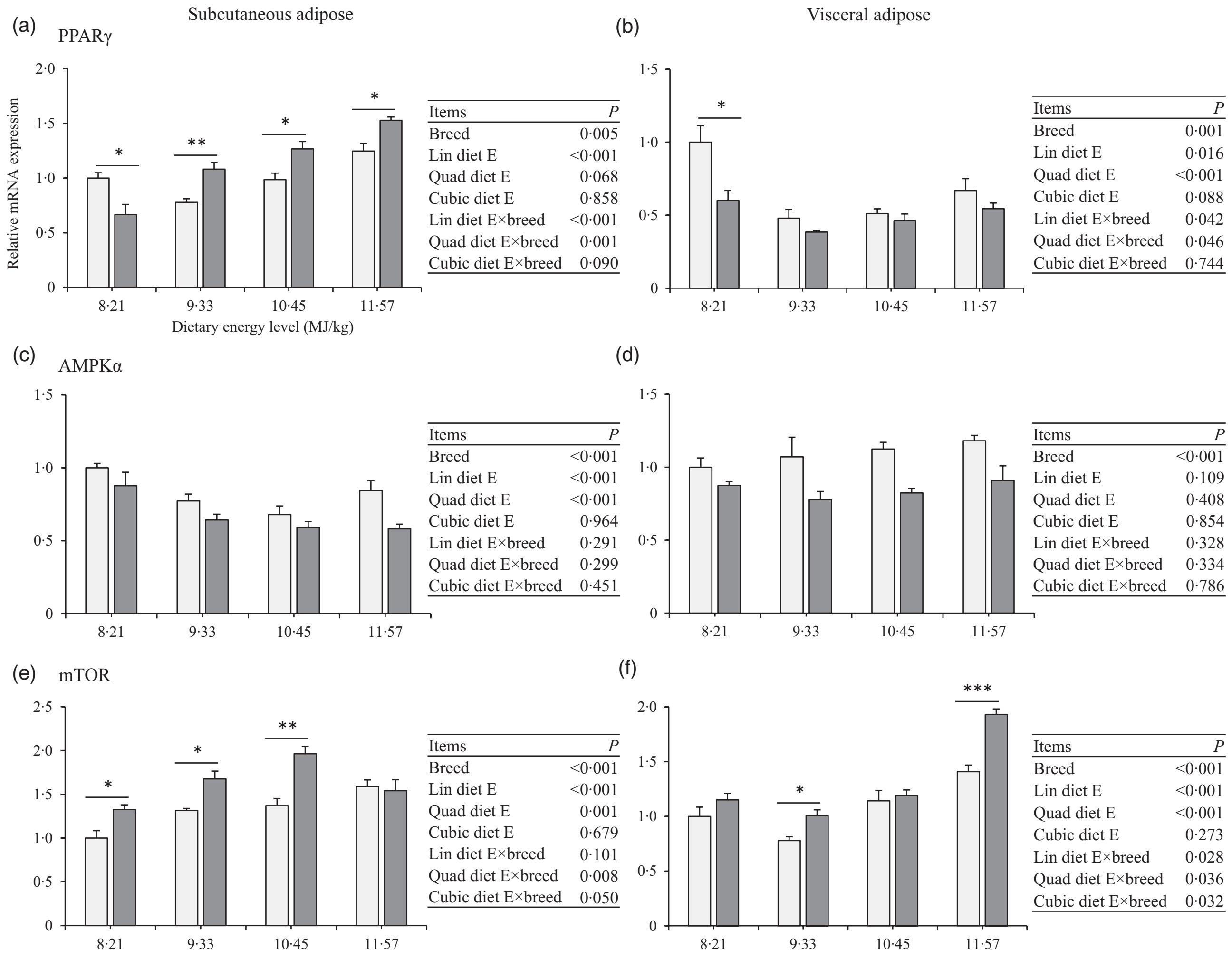


Items	*P*
Breed	<0·001
Lin diet E	<0·001
Quad diet E	<0·001
Cubic diet E	0·004
Lin diet E×breed	<0·001
Quad diet E×breed	<0·001
Cubic diet E×breed	<0·001



Items	*P*
Breed	<0·001
Lin diet E	<0·001
Quad diet E	<0·001
Cubic diet E	<0·001
Lin diet E×breed	<0·001
Quad diet E×breed	0·287
Cubic diet E×breed	0·085

Fig. 5. Expression of fatty acid binding protein 4 (FABP4) and lipoprotein lipase (LPL) mRNA in the subcutaneous and visceral adipose tissues of Tibetan (T, ■) and Small-tailed Han (H, □) sheep offered diets of different energy densities. The dietary energy levels are digestible energy on a DM basis. Values are means with their standard errors. Lin, linear; Quad, quadratic. * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.



