Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Paper type: Original Research

Fat-tailed and thin-tailed lambs responses to glucose and insulin challenges during different energy balances

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Received: 30 Aug 2022,
Accepted: 25 Dec 2022,
Published online: 04 May 2023,

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Abstract The proportion of adipose depots varies considerably between fat-tailed (FT) and thin-tailed (TT) sheep breeds. FT breeds accumulate majority of body fat in fat-tail depot, whereas TT breeds deposit considerable proportion of body fat in visceral depots. These differences in proportion of adipose depots seem to affect body metabolism due to differences in metabolism of different depots. Hence, the current research aimed to evaluate the response of fat-tailed lambs (FTL) and thin-tailed lambs (TTL) to glucose and insulin challenges during negative energy balance (NEB) and positive energy balance (PEB). Glucose tolerance and insulin challenge tests were conducted on randomly selected lambs from each genotypes at the end of induced NEB and PEB. Glucose injection during NEB caused greater plasma glucose concentration in TTL, whereas in PEB, the enhancement in glucose concentration as a consequence of glucose injection was higher in FTL (P≤0.09). The area under the glucose curve was higher in FT compared to TT lambs during glucose tolerance test regardless of energy balance (EB; P≤0.03). The clearance rates of insulin (P≤0.09) and glucose (P≤0.006) in the respective insulin and glucose tolerance tests were higher during PEB compared to NEB regardless of the genotypes. These results demonstrated that induced NEB can enhance insulin resistance in both FT and TT lambs, severity of which is greater in FT than in TT lambs.

Keywords: adipose depots, energy balance, fat-tailed lambs, glucose tolerance test, insulin sensitivity

Introduction

Adipokines, derived from adipose tissues, can modulate insulin sensitivity (Lee et al., 2019). Stimulation of inflammatory -pathways in adipose tissue and release of cytokines such as tumor necrosis factor alpha (TNF-α) and interlukine-6 (IL-6) -can affect insulin sensitivity at the level of insulin sensing through insulin receptor phosphorylation leading to reduced glucose uptake (Lee et al., 2019). High plasma level of non-esterified fatty acid (NEFA) in dairy cows as a consequence of body fat mobilization during early lactation was negatively associated with insulin sensitivity (Kerestes et al., -2009). The effectiveness of different adipose depots on whole body metabolism varies considerably (Marcadenti and de Abreu-Silva, 2015). It has been reported that various adipose depots have different affinity to insulin and the concentration of insulin receptors differs among various type of tissues and also between adipose depots. Visceral adipose depots have been reported to be more re-

sponsive to fluctuation in body energy balance (Guilherme et al., 2019) and in humans and rodents, accumulation of fat in visceral adipose depots was related to metabolic disorders such as liver steatosis, insulin resistance and diabetes (Duwaerts and Maher, 2019) as these adipose depots mobilize more NEFA compared to subcutaneous adipose depot (Spalding et al., 2017). Moreover, various adipose depots has been reported to have different effects on insulin sensitivity and responsiveness in dairy cows due to different adipokine secretory capacity, mobilization of fatty acids and their location in body (Drackley et al., 2014; Saremi et al., 2014; De Koster et al., 2015). Fat-tailed (FT) sheep breeds are characterized by their high body fat content with considerable amount of fat deposited in the tail region than in thin-tailed (TT) sheep breeds which deposit majority of body fat in visceral adipose depots. It has been reported that fat-tail as a body reserve is less responsive to negative energy balance (NEB) and lipolytic stimuli (Khachadurian et al., 1966). According to above mentioned differences in metabolism of various adipose depots, metabolic responses of FT and thin-tailed lambs (TTL) was hypothesized to be different during various energy balances and this difference might affect metabolism of glucose, insulin and fatty acids. Hence, the objective of the current study was to evaluate the glucose and fatty acid metabolic responses to periods of NEB and positive energy balance (PEB) in fat-tailed lambs (FTL) and thin-tailed lambs (TTL).

Materials and methods

Animals, housing and diet

The experiment was carried out at the Natural Resources and Agricultural Research Farm of Tehran University, Karaj, Iran. Thirty-six male lambs with average body weight (BW) of 41 ± 4.59 kg and age of 5 to 6 months were divided into three groups each containing six FT (Lori-Bakhtiari pure breed) and six TT (Lori-Bakhtiari × Romanov F_1 cross-bred) lambs according to their BW and housed in individual pens. The experiment began after 2 weeks of adaptation period to pen and lasted for 42 d. Lambs were fed a balanced total mixed ration (TMR**)** formulated by Cornell net protein and carbohydrate system (CNCPS) software program, 1.5 fold of their maintenance requirement during the adaptation period. The diet was consisted of 56 % forage (alfalfa hay and wheat straw) and 44 % concentrate (corn and barley grains, soybean and canola meal and wheat bran) with mineral and vitamin supplement. Blood sampling was done weekly to measure plasma metabolites including glucose, insulin, NEFA and TNF-α. At the end of the adaptation period, one group of lambs was randomly selected and slaughtered after 16 h of feed deprivation to record carcass characteristics and adipose depots mass. To induce NEB, the remaining two groups were fed under their maintenance requirements for 3 wk. The amount of feed provided to lambs was 90, 80 and 70 % of their maintenance requirements respectively, during weeks one, two and three of the experiment, being adjusted weekly according to the lamb's body weight changes. At the end of the third week, the second group of lambs was randomly selected and slaughtered for data collection. During the last 21 d of the experiment, the remaining groups of lambs had ad-libitum access to the diet to experience a period of PEB and then were slaughtered for data recording.

