Association Between Plasma Metabolites and Insulin Sensitivity Indexes in Fat-Tailed and Thin-Tailed Lambs During Negative and Positive Energy Balances

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Abstract:

BACKGROUND: Fatty acid mobilization and inflammatory response of adipose tissues vary in various depots, hence the response of fat-tailed and thin-tailed sheep breeds to different energy balances was hypothesized to be different due to differences in proportion of adipose depots.

OBJECTIVES: Current study aimed to evaluated the changes in plasma metabolites in response to negative and positive energy balances and their correlation with insulin sensitivity indexes in Lori-Bakhtiari fat-tailed and Lori-Bakhtiari × Romanov cross breed thin-tailed lambs.

METHODS: Lambs experienced periods of negative (21 d) and positive (21 d) energy balances. Lambs were bled weekly to measure plasma metabolites. Pearson correlation coefficients among variables were generated using Proc Corr of SAS. Results: In thin-tailed lambs, plasma NEFA showed a negative correlation with plasma glucose (R = -0.47; P<0.0003) and insulin (R = -0.46; P<0.0005) content. Plasma NEFA negatively correlated with revised quantitative insulin sensitivity check index (RQUICKI) and severity of the correlation was higher in fat-tailed (R = -0.58; P<0.0001) comparing to thin-tailed (R = -0.40; P<0.003) lambs. In fat-tailed lambs, plasma NEFA and insulin were the most influential factors affecting RQUICKI, whereas in thin-tailed lambs, insulin was the main factor affecting RQUICKI.

CONCLUSIONS: The results demonstrate that despite higher basal and negative energy balance induced plasma NEFA content in thin-tailed lambs, the contribution of plasma NEFA to insulin resistance was higher in fat-tailed lambs, whereas negative correlation between plasma NEFA and insulin content in thin-tailed lambs demonstrates higher susceptibility of insulin secretion capacity to negative energy balance.

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Introduction

Mobilization of NEFA and inflammatory cytokines secretion from adipose tissues during negative energy balance are considered as the main contributor of metabolic disorders including fatty liver, ketosis and insulin resistance in ruminants (Schönfeld and Wojtczak, 2008; Bradford et al., 2009; Xu et al., 2014). Subcutaneous injection of TNF-α caused insulin resistance in steers (Kushibiki et al., 2001a). Various adipose depots have different contribution to whole body metabolism. Visceral adipose depots are more responsive to fluctuation in energy balance and accumulation of fat in visceral depots in human and rodents has been related to metabolic disorders such as liver steatosis, insulin resistance and diabetes (Catalano et al., 2010; Bjørndal et al., 2011). Moreover, various adipose depots were shown to differently affect insulin sensitivity in dairy cows due to differences in adipokine secretory capacity, mobilization of fatty acids (Drackley et al., 2014; Saremi et al., 2014; De Koster et al., 2015). Fat-tail as a body reserve was less responsive to negative energy balance and lipolytic stimulus (Khachadurian et al., 1966; Almeida et al., 2013) and due to its great mass, low and constant release of NEFA from this site can guarantee the viability of animal through periods of feed shortage and negative energy balance (Atti et al., 2004). Proportion of various adipose depots varies considerably in fat-tailed and thin-tailed lambs. Fat-tailed sheep breeds are characterized by their high body fat content which is mainly deposited in fat-tail region (Atti et al., 2004), whereas in thin-tailed breeds, majority of body fat is deposited in visceral depots. According to the above mentioned differences in metabol-ic response of adipose depots and also considerable difference in proportion of various depots in fat-tailed and thin-tailed lambs, NEFA mobilization and inflammatory response of fat-tailed and thin-tailed lambs and their contribution to whole body insulin sensitivity was hypothesized to be different during different energy balances. Hence, current study aimed to evaluate the associations between plasma metabolites related to insulin sensitivity and their contribution to insulin sensitivity index in Lori-Bakhtiari fat-tailed and Lori-Bakhtiari × Romanov cross breed thin-tailed lambs during negative and positive energy balances.