Items	P
Breed	0·005
Lin diet E	<0·001
Quad diet E	0·068
Cubic diet E	0·858
Lin diet E×breed	<0·001
Quad diet E×breed	0·001
Cubic diet E×breed	0·090

Items	P
Breed	0·001
Lin diet E	0·016
Quad diet E	<0·001
Cubic diet E	0·088
Lin diet E×breed	0·042
Quad diet E×breed	0·046
Cubic diet E×breed	0·744

Items	P
Breed	<0·001
Lin diet E	<0·001
Quad diet E	<0·001
Cubic diet E	0·964
Lin diet E×breed	0·291
Quad diet E×breed	0·299
Cubic diet E×breed	0·451

Items	P
Breed	<0·001
Lin diet E	0·109
Quad diet E	0·408
Cubic diet E	0·854
Lin diet E×breed	0·328
Quad diet E×breed	0·334
Cubic diet E×breed	0·786

Items	P
Breed	<0·001
Lin diet E	<0·001
Quad diet E	0·001
Cubic diet E	0·679
Lin diet E×breed	0·101
Quad diet E×breed	0·008
Cubic diet E×breed	0·050

Items	P
Breed	<0·001
Lin diet E	<0·001
Quad diet E	<0·001
Cubic diet E	0·273
Lin diet E×breed	0·028
Quad diet E×breed	0·036
Cubic diet E×breed	0·032

Fig. 6. Expression of the related regulation factors mRNA of energy homoeostasis in the subcutaneous and visceral adipose tissues of Tibetan (T, ▨) and Small-tailed Han (H, ☐) sheep offered diets of different energy densities. The dietary energy levels are digestible energy on a DM basis. AMPK*α*, AMP-activated protein kinase-*α*; mTOR, mammalian target of rapamycin. Values are means with their standard errors. Lin, linear; Quad, quadratic. * $P < 0·05$; ** $P < 0·01$; *** $P < 0·001$.

Regulation of hormones and adipokines on lipid metabolism and energy homoeostasis of Tibetan and Small-tailed Han sheep

Adipose tissue plays a central role in the regulation of energy metabolism and maintenance of whole-body energy homoeostasis through lipolysis or lipogenesis. During the process, adipocytes are responsive to multiple signals from peripheral hormones and release adipokines, which influence each other and form a metabolic regulatory network(10). Among them, insulin plays a central role in the metabolic regulatory network, as it is the key anabolic hormone of adipose tissues by increasing lipogenesis and LPL activity and depressing lipolysis; in contrast, catecholamines inhibit lipogenesis and stimulate lipolysis, as a potent regulator of lipolysis(22). In the present study, the higher pre-prandial serum concentration of insulin and lower insulin receptor mRNA expression in both subcutaneous and visceral adipose tissues of Small-tailed Han sheep suggested lower insulin action and higher catabolism in Small-tailed Han than in Tibetan sheep, and higher catabolism in Small-tailed Han sheep was consistent with higher BW loss than Tibetan sheep. Furthermore, the lower serum concentrations of catecholamines (adrenaline and noradrenaline) in Tibetan than in Small-tailed Han sheep were also consistent with the lower BW lost in Tibetan sheep. High insulin action stimulates glucose uptake and results in increased mRNA expression of GLUT4, which is required for insulin-stimulated glucose uptake and oxidation(23–26). However, in the present study, the GLUT4 mRNA expression did not increase with the higher insulin receptor mRNA expression in Tibetan sheep at the lowest energy level, which suggested that when Tibetan sheep were in negative energy balance, the adipocytes reduced glucose uptake and oxidation to preserve glucose for regulating and maintaining glucose homoeostasis. We reasoned that this was due to the higher concentrations of resistin and TNF-*α* in Tibetan than in Small-tailed Han sheep at the lowest energy level, as resistin and TNF-*α* were reported to decrease insulin-stimulated glucose uptake by reducing the expression of GLUT4(27–30). Adipose tissue is also a major site for metabolism of glucocorticoids and, in contrast to insulin, functions as a catabolic hormone by increasing lipolysis in adipocytes to supply the increased metabolic demand of an animal(31–33). In the present study, the lower concentration of glucocorticoid in Tibetan than in Small-tailed Han sheep was also consistent with the lower BW lost by Tibetan sheep. It was reported that visceral adipocytes were more responsive than subcutaneous adipocytes to catecholamine-induced lipolysis and less responsive to insulin's antilipolytic effects(11,34). Consequently, the greater change in the size of adipocytes in visceral than in subcutaneous tissue in Small-tailed Han sheep was consistent with the higher serum concentration of catecholamine and lower insulin receptor mRNA expression in visceral adipose tissue. In the present study, insulin receptor mRNA expression was higher and the concentrations of catabolic hormones (adrenaline, noradrenaline and glucocorticoids) were lower in the Tibetan than in the Small-tailed Han sheep, which would mean a lower energetic demand in Tibetan sheep and could be explained by the lower maintenance energy requirement in Tibetan than in Small-tailed Han sheep(9).