Insulin and glucose tolerance tests

Glucose (GTT) and insulin (ITT) tolerance tests were performed during the last two d of the NEB and PEB periods using four randomly selected lambs from each genotypes according to their body weight. The same lambs were used for GTT and ITT in both NEB and PEB. Lambs were deprived of feed 16 hours before the tests started. Blood samples were collected through sterile catheters (14G×5.1cm; Jelco™, Johnson and Johnson, Mumbai, India) inserted into the jugular vein. Samples of times -10 and 0 were collected for determination of basal metabolite concentration. At time of 0, a glucose solution (500 mg of glucose/kg body weight as sterile 50 % solution) was injected through the catheter and blood samples were taken into heparinized vacuum tubes at 5, 10, 20, 30, 40, 60, 90, 120, and 150 min after glucose injection. Insulin challenge was done the day after GTT by jugular vein injection of insulin (0.1 U/kg BW) and blood samples were collected at the same intervals as in GTT. Tubes were placed in an ice bath and then centrifuged for 15 min at 3000 g. Plasma samples were separated and stored at -20 °C until subsequent analysis. Glucose and insulin clearance rates were calculated using incremental concentration of glucose and insulin above the baseline between time **a** (time of maximum concentration) and time **b** (time of minimum concentration) using the following formula (Kaneko 1989):

$$CR\ (\%\ /\ min) = (Ln\ (ta) - Ln\ (tb)) / (tb\text{-}ta) \times 100$$

where, ta and tb are, respectively, the maximum and minimum concentrations of metabolites after glucose and insulin injection.

The time needed for plasma metabolites to return to half of the maximal concentration ($T_{1/2}$) of glucose, insulin and NEFA was calculated using the following formula:

$$T_{1/2}\ (min) = (Ln\ (2) / CR) \times 100$$

The time needed for plasma metabolites to return to their basal level (T_{basal}) was calculated as; according to following formula:

$$T_{basal}\ (min) = ((Ln\ (ta) - Ln\ (tb)) / CR) \times 100$$

where, ta and tb are, respectively, the maximum and minimum concentrations of metabolites after glucose and insulin injection.

The AUC for glucose and insulin was calculated after drawing the curve for glucose and insulin, using the trapezoidal rule (Shiang 2004).

Plasma metabolites including glucose, insulin, NEFA and TNF-α were measured using commercial human kits (Pars Azmoon CO, Iran for glucose, Randox Laboratories,

Ardmore, UK for insulin and NEFA and Shanghai Crystal day Biotech CO. for TNF-α). Glucose were quantified using spectrophotometer (Jasco V-570, Japan) according to kit instructions with the wave length of 500-546 and intra- and inter-assay coefficients of variation of 7.9 and 8.1 respectively. The insulin, NEFA and TNF-α were quantified using enzymatically by ELISA Reader (ELx 808-Ultramicroplate Reader Bio-Tek. U.S.A). The intra- and inter-assay coefficients of variation for insulin, NEFA and TNF-α were 7.9 and 9.8, 6.3 and 6.6, 8.2 and 8.5 respectively.

Statistical analysis

Data were analyzed by MIXED procedure of SAS (SAS, 2008). The mixed model included the fixed effects of genotype, energy balance and their interaction and random effect of animal. Least-squares means were computed and tested for difference by the Tukey's test.

Differences between means were considered to be significant at P≤0.05 for the main effects, and at P≤0.10 for the interaction effects. Trends were discussed when P≤0.10 for the main effects and P≤0.16 for the interaction effects.

Results

Carcass characteristics

The weight of liver was decreased in response to NEB regardless of genotype (Table 1). The weight of perirenal fat was lower in FTL regardless of EB (0.074 and 0.229 kg of absolute weights for FTL and TTL respectively; P<0.001). The weight of OM fat was higher in TTL regardless of EB (0.17 versus 0.46 kg for FTL and TTL respectively; P<0.004). The weight of visceral adipose depots was higher in TTL regardless of EB (0.250 versus 0.698 kg for FTL and TTL respectively; P<0.01). The weight of fat-tail adipose depot was significantly higher in FTL regardless of EB.

Table 1. The weight of internal organs and fat depots affected by genotype, EB and their interaction

	FTL			TTL				P-value		
Item (kg)	NUEB	NEB	PEB	NUEB	NEB	PEB	SEM	G	EB	G × EB
Body weight	42.84	37.16	46.33	40.60	36.03	44.41	3.00	0.47	0.02	0.98
Carcass	17.66	15.96	17.50	17.84	16.11	18.20	1.49	0.78	0.40	0.97
Heart	0.142	0.157	0.158	0.156	0.157	0.193	0.015	0.20	0.24	0.52
Liver	0.55	0.45	0.591	0.61	0.44	0.601	0.035	0.48	0.0003	0.63
Lung	0.60	0.60	0.60	0.63	0.59	0.58	0.050	0.97	0.87	0.82
Kidney	0.10	0.098	0.10	0.10	0.093	0.10	0.006	0.60	0.33	0.81
Perirenal fat	0.062	0.070	0.089	0.239	0.179	0.270	0.045	0.0003	0.48	0.67
Omental plus mesenteric fat	0.202	0.134	0.194	0.524	0.397	0.484	0.11	0.004	0.67	0.96
Visceral fat	0.264	0.204	0.283	0.764	0.576	0.755	0.27	0.0001	0.16	0.39
Fat-tail	2.88	1.97	2.44	0.41	0.25	0.31	0.15	0.001	0.65	0.91
Visceral plus fat-tail	3.14	2.18	2.73	1.17	0.83	1.06	0.32	0.0001	0.14	0.64

NEUB; neutral energy balance, NEB; negative energy balance, PEB; positive energy balance, G; genotype, EB; energy balance, G × EB; interaction of genotype and energy balance. a,b: Within rows, mean values with common superscripts are not different (P>0.05; Tukey's test).