Material and Methods

Animals, diet and housing: The experiment was carried out at Natural Resources & Agricultural Research farm of Tehran University, Karaj, Iran. Thirty-six male lambs (18 from Lori-Bakhtiari pure breed and 18 from Lori-Bakhtiari and Romanov F1 cross breed) with average body weight of 41.08 ± 4.59 and age of 5-6 months were divided into 3 groups of 12 lambs (6 fat-tailed and 6 thin-tailed lambs) in each according to their body weight and were placed in individual pens. The experiment began after two weeks of adaptation to pens and lasted for 42 days. Lambs were fed a balanced total mixed ration (TMR) 150% of their maintenance requirement during adaptation period. Lambs were fed twice daily at 8:00 and 17:00 in equal amount and had free access to water across the experiment. At the end of adaptation period, the first group was randomly selected and slaughtered. For induction of negative energy balance, the remained 2 groups were fed 90, 80 and 70% of

their maintenance requirement respectively during weeks 1, 2 and 3 of the experiment. At the end of week 3, the second group was randomly selected and slaughtered and the remained group was fed ad-libitum until the end of experiment (day 42) to experience a period of positive energy balance and then slaughtered for sample collection.

Blood sampling and glucose and insulin tolerance tests: Samples of blood were taken weekly through jugular vein by heparinized vacuum tubes. Plasma was separated by centrifuging the tubes at 3000 RPM for 15 min at 4 ºC and stored at -20 ºC until analysis. Plasma metabolites including glucose, insulin, NEFA, triglyceride and TNF-α were measured using commercial kits (PARS AZMOON CO, Iran for glucose and triglyceride, Randox Laboratories Ltd., Ardmore, UK for insulin and NEFA, SHANGHAI CRYSTAL DAY BIOTECH CO. LTD for TNF-α). The revised quantitative insulin sensitivity check index (RQUICKI) was calculated by the following formula (Perseghin et al., 2001):

RQUICKI = 1/[log glucose (mg/dl) + log insulin (µU/ml) + log NEFA (mmol/l)].

Glucose and insulin tolerance tests: Glucose and insulin tolerance tests were performed on four randomly selected lambs from each genotype in two consecutive days at the end of negative and positive energy balances to measure insulin sensitivity according to (Salin et al., 2012). The same lambs were used for glucose and insulin tolerance tests in both negative and positive energy balances. Lambs were deprived of feed 16 hours before the beginning of the tests. Blood samples were collected through sterile catheter (14G×5.1 cm; Jelco™, Johnson and Johnson, Mumbai, India) inserted into jugular vein of the lambs. Samples of times

-10 and 0 were collected for determination of basal concentration of metabolites. At the time of 0, glucose solution (500 mg of glucose/kg body weight as sterile 50 % solution) was injected through catheter and subsequent blood sampling was done at 5, 10, 20, 30, 40, 60, 90, 120, and 150 min after glucose injection collecting in heparinized vacuum tubes. Insulin challenge was done the day after the glucose tolerance test by jugular vein injection of insulin (0.1 U/kg of body weight) and blood samples were collected during time intervals same as glucose tolerance test. Tubes were placed in ice bath and then centrifuged for 15 min at 3000 RPM. Plasma samples were separated and stored at -20 ºC until subsequent analysis. Glucose and insulin clearance rates (CR) 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.

Statistical analysis: Pearson correlation coefficients among variables were generated using Proc Corr of SAS. Regression analysis was done by using proc GLM in SAS (Institute, 2002) software for determination of the relationship among variable including plasma metabolites and insulin sensitivity index. Differences between least-squared means were considered to be significant at $P < 0.05$.

Results

The data related to performance and

Table 1. Pearson correlation coefficients among plasma metabolites and RQUICKI in fat-tailed (over diagonal line) and thin-tailed (under diagonal line) lambs negative and positive energy balances. NEFA: Non-esterified fatty acid, Glucose/Insulin: Glucose to insulin ratio, RQUICKI: Revised quantitative insulin sensitivity check index, TG: Triglyceride, TNF-α: Tumor necrosis factor alpha.