In addition to glucose, adipocytes also uptake fatty acids. During this process, FABP4 plays a key role, as it activates HSL in adipocytes to regulate lipolysis(35–37) and transports fatty acids to the endoplasmic reticulum and cell nucleus to regulate transcription(38,39). In the present study, the higher mRNA expression of FABP4 in Small-tailed Han than in Tibetan sheep was consistent with its higher HSL activity in visceral adipose tissue and greater body reserve mobilisation, which resulted in Small-tailed Han sheep losing more BW than Tibetan sheep. Adipose tissue secretes LPL to obtain fatty acids from VLDL, which is a key regulator of fat accumulation in adipose tissues(11). In the present study, the higher mRNA expression of LPL in Tibetan than in Small-tailed Han sheep in both subcutaneous and visceral adipose tissues at the three highest dietary energy levels was consistent with its higher insulin receptor mRNA expression than in Small-tailed-Han sheep. However, the mRNA expression of LPL was lower in Tibetan than in Small-tailed Han sheep at the lowest dietary energy level, which suggested that Tibetan sheep had a lower capability for the uptake of fatty acids than Small-tailed Han sheep when they were in negative energy balance to maintain energy homoeostasis. This was consistent with the lower VLDL concentration in Tibetan than in Small-tailed Han sheep at the lowest dietary energy level(9). In addition, an inverse relationship was found between TNF-α and LPL expression in the present study, which was supported by previous research(40).

Leptin, IL-6 and adiponectin are peptide hormones secreted by adipose tissue, which have an important role in regulating energy metabolism. It was reported that the secretion of leptin was regulated by peripheral hormones and other adipokines and that its serum concentration was increased by insulin, glucocorticoids and TNF-α(41). These relationships also occurred in the present study, as the Small-tailed Han sheep had higher leptin, insulin, glucocorticoids and TNF-α concentrations than Tibetan sheep. A higher leptin level indicated higher energy expenditure(42,43) in Small-tailed Han than in Tibetan sheep, which fit in well with the higher energy requirements of the Small-tailed Han sheep(9). It was reported that IL-6 decreased insulin action by reducing insulin receptor expression and also inhibited adipogenesis and decreased adiponectin secretion(44) and that there was a positive association between adiponectin and insulin action(45,46). These findings supported results in the present study, where Small-tailed Han sheep had higher IL-6 concentration and lower adiponectin concentration and insulin receptor expression than Tibetan sheep. These results also explained the lower loss in BW in Tibetan than in Small-tailed Han sheep.

In the regulation of energy homoeostasis, adipocytes of Tibetan sheep demonstrated higher insulin receptor mRNA expression and fatty acid uptake capacity when they were in positive energy balance, but lower glucose and fatty acid uptake capacity when they were in negative energy balance to maintain energy homoeostasis and cope with the lower energy intake. The Small-tailed Han sheep had higher concentrations of catabolic hormones and higher concentrations of adipokines, which inhibited insulin action and adipogenesis, and also higher fatty acid transportation and oxidation capacity to cope with the higher maintenance energy requirement.