Plasma metabolites

Plasma glucose concentration was higher in FTL regardless of EB (67.64 versus 64.36 mg/dL for FTL and TTL respectively; P<0.01; Figure 1). Plasma glucose was decreased at the end of NEB regardless of genotype (64.22, 66.44, 65.50, 61.27, 64.33, 68.91 and 71.33 mg/dL for weeks 1 to 7 of the experiment respectively; P<0.01). When data related to various weeks were analyzed separately, the difference in plasma glucose content between two genotypes was significant only at the end of weeks 1 and 3 of the experiment. Plasma NEFA content was affected by genotype, EB and their interaction (P<0.004). Plasma NEFA was higher in TTL regardless of EB (0.132 versus 0.192 mmol/l for FTL and TTL respectively; P<0.001). Induced NEB and PEB respectively increased and decreased plasma NEFA content regardless of genotype (0.10, 0.12, 0.18, 0.44, 0.11, 0.08 and 0.09 mmol/l for weeks 1 to 7 of the experiment respectively; P<0.0001). At the end of NEB, plasma concentration of NEFA was 65 % higher (P< 0.01) in TTL compared to FTL. The concentration of TNF-α was not affected by EB and interaction of genotype and EB, however it was considerably higher in FTL (174.48 versus 82.45 ng/L for FTL and TTL, respectively; P<0.0001).

Glucose tolerance test

The maximum plasma glucose concentration caused by glucose injection was affected by the interaction of genotype and EB (P≤0.09). Glucose injection during NEB increased plasma glucose concentration more in TTL, whereas during PEB, the enhancement in plasma glucose concentration as a consequence of glucose injection was higher in FTL (Figure 2). Basal concentration of NEFA was higher in TTL during the tests conducted in NEB (P≤0.01). Plasma NEFA level started to decline 10 min after glucose injection in both genotypes to a constant level 60 min after glucose injection.

The interaction of genotype and EB influenced the AUC30 (P≤0.05) and AUC30-60 (P≤0.07) of glucose during GTT (Table 2). During NE, the AUC30 and AUC30-60 of glucose in TTL were higher than FTL, whereas during PEB, these values in FTL were higher than TTL. The FTL had numerically higher AUC60-150 of glucose regardless of EB (16305 versus 14766, respectively, in FTL and TTL; P≤0.10). The AUC60-150 of glucose was affected by the interaction of genotype and EB (P≤0.10). During NEB, the AUC60-150 of glucose was not different between the 2 genotypes, whereas during PEB, it was higher in FTL. The FTL had higher AUC150 of glucose regardless of EB (16350 versus 13538, respectively, for FTL and TTL; P≤0.03). The, clearance rate of insulin was numerically lower during NEB compared to PEB (Table 3; 0.36 versus 1.48 for NEB and PEB, respectively; P≤0.09).

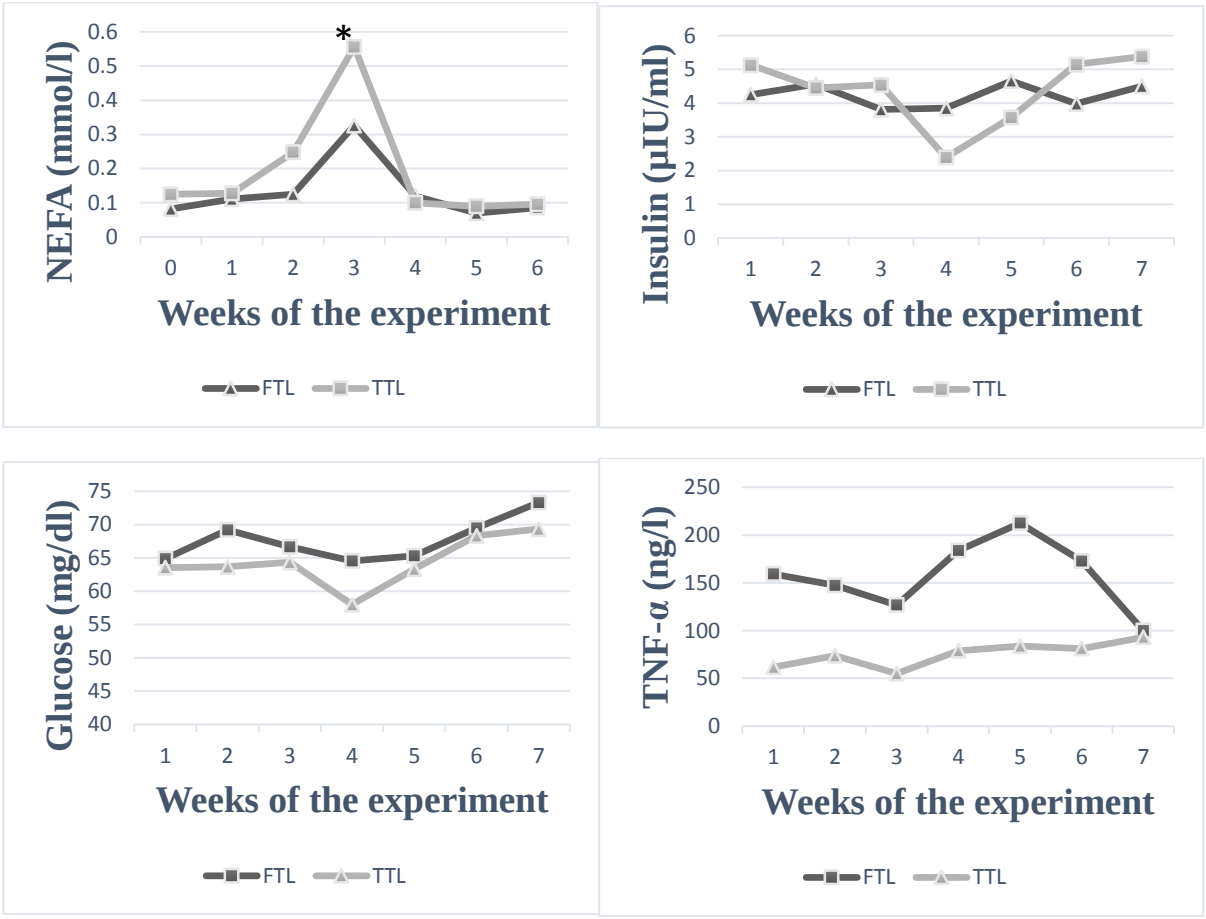

Figure 1. Changes in plasma insulin, NEFA, TNF-α and glucose in FTL and TTL during NEB and PEB.