Item	NEFA	Glucose	Insulin	Glucose/Insulin	RQUICKI	TG	TNF-α
NEFA	1	-0.23	-0.08	0.05	-0.58	0.03	-0.03
P-value		0.08	0.55	0.68	0.0001	0.82	0.80
Glucose	-0.47	1	0.20	0.06	-0.13	0.13	-0.35
P-value	0.0003		0.15	0.64	0.35	0.36	0.01
Insulin	-0.46	0.33	1	-0.81	-0.55	-0.07	-0.002
P-value	0.0005	0.01		0.0001	0.0001	0.64	0.99
Glucose/Insulin	0.45	-0.39	-0.86	1	0.58	0.05	-0.17
P-value	0.0008	0.004	0.0001		0.0001	0.74	0.29
RQUICKI	-0.40	0.07	-0.45	0.43	1	0.02	0.002
P-value	0.003	0.59	0.0008	0.001		0.88	0.98
TG	-0.07	-0.04	0.03	-0.02	0.07	1	-0.14
P-value	0.60	0.75	0.80	0.86	0.59		0.37
TNF-α	0.05	0.10	0.002	-0.008	-0.18	-0.12	1
P-value	0.71	0.49	0.98	0.95	0.24	0.43	

plasma metabolites are reported elsewhere, hence a brief comparison is made here. The aim for induction of negative energy balance was to simulate a situation like transition period that is happening in female animals. Although there are some studies inducing negative energy balance in sheep (Almeida et al., 2013), as the breeds used in current study were totally different from the breeds used in those researches, we had to set our own feeding program for induction of negative and positive energy balances. Hence, plasma NEFA content was defined weekly in order to be sure that the lambs were experiencing negative and positive energy balances properly. Plasma NEFA content was increased in response to negative energy balance (P<0.0001) and the enhancement was considerably higher in thin-tailed lambs (P<0.004) despite higher body weight and fat loss in fat-tailed lambs during negative energy balance. Fat-tailed lambs showed higher plasma TNF-α content comparing to thin-tailed lambs regardless of energy balance (P<0.0001). Induced

negative energy balance reduced plasma insulin (P<0.11) and glucose (P<0.0001) content and severity of this reduction was more in thin-tailed lambs. Negative energy balance significantly increased glucose to insulin ratio in thin-tailed but not fat-tailed lambs during the third week of negative energy balance (P<0.04). Plasma TG content was decreased and increased respectively in thin-tailed and fat-tailed lambs during the second and third weeks of negative energy balance (P<0.14). Induced negative energy balance reduced RQUICKI regardless of genotype (P<0.03). During the second week of negative energy balance, RQUICKI was significantly higher in fat-tailed comparing to thin-tailed lambs (P<0.04), where lower RQUICKI at the end of third week of negative energy balance in fat-tailed lambs was not statistically significant.

Correlation among plasma metabolites and insulin sensitivity index: The correlation among plasma metabolites and RQUICKI in fat-tailed and thin-tailed lambs is presented in Table 1. In thin-tailed but

Table 2. Pearson correlation coefficients among plasma metabolites, CR of Glucose, NEFA and insulin, and RQUIC-KI in fat-tailed (over diagonal line) and thin-tailed (under diagonal line) lambs during glucose tolerance test in negative and positive energy balances. CR: Clearance rate, NEFA: Non-esterified fatty acid, TNF-α: Tumor necrosis factor alpha, RQUICKI: Revised quantitative insulin sensitivity check index, Glu/Insu: Glucose to insulin ratio.