Lipid metabolism and energy homoeostasis of Tibetan and Small-tailed Han sheep

To coordinate and regulate energy metabolism and to maintain energy homoeostasis at the cellular and whole-body levels, adipocytes respond to signals not only from peripheral hormones and adipokines but also to various regulators and sensors which detect changes of energy levels in the intra- and extra-cellular environment, for example, PPARγ, mTOR and AMPKα(47). It was reported that in mice lacking leptin, PPARγ activity was increased by the overexpression of adiponectin, which led to a massive expansion of the subcutaneous adipose tissue and to an increase in insulin action(48). In the present study, the higher PPARγ mRNA expression in subcutaneous adipose tissue of Tibetan than in Small-tailed Han sheep was consistent with its higher adiponectin but lower leptin levels and also with the higher insulin receptor mRNA expression and higher lipid metabolic activity in the subcutaneous adipose tissue. The higher PPARγ mRNA expression in visceral adipose tissue in Small-tailed Han than in Tibetan sheep was consistent with the higher lipid metabolic activity in the visceral adipose tissue of Small-tailed Han sheep. Moreover, the products of partial hydrogenation of unsaturated fatty acids in ruminants, conjugated linoleic acids, for instance *trans*10, *cis*12-conjugated linoleic acid, have potent physiological effects in reducing lipid synthesis in mammary gland and adipose tissues(49,50). In the present study, the unsaturated fatty acid content increased with an increase in dietary energy level due to the increased soyabean oil contents in the diets and, consequently, the yield of conjugated linoleic acid also increased. This led to an increase in oxidation of fatty acids and reduction in lipid synthesis, which was consistent with the increased PPARγ mRNA expression in both subcutaneous and visceral adipose tissues in both sheep breeds. The adipocyte diameter in the subcutaneous adipose tissue being smaller in Tibetan than in Small-tailed Han sheep, and the reverse in the visceral adipose tissue was due to the higher PPARγ mRNA expression.

AMPK acts as an energy monitor by sensing the depletion of intracellular energy and promotes fatty acid oxidation and also increases energy expenditure(47,51–54). It is regulated by the intracellular AMP:ATP ratio and responds by stimulating catabolic pathways that generate ATP. The higher AMPK*α* mRNA expression in both subcutaneous and visceral adipose tissues in Small-tailed Han sheep than in Tibetan sheep in the present study suggested a higher fatty acid oxidation activity and energy expenditure in the Small-tailed Han sheep, which was supported by the greater BW lost when in negative energy balance and higher maintenance energy requirement by Small-tailed Han than Tibetan sheep(9). Another important pathway in maintaining energy homoeostasis is the mTOR signalling pathway, which regulates cellular energy metabolism in response to nutrient availability and energy status, and affects the rate of cell growth and proliferation; however, unlike AMPK, mTOR is activated during favourable energy conditions(55,56). In the mTOR signalling pathway, mTOR itself is the central component. In the present study, Tibetan sheep had higher mRNA expression of mTOR in both subcutaneous and visceral adipose tissues than Small-tailed Han sheep, which suggested a higher rate of cell growth and proliferation of adipose tissue in Tibetan than in Small-tailed Han sheep, and supported the higher anabolism in Tibetan than in Small-tailed Han sheep.

The interplay of mTOR and AMPK signalling pathways provides a more precise mechanism for mammals to coordinate with environmental conditions(56). In the present study, the Tibetan sheep had a lower AMPK*α* expression but higher mTOR expression in the adipocytes than the Small-tailed Han sheep, which suggested a higher anabolic rate but lower catabolic rate to conform with the lower energy requirements of the Tibetan sheep when compared with the Small-tailed Han sheep.