Table 2. Glucose and insulin kinetics of FTL and TTL in response to GTT during NEB and PEB

	Fat-tailed		Thin-tailed			P-value		
Item	NEB	PEB	NEB	PEB	SEM	G	EB	G × EB
Glucose								
AUC30	3990	4745	4805	3843	392	0.91	0.79	0.05
AUC30-60	5767	7195	7190	5940	641	0.9	0.89	0.07
AUC60-150	15465	17145	15480	14051	843	0.10	0.88	0.10
AUC150	16275	16425	14138	12938	1333	0.03	0.65	0.56
Insulin								
AUC30	165.5	184.2	182.2	99.26	41.8	0.43	0.46	0.25
AUC30-60	235.5	239.7	210.8	148.3	36.5	0.14	0.44	0.38
AUC60-150	622	692	529	459	93.5	0.12	0.99	0.47
AUC150	686	998	660	519	169	0.17	0.62	0.22

NEB; Negative energy balance, PEB; Positive energy balance, G; genotype, EB; Energy balance, G × EB; Interaction of genotype and energy balance, AUC; area under the curve. a,b: Within rows, mean values with common superscripts are not different (P>0.05; Tukey's test).

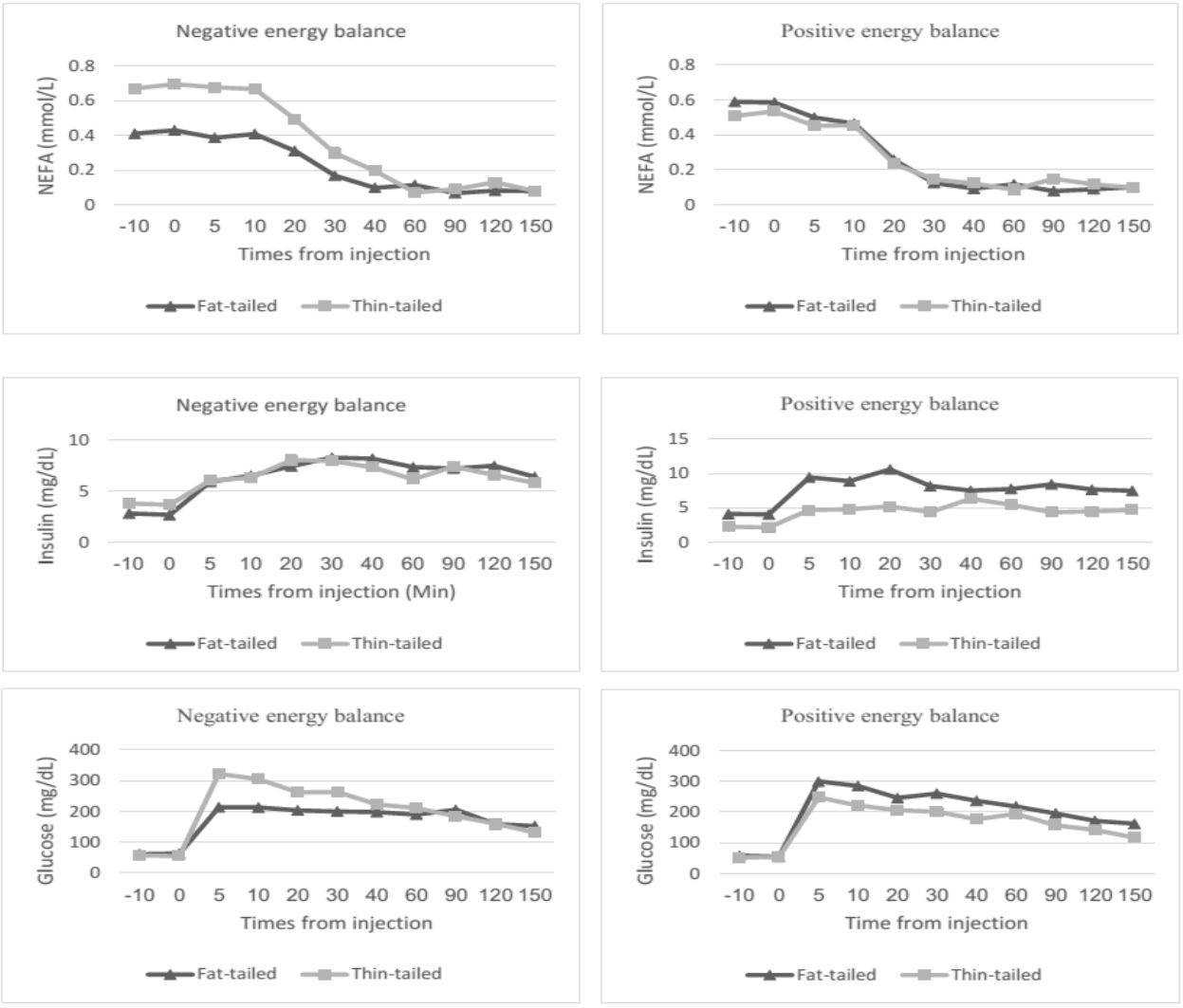

Figure 2. Plasma glucose, insulin and NEFA responses of FTL and TTL during GTT in NEB and PEB

Table 3. Glucose, insulin and NEFA kinetics of FTL and TTL in GTT during NEB and PEB