Item	Glucose CR	NEFA CR	Insulin CR	TNF-α	RQUICKI	NEFA	Glucose	Insulin	Glu/Insu
Glucose CR	1	0.48	-0.18	0.08	-0.31	-0.43	0.64	0.83	-0.72
P-value		0.32	0.72	0.87	0.54	0.38	0.16	0.03	0.10
NEFA CR	-0.04	1	-0.62	0.15	-0.58	0.17	0.05	0.82	-0.68
P-value	0.91		0.18	0.76	0.21	0.73	0.91	0.04	0.13
Insulin CR	-0.58	0.89	1	-0.49	0.93	-0.65	0.29	-0.54	0.71
P-value	0.16	0.006		0.31	0.005	0.15	0.56	0.26	0.10
TNF-α	0.21	0.33	0.46	1	-0.54	0.31	-0.67	0.38	-0.62
P-value	0.63	0.46	0.35		0.25	0.54	0.14	0.45	0.18
RQUICKI	0.13	0.48	0.26	-0.38	1	-0.67	0.26	-0.63	0.82
P-value	0.76	0.26	0.60	0.39		0.13	0.61	0.17	0.04
NEFA	-0.08	-0.38	-0.47	-0.16	-0.51	1	-0.64	-0.05	-0.18
P-value	0.85	0.38	0.34	0.71	0.24		0.16	0.91	0.72
Glucose	-0.05	0.23	0.56	0.65	-0.10	-0.68	1	0.21	0.01
P-value	0.90	0.61	0.24	0.11	0.82	0.09		0.68	0.97
Insulin	0.14	0.08	0.28	0.62	-0.17	-0.69	0.76	1	-0.92
P-value	0.75	0.86	0.58	0.13	0.70	0.08	0.04		0.008
Glu/Insu	0.16	0.0008	-0.34	-0.64	0.48	0.45	-0.74	-0.91	1
P-value	0.72	0.99	0.50	0.12	0.27	0.30	0.05	0.004	

not fat-tailed lambs, there was a negative correlation between plasma NEFA and insulin content. Plasma NEFA was negatively correlated with plasma glucose in both fat-tailed and thin-tailed lambs, however the severity of this negative association was 2-fold higher in thin-tailed comparing to fat-tailed lambs. Plasma insulin was positively correlated with plasma glucose in both fat-tailed (not statistically significant) and thin-tailed lambs and severity of this positive correlation was higher in thin-tailed lambs. There was a positive correlation between plasma NEFA and glucose to insulin ratio in thin-tailed but not fat-tailed lambs. Plasma concentration of glucose negatively correlated with glucose to insulin ratio in thin-tailed but not fat-tailed lambs. In addition, plasma insulin showed a strong negative correlation with glucose to insulin ratio in both fat-tailed and thin-tailed lambs. Plasma

TNF-α was negatively correlated with glucose in fat-tailed but not thin-tailed lambs. There was a significant negative correlation between plasma NEFA and RQUICKI and severity of this correlation was higher in fat-tailed comparing to thin-tailed lambs. There were significant positive and negative correlations respectively between glucose to insulin ratio and insulin with RQUICKI in both fat-tailed and thin-tailed lambs, however severity of these correlations was higher in fat-tailed lambs.

Correlations during glucose tolerance test: During glucose tolerance test, glucose CR was positively correlated with plasma insulin concentration in fat-tailed but not thin-tailed lambs (Table 2). There was a positive correlation between CR of NEFA and plasma insulin in fat-tailed but not thin-tailed lambs. Insulin CR was positively correlated with RQUICKI and glucose to

Table 3. Pearson correlation coefficients among plasma metabolites, CR of Glucose, NEFA and insulin, and RQUICKI in fat-tailed (over diagonal line) and thin-tailed (under diagonal line) lambs during insulin challenge in negative and positive energy balances. CR: Clearance rate, NEFA: Non-esterified fatty acid, TNF-α: Tumor necrosis factor alpha, RQUICKI: Revised quantitative insulin sensitivity check index, Glu/Insu: Glucose to insulin ratio.