Conclusion

Tibetan sheep demonstrated higher metabolic activity of both lipolysis and lipogenesis in subcutaneous adipose tissue than Small-tailed Han sheep, but this was reversed between breeds in the visceral adipose tissue. Tibetan sheep had higher insulin receptor mRNA expression and lower concentration of catabolic hormones; however, Small-tailed Han sheep had higher concentrations of catabolic hormones and higher concentrations of adipokines which inhibited insulin action and adipogenesis. In addition, the adipocytes of Tibetan sheep had a higher capacity for fatty acid uptake when they were in positive energy balance, but a lower capacity for glucose and fatty acid uptake when in negative energy balance. Moreover, Tibetan sheep had lower AMPK*α* expression but higher mTOR expression in the adipocytes than Small-tailed Han sheep. These findings suggested that Tibetan sheep had lower tissue catabolism and energy expenditure but higher anabolism in adipose tissue and reduced capability for uptake of glucose and fatty acids when in negative energy balance. These responses provided the Tibetan sheep an advantage over Small-tailed Han sheep to cope with low energy intake and the harsh environment of the QTP. The present study examined the responses of sheep to low energy intake under laboratory conditions, and based on the results, predictions were then made on their abilities to cope with low energy intake under harsh field conditions. Future research should be done under natural grazing conditions where the sheep may experience a number of limiting nutrients, not only energy, and may be exposed to harsh environmental conditions. The penned conditions inhibited behavioural responses, and these responses may be as important as physiological responses to the grazing sheep in coping with the harsh conditions.

Acknowledgements

The authors are grateful to two anonymous reviewers for their very helpful suggestions. Chao Yang, Fuyu Shi, Na Guo, Qi Yan, Mingjie Hou, Jiaojiao Zhang, Weixing Xu and Sisi Bi helped in collecting samples.

This work was funded by the National Nature Science Foundation of China (31601960, 31672453 and 31661143020), a grant from State Key Laboratory of Grassland Agro-Ecosystems (Lanzhou University) and key Research, Development and Conversion Program of Qinghai Province, China (2018-SF-145). X. J. is supported by the Chinese Scholarship Council (CSC, China).

R. L., J. Z. and X. J. conceived and designed the experiment. J. Z., X. J., W. W., Y. G., J. K., P. L. and X. D. performed the experiment. X. J., A. D., L. D., Z. S. and Q. Q. contributed to the writing and revising of the manuscript. All authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520001701

References

1. Qiu J (2008) China: the third pole. *Nature* **454**, 393–396.
2. Chen H, Zhu Q, Peng C, *et al.* (2013) The impacts of climate change and human activities on biogeochemical cycles on the Qinghai-Tibetan Plateau. *Glob Chang Biol* **19**, 2940–2955.
3. Miller DJ (1999) Nomads of the Tibetan Plateau rangelands in western China. Part Two. Pastoral production practices. *Rangelands Arch* **21**, 16–19.
4. Long R, Ding L, Shang Z, *et al.* (2008) The yak grazing system on the Qinghai-Tibetan plateau and its status. *Rangeland J* **30**, 241–246.
5. Long R, Apori S, Castro F, *et al.* (1999) Feed value of native forages of the Tibetan Plateau of China. *Anim Feed Sci Technol* **80**, 101–113.
6. Ding K, Shi H, Zhang Y, *et al.* (2011) Cold season supplementary feeding experiment of Gannan Tibetan sheep in cold grazing region. *Anim Sci Vet Med* **6**, 28–30.
7. Zhang Z, Xu D, Wang L, *et al.* (2016) Convergent evolution of rumen microbiomes in high-altitude mammals. *Curr Biol* **26**, 1873–1879.
8. Zhou J, Guo Y, Kang J, *et al.* (2019) Tibetan sheep require less energy intake than small-tailed Han sheep for N balance when offered a low protein diet. *Anim Feed Sci Technol* **248**, 85–94.
9. Jing X, Zhou J, Wang W, *et al.* (2019) Tibetan sheep are better able to cope with low energy intake than Small-tailed Han sheep due to lower maintenance energy requirements and higher nutrient digestibilities. *Anim Feed Sci Technol* **254**, 11420.