	Fat-tailed		Thin-tailed			P-value		
Item	NEB	PEB	NEB	PEB	SEM	G	EB	G × EB
Glucose CR (%)	0.28	0.45	0.60	0.47	0.16	0.33	0.88	0.39
Glucose $T_{1/2}$ (min)	291.68	358.90	125.09	413.19	0.16	0.73	0.30	0.51
Glucose T_{basal} (min)	476.24	645.15	306.49	616.19	200	0.63	0.26	0.73
NEFA CR %	2.88	2.89	3.82	4.48	1.29	0.35	0.80	0.80
NEFA $T_{1/2}$ (min)	29.60	35.27	22.12	25.73	10.08	0.42	0.65	0.92
NEFA T_{basal} (min)	73.98	92.02	81.24	66.14	23.92	0.70	0.95	0.50
Insulin CR (%)	0.41	1.10	0.30	1.86	0.60	0.60	0.09	0.49
Insulin $T_{1/2}$ (min)	173.04	101.67	312.53	160.67	0.97	0.33	0.28	0.69
Insulin T_{basal} (min)	327.52	139.35	431.97	332.60	175	0.41	0.43	0.80

NEB; negative energy balance, PEB; Positive energy balance, G; genotype, EB; Energy balance, G × EB; Interaction of genotype and energy balance, CR; clearance rate, $T_{1/2}$; the time needed for metabolites to return to half of their maximum concentration, T_{basal}; the time needed for metabolites to return to their basal level, NEFA; None esterified fatty acid. a,b: Within rows, mean values with common superscripts are not different (P>0.05; Tukey's test).

Insulin challenge

During ITT, basal glucose concentration in PEB was higher than NEB regardless of the genotypes (P≤0.01). Plasma glucose concentration started to decline immediately after insulin injection to reach a nadir at 40 min post-injection (Figure 3). During NEB, plasma glucose concentration returned to basal level at the end of the test, whereas during PEB, plasma glucose concentration did not return to basal level until the end of the test. Maximum insulin concentration caused by insulin injection in FTL was numerically higher than TTL regardless of EB (41.19 versus 37.54 µIU/mL for FTL and TTL, respectively; P≤0.12). Injection of insulin during NEB caused lower maximum plasma insulin concentration compared to PEB (37.02 versus 41.72

µIU/mL in NEB and PEB, respectively; P≤0.05), regardless of the genotypes. Plasma insulin was reduced to minimum concentration 90 min after insulin injection in both genotypes; however, this minimum concentration was numerically lower in FTL. The AUC30 of glucose was lower in NEB compared to PEB regardless of genotype (Table 4; 1512.5 versus 1728.75 during NEB and PEB, respectively; P≤0.007). The AUC60-150 of glucose was affected by the interaction of genotype and EB (P≤0.07). During NEB, AUC60-150 of glucose was higher in FTL, whereas in PEB, it was higher in TTL. Moreover, the AUC150 of glucose was affected by the EB and interaction of genotype and EB (P≤0.05). The AUC150 of glucose was enhanced from 9212 in NEB to 10388 in PEB regardless of genotype (P≤0.02). During NEB, AUC150 of glucose was higher in FTL, whereas during PEB, there was no difference in AUC150 of glucose between the 2 genotypes. Glucose CR was affected by the EB and interaction of genotype and EB (Table 5; P≤0.09). Glucose CR was considerably higher during PEB compared to NEB regardless of genotype (2.39 versus 1.44 for PEB and NEB, respectively; P≤0.006). During NEB, the CR of glucose was numerically higher in FTL and this difference was reversed during the PEB. During insulin challenge, the CR of insulin was numerically higher in PEB (2.19 versus 3.66 for NEB and PEB, respectively; P≤0.12). The clearance of NEFA was higher during PEB compared to NEB regardless of the genotype (3.62 versus 6.34 respectively for NEB and PEB; P≤0.03). The $T_{1/2}$ of glucose was higher during NEB regardless of the genotypes (51.92 versus 30.35 for NEB and PEB, respectively; P≤0.001). During the NEB, FTL showed lower $T_{1/2}$ of glucose, whereas in PEB, $T_{1/2}$ of glucose was higher in FTL (P≤0.02). In addition, $T_{1/2}$ of NEFA was higher during NEB (20.82 versus 12.27 for NEB and PEB, respectively; P≤0.04). FTL had numerically higher T_{basal} of NEFA regardless of the EB (34.80 versus 25.83 for FTL and TTL, respectively; P≤0.08). The T_{basal} of NEFA was higher during NEB regardless of the genotypes (34.63 versus 26.01 for NEB and PEB, respectively; P≤0.09).