Item	Glucose CR	NEFA CR	Insulin CR	TNF-α	RQUICKI	NEFA	Glucose	Insulin	Glu/Insu
Glucose CR	1	-0.18	-0.53	-0.54	0.02	-0.15	0.48	0.03	0.12
P-value		0.72	0.27	0.25	0.95	0.77	0.33	0.94	0.80
NEFA CR	0.52	1	0.74	-0.42	0.82	-0.61	0.20	-0.25	0.53
P-value	0.23		0.09	0.39	0.04	0.19	0.69	0.62	0.27
Insulin CR	0.33	0.74	1	-0.32	0.72	-0.48	0.23	-0.43	0.51
P-value	0.46	0.05		0.52	0.10	0.33	0.65	0.38	0.29
TNF-α	0.28	0.16	0.02	1	-0.54	0.31	-0.67	0.38	-0.62
P-value	0.58	0.75	0.96		0.25	0.54	0.14	0.45	0.18
RQUICKI	0.002	-0.07	0.44	-0.38	1	-0.67	0.26	-0.63	0.82
P-value	0.99	0.88	0.38	0.45		0.13	0.61	0.17	0.04
NEFA	-0.81	-0.40	-0.52	-0.14	-0.52	1	-0.64	-0.05	-0.18
P-value	0.04	0.43	0.28	0.78	0.28		0.16	0.91	0.72
Glucose	0.71	0.55	0.50	0.66	-0.10	-0.68	1	0.21	0.01
P-value	0.11	0.25	0.30	0.14	0.84	0.13		0.68	0.97
Insulin	0.89	0.31	0.10	0.60	-0.17	-0.71	0.86	1	-0.92
P-value	0.01	0.54	0.84	0.19	0.73	0.11	0.02		0.008
Glu/Insu	-0.78	-0.54	-0.14	-0.62	0.52	0.44	-0.84	-0.88	1
P-value	0.06	0.26	0.78	0.18	0.28	0.38	0.03	0.01	

insulin ratio (P<0.10) in fat-tailed but not thin-tailed lambs. In thin-tailed lambs, there was a positive correlation between CR of insulin and NEFA, whereas this correlation was negative in fat-tailed lambs but not statistically significant.

Correlations during insulin challenge: The CR of NEFA was positively correlated with insulin CR in both fat-tailed and thin-tailed lambs (Table 3). The variation in RQUICKI was positively associated with variation in CR of NEFA in fat-tailed but not thin-tailed lambs. The CR of NEFA was negatively correlated with plasma NEFA in both fat-tailed and thin-tailed lambs (not statistically significant) and the severity of this correlation was higher in fat-tailed lambs. There was a positive correlation between CR of insulin and RQUICKI in fat-tailed lambs, however it was not statistically significant. There was a negative correlation between plasma NEFA and glucose CR in thin-tailed but not fat-tailed lambs. There was a not significant positive correlation between plasma glucose and glucose CR in thin-tailed but not fat-tailed lambs. In thin-tailed but not fat-tailed lambs, there was a strong positive correlation between plasma insulin and glucose CR. In thin-tailed lambs, there was a negative correlation between glucose to insulin ratio and glucose CR.

Regression analysis: The regression analysis of RQUICKI versus plasma metabolites including NEFA, glucose, insulin, TG and TNF-α in fat-tailed and thin-tailed lambs is presented in Table 4. Stepwise inclusion of factors in the model showed that plasma NEFA and insulin were the main factors describing the variation in RQUICKI. In fat-tailed lambs, plasma NEFA alongside with plasma insulin were the most influen-

Table 4. Regression of RQUICKI from plasma NEFA, glucose, insulin, triglyceride and TNF-α in fat-tailed and thin-tailed lambs during negative and positive energy balances. Y = Revised quantitative insulin sensitivity check index (RQUICKI). NEFA: Non-esterified fatty acid, TG: Triglyceride, TNF-α: Tumor necrosis factor alpha.