10. Rajala MW & Scherer PE (2003) Minireview: the adipocyte-at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology* **144**, 3765–3773.
11. Wajchenberg BL (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* **21**, 697–738.
12. Konige M, Wang H & Sztalryd C (2014) Role of adipose specific lipid droplet proteins in maintaining whole body energy homeostasis. *Biochim Biophys Acta Mol Basis Dis* **1842**, 393–401.
13. Miao X & Luo Q (2013) Genome-wide transcriptome analysis between small-tail Han sheep and the Surabaya fur sheep using high-throughput RNA sequencing. *Reproduction* **145**, 587–596.
14. Agriculture Mo (2004) *Feeding Standard of Meat-producing Sheep and Goats (NY/T 816–2004)*. Beijing: China Agricultural Press.
15. Sullivan GM & Feinn R (2012) Using effect size – or why the *P* value is not enough. *J Grad Med Educ* **4**, 279–282.
16. Xie AY, Chai ST, Wang WB, *et al.* (1996) The herbage yield and the nutrient variation in mountain meadow. *Qinghai J Anim Sci Vet Med* **26**, 8–10.
17. Metzler-Zebeli B, Hollmann M, Sabitzer S, *et al.* (2013) Epithelial response to high-grain diets involves alteration in nutrient transporters and Na^+/K^+-ATPase mRNA expression in rumen and colon of goats. *J Anim Sci* **91**, 4256–4266.
18. Jing X, Wang W, Degen A, *et al.* (2020) Tibetan sheep have a high capacity to absorb and to regulate metabolism of short chain fatty acids in the rumen epithelium to adapt to low energy intake. *Br J Nutr* **123**, 1–35.
19. Livak KJ & Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**, 402–408.
20. Cui G, Degen AA, Wei X, *et al.* (2016) Trolox equivalent antioxidant capacities and fatty acids profile of 18 alpine plants available as forage for yaks on the Qinghai-Tibetan Plateau. *Rangeland J* **38**, 373–380.
21. Wei X, Wang J, Ma X, *et al.* (2005) Seasonal dynamics of carbohydrate and amino acid in alpine meadow of Qinghai-Tibetan Plateau. *Acta Prataculturae Sinica* **14**, 94–99.
22. Vernon RG (2005) Lipid metabolism during lactation: a review of adipose tissue-liver interactions and the development of fatty liver. *J Dairy Res* **72**, 460–469.
23. Li Z, Frey JL, Wong GW, *et al.* (2016) Glucose transporter-4 facilitates insulin-stimulated glucose uptake in osteoblasts. *Endocrinology* **157**, 4094–4103.
24. Khan A & Pessin J (2002) Insulin regulation of glucose uptake: a complex interplay of intracellular signalling pathways. *Diabetologia* **45**, 1475–1483.
25. Zisman A, Peroni OD, Abel ED, *et al.* (2000) Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat Med* **6**, 924.
26. Shepherd PR & Kahn BB (1999) Glucose transporters and insulin action – implications for insulin resistance and diabetes mellitus. *N Engl J Med* **341**, 248–257.
27. Banerjee RR & Lazar MA (2003) Resistin: molecular history and prognosis. *J Mol Med* **81**, 218–226.
28. Steppan CM, Bailey ST, Bhat S, *et al.* (2001) The hormone resistin links obesity to diabetes. *Nature* **409**, 307.
29. Szalkowski D, White-Carrington S, Berger J, *et al.* (1995) Antidiabetic thiazolidinediones block the inhibitory effect of tumor necrosis factor-alpha on differentiation, insulin-stimulated glucose uptake, and gene expression in 3T3-L1 cells. *Endocrinology* **136**, 1474–1481.
30. Stephens JM & Pekala P (1991) Transcriptional repression of the GLUT4 and C/EBP genes in 3T3-L1 adipocytes by tumor necrosis factor-alpha. *J Biol Chem* **266**, 21839–21845.
31. Kershaw EE & Flier JS (2004) Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* **89**, 2548–2556.
32. Peckett AJ, Wright DC & Riddell MC (2011) The effects of glucocorticoids on adipose tissue lipid metabolism. *Metabolism* **60**, 1500–1510.
33. Xu C, He J, Jiang H, *et al.* (2009) Direct effect of glucocorticoids on lipolysis in adipocytes. *Mol Endocrinol* **23**, 1161–1170.
34. Bolinder J, Kager L, Östman J, *et al.* (1983) Differences at the receptor and postreceptor levels between human omental and subcutaneous adipose tissue in the action of insulin on lipolysis. *Diabetes* **32**, 117–123.
35. Shen W-J, Sridhar K, Bernlohr DA, *et al.* (1999) Interaction of rat hormone-sensitive lipase with adipocyte lipid-binding protein. *PNAS* **96**, 5528–5532.
36. Smith AJ, Sanders MA, Juhlmann BE, *et al.* (2008) Mapping of the hormone-sensitive lipase binding site on the adipocyte fatty acid-binding protein (AFABP) identification of the charge quartet on the AFABP/aP2 helix-turn-helix domain. *J Biol Chem* **283**, 33536–33543.
37. Coe NR, Simpson MA & Bernlohr DA (1999) Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J Lipid Res* **40**, 967–972.
38. Ayers SD, Nedrow KL, Gillilan RE, *et al.* (2007) Continuous nucleocytoplasmic shuttling underlies transcriptional activation of PPARγ by FABP4. *Biochemistry* **46**, 6744–6752.
39. Garin-Shkolnik T, Rudich A, Hotamisligil GS, *et al.* (2014) FABP4 attenuates PPARγ and adipogenesis and is inversely correlated with PPARγ in adipose tissues. *Diabetes* **63**, 900–911.
40. Kern PA, Saghizadeh M, Ong JM, *et al.* (1995) The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. *J Clin Investig* **95**, 2111–2119.
41. Margetic S, Gazzola C, Pegg G, *et al.* (2002) Leptin: a review of its peripheral actions and interactions. *Int J Obes* **26**, 1407.
42. Scarpace PJ, Matheny M, Pollock BH, *et al.* (1997) Leptin increases uncoupling protein expression and energy expenditure. *Am J Physiol Endocrinol Metab* **273**, E226–E230.
43. Hwa JJ, Fawzi AB, Graziano MP, *et al.* (1997) Leptin increases energy expenditure and selectively promotes fat metabolism in ob/ob mice. *Am J Physiol Regul Integr Comp Physiol* **272**, R1204–R1209.
44. Fernández-Real JM & Ricart W (2003) Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev* **24**, 278–301.
45. Kubota N, Terauchi Y, Yamauchi T, *et al.* (2002) Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* **277**, 25863–25866.
46. Combs TP, Pajvani UB, Berg AH, *et al.* (2004) A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. *Endocrinology* **145**, 367–383.
47. Marshall S (2006) Role of insulin, adipocyte hormones, and nutrient-sensing pathways in regulating fuel metabolism and energy homeostasis: a nutritional perspective of diabetes, obesity, and cancer. *Sci STKE* **2006**, re7.
48. Kim J-Y, Van De Wall E, Laplante M, *et al.* (2007) Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clini Investig* **117**, 2621–2637.
49. Bauman DE, Perfield JW, Harvatine KJ, *et al.* (2008) Regulation of fat synthesis by conjugated linoleic acid: lactation and the ruminant model. *J Nutr* **138**, 403–409.