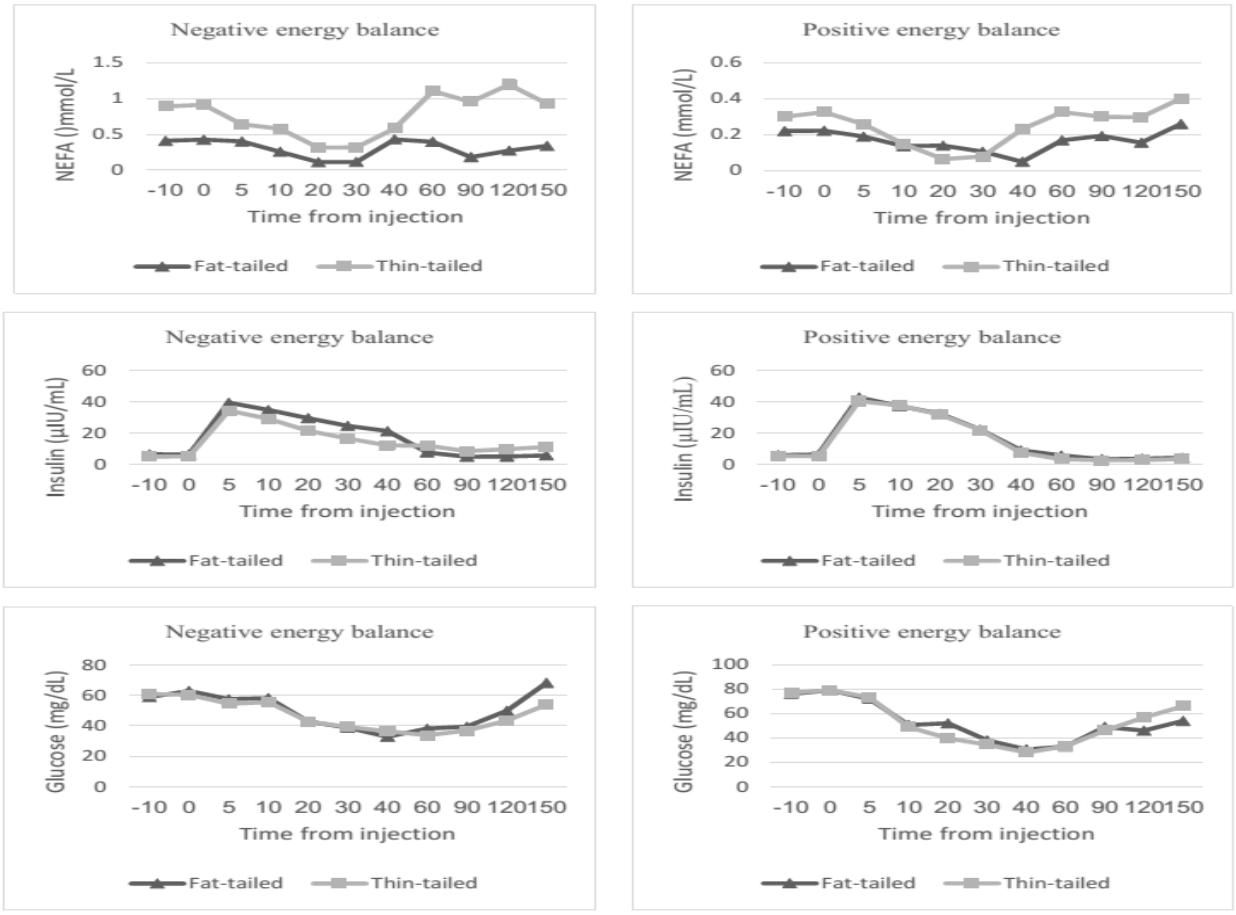

Figure 3. Plasma glucose, insulin and NEFA responses of FTL and TTL during ITT in NEB and PEB

Discussion

There are 27 breeds of sheep in Iran which 26 of them are fat-tailed. These breeds are characterized by high body fat content enabling them to survive periods of feed scarcity as Iran located in arid and semi-arid region of the world and experiences periods of feed abundant and scarcity during the year. Lori-Bakhtiari breed was used for cross-breeding with thin-tailed Romanov breed in or-

der to reduce its body fat content and increase the reproductive performance. This cross-breeding caused considerable reduction and/or elimination in mass of fat-tail and enhancement in visceral adipose depots (omental, mesenteric and perirenal depots), whereas total body fat content was decreased. These changes in proportion of adipose depots was hypothesized to affect whole body metabolism including glucose metabolism.

Table 4. Glucose and insulin kinetics of FTL and TTL in ITT during NEB and PEB

	Fat-tailed		Thin-tailed			P-value		
Item	NEB	PEB	NEB	PEB	SEM	G	EB	G × EB
Glucose								
AUC30	1530	1755	1495	1702	63.5	0.5	0.007	0.89
AUC30-60	1160	1065	1100	1016	95	0.58	0.37	0.95
AUC60-150	4800	3915	3960	4612	358	0.84	0.75	0.07
AUC150	9850^{ab}	9975^{ab}	8575^{b}	10800^{a}	415	0.60	0.02	0.04
Insulin								
AUC30	466	429	331	407	65	0.25	0.77	0.40
AUC30-60	486	420	430	379	69	0.49	0.42	0.91
AUC60-150	608	454	1043	300	239	0.57	0.09	0.25
AUC150	916	801	1245	767	396	0.71	0.47	0.65

NEB; Negative energy balance, PEB; Positive energy balance, G; genotype, EB; Energy balance, G × EB; Interaction of genotype and energy balance, AUC; area under the curve. a,b: Within rows, mean values with common superscripts are not different (P>0.05; Tukey's test).

Table 5. Glucose, insulin and NEFA kinetics of FTL and TTL in ITT during NEB and PEB

	Fat-tailed		Thin-tailed			P-value		
Item	NEB	PEB	NEB	PEB	SEM	G	EB	G × EB
Glucose CR (%)	1.76^{ab}	2.20^{ab}	1.13^{b}	2.59^{a}	0.26	0.66	0.006	0.09
Glucose $T_{1/2}$ (min)	42.00^{ab}	33.62^{b}	61.85^{a}	27.08^{b}	4.79	0.19	0.001	0.02
Glucose T_{basal} (min)	46.76	46.56	53.33	40.00	5.25	0.96	0.23	0.23
NEFA CR (%)	3.61	6.19	3.63	6.49	1.13	0.89	0.03	0.90
NEFA $T_{1/2}$ (min)	20.93	11.93	20.71	12.61	3.61	0.95	0.04	0.90
NEFA T_{basal} (min)	42.59	27.02	26.66	25.00	4.61	0.08	0.09	0.16
Insulin CR (%)	2.43	3.26	1.95	4.05	0.85	0.85	0.12	0.47
Insulin $T_{1/2}$ (min)	28.64	25.65	40.97	22.28	7.27	0.55	0.17	0.30
Insulin T_{basal} (min)	81.32	72.41	111.09	82.46	25.24	0.45	0.47	0.70

NEB; Negative energy balance, PEB; Positive energy balance, G; genotype, EB; Energy balance, G × EB; Interaction of genotype and energy balance, CR; clearance rate, $T_{1/2}$; the time needed for metabolites to return to half of their maximum concentration, T_{basal}; the time needed for metabolites to return to their basal level. a,b: Within rows, mean values with common superscripts are not different (P>0.05; Tukey's test).