Genotype	Regression equations	R2
Fat-tailed lambs	Y = 0.853 - 1.072 (NEFA)	0.34
	Y = 1.306 - 1.167 (NEFA) - 0.0065 (glucose)	0.40
	Y = 1.360 - 1.219 (NEFA) - 0.0036 (glucose) - 0.057 (insulin)	0.73
	Y = 1.357 - 1.277 (NEFA) - 0.0034 (glucose) - 0.057 (insulin) - 0.0001 (TG)	0.73
	Y = 1.388 - 1.255 (NEFA) - 0.0038 (glucose) - 0.058 (insulin) + 0.0002 (TG) - 0.000034 (TNF-α)	0.73
Thin-tailed lambs	Y = 0.726 - 0.283 (NEFA)	0.16
	Y = 1.035 - 0.344 (NEFA) - 0.0046 (glucose)	0.19
	Y = 1.172 - 0.57 (NEFA) - 0.002 (glucose) - 0.061 (insulin)	0.69
	Y = 1.159 - 0.606 (NEFA) - 0.002 (glucose) - 0.062 (insulin) +0.00094 (TG)	0.67
	Y = 1.086 - 0.588 (NEFA) - 0.00019 (glucose) - 0.065 (insulin) + 0.00089 (TG) - 0.00036 (TNF-α)	0.69

tial factors, whereas in thin-tailed lambs, plasma insulin was the most influential factor contributing in RQUICKI variation.

Discussion

The data related to performance and blood metabolites were discussed elsewhere, hence they are not further considered here. The negative correlation observed between plasma NEFA and insulin contents in thin-tailed lambs originated from, respectively, lowered and enhanced plasma insulin and NEFA content during negative energy balance which were more severe in thin-tailed comparing to fat-tailed lambs. Lowered insulin content can be related to detrimental effect of elevated concentration of plasma NEFA on pancreas insulin secretion as enhanced concentration of plasma NEFA in dairy cows has been reported to hamper the insulin-secretory capacity of pancreas (Jaakson, 2012). Moreover, higher plasma insulin in fat-tailed lambs might be related to higher degree of insulin resistance in fat-tailed lambs and/or lack of detrimental effect of NEFA on insulin secretory capacity due to lower plasma NEFA content in fat-tailed comparing to thin-tailed lambs. High-

er negative association of plasma NEFA with plasma glucose content in thin-tailed lambs can be related to more reduction and enhancement respectively in plasma glucose and NEFA concentrations during negative energy balance. The lower reduction in glucose content in fat-tailed lambs in response to negative energy balance can be explained by higher insulin resistance in fat-tailed comparing to thin-tailed lambs. The stronger positive correlation between plasma insulin and glucose content in thin-tailed lambs is related to more coordinated changes of these metabolites in thin-tailed comparing to fat-tailed lambs during different energy balances. Higher plasma glucose and insulin in fat-tailed lambs during the negative energy balance are indicators of higher degree of insulin resistance as plasma insulin and glucose content has been reported to positively correlate with insulin resistance (Olefsky et al., 1973). The positive and negative associations of plasma NEFA and glucose with glucose to insulin ratio in thin-tailed lambs can be explained by the negative correlation of plasma NEFA with insulin content, as each unit of reduction in insulin (in response to elevated NEFA) can enhance glucose to insulin ratio

more than the decline in glucose to insulin ratio as a consequence of each unit of reduction in glucose content (in response to elevated NEFA). The negative correlation between plasma insulin and glucose to insulin ratio in both genotypes can be explained by the effect of insulin on both denominator and numerator of the fraction as insulin reduces plasma glucose concentration and more severe negative correlation in thin-tailed lambs might be related to higher degree of insulin resistance in fat-tailed lambs. The negative correlation between plasma TNF-α and glucose in fat-tailed lambs is related to lower and enhanced concentration of glucose and TNF-α respectively during negative energy balance. The body fat loss was considerably higher in fat-tailed comparing to thin-tailed lambs, whereas elevation in plasma NEFA in response to negative energy balance was considerably higher in thin-tailed lambs (data shown elsewhere). As plasma NEFA showed more negative association with RQUICKI and body fat mobilization was also more in fat-tailed lambs, lower NEFA concentration in fat-tailed comparing to thin-tailed lambs during negative energy balance seems to be caused by some post-mobilization strategies. Higher liver TG formation and VLDL secretion might be the likely contributor, since there was an elevation in plasma TG in fat-tailed lambs in response to negative energy balance. Glucose to insulin ratio and RQUICKI are indicators of insulin sensitivity, hence positive association between these variables in fat-tailed and thin-tailed lambs seems to be logical as glucose to insulin ratio has been reported to be positively correlated with insulin sensitivity index (Vuguin et al., 2001). The lower severity of the positive correlation between glucose to