50. Kramer R, Wolf S, Petri T, *et al.* (2013) A commonly used rumen-protected conjugated linoleic acid supplement marginally affects fatty acid distribution of body tissues and gene expression of mammary gland in heifers during early lactation. *Lipids Health Dis* **12**, 96.
51. Long YC & Zierath JR (2006) AMP-activated protein kinase signaling in metabolic regulation. *J Clini Investig* **116**, 1776–1783.
52. Viollet B, Andreelli F, Jørgensen SB, *et al.* (2003) The AMP-activated protein kinase α2 catalytic subunit controls whole-body insulin sensitivity. *J Clini Investig* **111**, 91–98.
53. O'Neill HM, Holloway GP & Steinberg GR (2013) AMPK regulation of fatty acid metabolism and mitochondrial biogenesis: implications for obesity. *Mol Cell Endocrinol* **366**, 135–151.
54. Hardie DG & Ashford ML (2014) AMPK: regulating energy balance at the cellular and whole body levels. *Physiology* **29**, 99–107.
55. Colombani J, Raisin S, Pantalacci S, *et al.* (2003) A nutrient sensor mechanism controls Drosophila growth. *Cell* **114**, 739–749.
56. Xu J, Ji J & Yan X-H (2012) Cross-talk between AMPK and mTOR in regulating energy balance. *Crit Rev Food Sci Nutr* **52**, 373–381.