In human, skeletal muscle contributes up to 80 % of whole body insulin-stimulated glucose uptake, whereas adipose tissue uptakes 5 % of plasma glucose (DeFronzo, 2004) and this proportion goes farther for skeletal muscle in ruminants as ruminant's adipose tissue preferentially use acetates for lipogenesis (Duhlmeier et al., 2005). Higher plasma glucose content in FTL can be related to their higher body fat content and possibly lower body muscle content. Adipose tissue prefers acetate as a substrate, whereas muscle preferentially uses glucose (De Koster and Opsomer, 2013). The other likely explanation for higher glucose concentration in FTL can be the higher insulin resistant demonstrated by glucose and insulin tolerance tests in FTL during the last week of NEB. Insulin resistance in liver can have inhibitory effect on ability of insulin to suppress glucose production (Kerouz et al., 1997) and subsequently cause incomplete suppression of liver glucose production (Lewis *et al.* 1996). Liver of human suffering from type 2 diabetes was resistant to inhibitory effect of insulin on hepatic glucose production which can lead to hyperglycemia (DeFronzo, 2004). Reduction of plasma insulin content in response to negative energy balance is in agreement with study of Almeida et al. (2016) in which restricted feeding reduced plasma concentration of insulin in Damara, Dorper and Merino lambs. Elevated concentration of plasma NEFA in dairy cows has been reported to hamper the insulin-secretory capacity of pancreas (Bossaert et al., 2008). The lower plasma insulin in TTL compared to FTL during the last week of NEB can be related to higher plasma concentration of NEFA in TTL. On the other hand, higher insulin concentration in FTL can be due to higher degree of insulin resistance at the end of NEB. This lower insulin sensitivity in FTL seems to be related to higher plasma concentration of TNF-α as subcutaneous injection of TNF-α has been reported to cause insulin resistance in young steers (Kushibiki et al., 2001). Secretion of pro-inflammatory cytokines has been reported to be considerably higher in visceral comparing to subcutaneous adipose depot (Fontana et al., 2007), hence in current study, TTL were expected to have more plasma TNF-α content as proportion of visceral adipose depots was considerably higher in TTL, but surprisingly both basal and NEB induced plasma TNF-α concentration were higher in fat-tailed lambs.

Higher and lower maximum glucose concentration caused by glucose injection in FTL, respectively, during NEB and PEB seem to originate from the differences in basal plasma insulin concentrations between 2 the geno-

types. The lack of significant difference in basal plasma insulin concentration in GTT and ITT during NEB can be related to limited number of lambs used for ITT and GTT as weekly measurement of plasma metabolites with higher number of animals per genotype showed lower plasma insulin in TTL compared to FTL during the last week of NEB. Lower plasma insulin concentration in TTL allowed plasma glucose to rise to higher extent compared to FTL. This indicates that presence of insulin in FTL even in insulin resistance state caused by NEB, prevents supra-physiological concentrations of glucose. Hence, the higher glucose AUC during the first 60 min after glucose injection in TTL can be related to their lower plasma insulin content rather than higher degree of insulin resistance as glucose stimulated secretion of insulin in TTL caused higher glucose CR, whereas there was no considerable change in plasma glucose concentration in FTL until 90 min after glucose injection. Lower enhancement of plasma insulin in response to glucose injection in TTL might be related to lower sensitivity of beta-cells to changes in glucose concentration or higher insulin resistance in FTL which induced beta-cells to secrete more insulin for elimination of enhanced plasma glucose as a consequence of glucose injection. This higher amount of insulin needed for reduction of plasma glucose in FTL can be due to higher and lower body fat and muscle content respectively. Skeletal muscle is quantitatively the most important tissue for glucose uptake and adipose tissue account only for a small portion of insulin-induced glucose disposal (Herdt, 2013). During NEB, the higher enhancement of plasma insulin concentration as a consequence of glucose injection in FTL did not lead to clearance of plasma glucose (higher CR of glucose in TTL compared to FTL), whereas during PEB, the enhancement in insulin concentration in response to glucose injection caused a glucose CR equal to those of TTL. This difference demonstrates that NEB increased the severity of insulin resistance more in FTL. The basal plasma insulin concentration and insulin response to glucose injection were 4 fold higher in dairy cows 17 d before parturition compared to 3 to 5 d postpartum (Zachut et al., 2013). During PEB, plasma insulin level was not different between the 2 genotypes which is consistent with Eryavuz et al. (2007) who reported no difference in plasma insulin between FTL and TTL. During PEB, higher responsiveness of TTL to insulin did not allow plasma glucose concentration to rise more than FTL and this difference in basal plasma insulin concentration between FTL and TTL during NEB and PEB caused AUC30 and AUC30-60 of glucose to be affected by the interaction of genotype and EB. In fact, the higher and lower AUC30 of glucose in TTL during NEB and PEB, respectively, is the consequence of higher and lower maximum glucose concentration caused by glucose injection during NEB and PEB, respectively.

During ITT, the higher AUC30 of glucose in PEB can be related to higher basal plasma glucose content. The higher AUC150 of glucose during NEB can be due to enhanced insulin resistance and reduced insulin secretion in FTL and TTL, respectively. The higher AUC60-150 of glucose in response to insulin challenge in FTL during NEB can be explained by lower AUC60-150 of insulin; however, there is no clear explanation for lowered glucose concentration in FTL during the last 60 min of ITT in PEB which caused the AUC60-150 of glucose in FTL to be lower than TTL. The higher AUC150 of glucose in FTL during NEB demonstrates their higher susceptibility to development of insulin resistance and lowered insulin sensitivity. The inhibitory effect of supra-physiological concentration of insulin (caused by insulin injection) on pancreatic insulin secretion may explain the lower insulin AUC60-150 in FTL.

The higher CR of glucose, insulin and NEFA during ITT in PEB demonstrates the detrimental effects of NEB on insulin sensitivity in both genotypes. The higher CR and lower $T_{1/2}$ of glucose in FTL during NEB can be related to higher plasma insulin concentration caused by insulin injection, as during the PEB, when insulin injection caused equal plasma insulin concentrations, the CR and $T_{1/2}$ of glucose were, respectively, higher and lower in TTL. Moreover, the higher plasma insulin content in FTL, caused by insulin injection during NEB, resulted in higher CR and lower $T_{1/2}$ of glucose in FTL, whereas it was not influential on CR and $T_{1/2}$ of NEFA, and the T_{basal} of NEFA was even numerically higher in FTL. These differences in response of glucose and fatty acid metabolism demonstrate that glucose metabolism, compared to fatty acid metabolism, needs higher plasma insulin concentration to be affected. The higher CR and lower $T_{1/2}$ of insulin in FTL during NEB were due to sudden reduction in plasma insulin concentration from 40 to 60 min after the beginning of the test. The insulin CR was almost the same in FTL and TTL until 40 min after insulin injection. From min 40 to 60 after insulin injection, plasma insulin concentration fell sharply in FTL, whereas it was considerably stable in TTL; this difference caused the CR of insulin to be numerically higher in FTL during NEB. This sudden decline in plasma insulin concentration can be explained by the short half-life of insulin, as this reduction was accompanied by enhancement (not reduction) in plasma glucose concentration, whereas in TTL, the majority of injected insulin was bond to its receptors before min 40 and this higher insulin CR was coincided with higher CR of NEFA but not glucose in TTL. It has been reported that FT as a body reserve is less responsive to lipolytic stimulus (Khachadurian et al., 1966). Moreover, visceral adipose depots are more vascular and have higher capacity to uptake preformed long-chain fatty acids, whereas subcutaneous adipose depot prefers *de novo* synthesis of fatty acids (Ibrahim 2010; Ji et al., 2014). The higher CR of glucose and NEFA, respectively, in response to glucose and insulin injection in TTL can be related to the higher proportion of muscle and visceral adipose depots, and possibly to higher insulin responsiveness.

The results from GTT and ITT during the periods of NEB and PEB demonstrate that NEB can impair insulin sensitivity and develop insulin resistance in both FTL and TTL and this detrimental effects are more severe in FTL. In FTL, induced NEB compared to PEB reduced the CR of glucose and insulin during ITT by 52 and 56 %, respectively, whereas in TTL the reduction was 25 and 20 %, respectively. These differences demonstrate that insulin CR and glucose metabolic response to ITT was affected more in FTL compared to TTL as a consequence of NEB which is not consistent with higher plasma NEFA concentration in TTL during NEB; however, it is in accordance with the higher plasma TNF-α concentration in FTL (174.48 versus 82.45 ng/l FTL and TTL respectively). In cows suffering from different degree of fatty liver, there was a negative association between plasma NEFA concentration and insulin-stimulated reduction in plasma glucose level during ITT (Ohtsuka et al., 2001). Higher plasma NEFA concentration as a consequence of NEB can cause insulin resistance, impaired insulin secretion and diabetes through production of oxygen radicals, including the reactive oxygen species and reactive nitrogen species, and subsequently oxidative stress (Xu et al., 2014). Moreover, plasma NEFA have been reported to stimulate the phosphorylation of insulin receptor substrate 1 (IRS-1) on serine residues which can reduce insulin-induced tyrosine phosphorylation of IRS-1, a necessary step for normal activation of the insulin signaling pathway (Newsholme et al., 2014). Glucose-fatty acid cycle is another underlying mechanism explaining the fatty acid-induced insulin resistance. According to this theory, when availability of fatty acids is abundant, their oxidation inhibits utilization of glucose as substrate for cellular metabolism. This process is mediated by different intracellular pathways, including inhibition of pyruvate dehydrogenase, phosphofructokinase activity, hexokinase activity, and decreased GLUT4 translocation in skeletal muscle (Sears and Perry, 2015). Increasing plasma concentration of NEFA through fasting or intravenous administration of tallow has been reported to hamper insulin-stimulated glucose uptake by insulin-sensitive tissues (Johnston et al., 2018), whereas reduction of plasma NEFA by abomasal delivery of nicotinic acid improved insulin-stimulated glucose uptake (Cincović et al., 2018). In the current study, higher degree of insulin resistance as a consequence of NEB was observed in FTL, whereas plasma NEFA concentration was higher in TTL. It seems that in addition to elevated plasma concentration of NEFA, elevated concentration of TNF-α plays a crucial role in the development of insulin resistance. Consecutive subcutaneous injection of TNF-α has been reported to lead to liver accumulation of triglycerides in dairy cows (Bradford et al., 2009).

When CR of NEFA during periods of NEB and PEB was compared, the reduction in CR of NEFA during NEB compared to PEB was not different between FTL (44 %) and TTL (41 %). This may indicate that fatty acids metabolic response to insulin was the same in FTL and TTL. Reduction of plasma NEFA concentration in response to glucose injection was equal in ewes with pregnancy toxemia, high risk of pregnancy toxemia and low risk of pregnancy toxemia (Duehlmeier et al. 2013). Moreover, in the current study, clearance of glucose in GTT and ITT during the NEB and PEB in both genotypes was lower compared to CR of NEFA which indicates that compared to glucose, metabolism of fatty acids is more responsive to insulin. It has been reported that the response of fatty acid metabolism occurs at lower plasma insulin concentration compared to glucose metabolism (De Koster et al. 2015).

Conclusions

The FTL were more insulin resistant than TTL, regardless of EB, and induced NEB increased the severity of insulin resistance more in FTL compared to TTL. The higher degree of insulin resistance in FTL can be the result of elevated plasma NEFA concentration in combination with enhanced inflammatory cytokines.

Acknowledgement

The authors would like to appreciate Engineer Rahmani for his help and guidance during GTT and ITT.

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