insulin ratio and RQUICKI in thin-tailed lambs can be related to the harsh enhancement in glucose to insulin ratio at the last week of negative energy balance which was in opposition to lowered RQUICKI in this week. The RQUICKI is an indicator of insulin sensitivity and high plasma insulin content represents lowered insulin sensitivity, hence the negative correlation between plasma insulin and RQUICKI in fat-tailed and thin-tailed lambs is logical and stronger correlation in fat-tailed lambs can be related to the fact that the reduction in RQUICKI during the last week of negative energy balance was accompanied by higher reduction in plasma insulin content in thin-tailed comparing to fat-tailed lambs.

Glucose tolerance test: In fat-tailed lambs, the positive correlation between plasma insulin and glucose CR can originate from lower and higher values of insulin and glucose CR respectively during negative and positive energy balances, whereas lack of this correlation in thin-tailed lambs can be related to the fact that lower insulin concentration during negative energy balance was associated with higher glucose CR (data shown elsewhere). These differences seem to originate from higher degree of insulin resistance in fat-tailed lambs as a consequence of negative energy balance. The positive correlation between insulin CR and RQUICKI in fat-tailed lambs can be related to coordinated decline and enhancement in these parameters respectively during negative and positive energy balances, however this correlation was not observed in thin-tailed lambs, possibly due to interanimal variation in response to glucose tolerance test. The negative correlation between insulin CR and glucose CR in thin-tailed lambs and the negative and positive

CR in thin-tailed lambs can be due to the harsh enhancement in glucose to insulin ratio during negative energy balance and also higher decline in glucose CR during negative energy balance. This negative correlation demostrates that when glucose to insulin ratio increases due to the harsh decline in insulin concentration, it can not be a suitable representative of insulin sensitivity as RQUICKI and the CR of glucose, insulin and NEFA were decreased in response to negative energy balance.

It has been reported that TNF-α can negatively affect insulin sensitivity (Kushibiki et al., 2001b; Nieto-Vazquez et al., 2007). In current study, although there was a negative correlation between plasma TNF-α and glucose to insulin ratio during insulin challenge, weekly measurement of plasma metabolites with higher number of animal per genotype during the whole experiment revealed no association between plasma TNF-α content and glucose to insulin ratio in both fat-tailed and thin-tailed lambs. The other likely explanation can be the fact that the negative effect of TNF-α on glucose to insulin ratio appears during the extreme negative energy balance when plasma concentration of TNF-α was highest.

Regression analysis demonstrated that in fat-tailed lambs, plasma NEFA and insulin content were the main factors affecting variation in RQUICKI, whereas in thin-tailed lambs insulin was the main factor influencing RQUICKI. In fat-tailed lambs, the effects of negative energy balance and elevated NEFA on insulin sensitivity might be through its detrimental effects on insulin receptors and their binding capacity (Le Marchand-Brustel et al., 2003), as there was a negative correlation (not statistically significant) between plasma NEFA and

insulin CR in fat-tailed lambs during glucose tolerance test. In thin-tailed lambs, although insulin was the main factor affecting RQUICKI, the effect of insulin seems to be mediated by NEFA as there was a negative correlation between plasma NEFA and insulin concentration in thin-tailed lambs. High plasma NEFA content can cause insulin resistance, imaired insulin secretion and diabetes through induction of oxidative stress (Schönfeld and Wojtczak, 2008; Xu et al., 2014). Plasma NEFA stimulates phosphorylation of insulin receptor substrate 1 (IRS-1) on serine residues which can reduce its insulin-induced tyrosine phosphorylation, a prerequisite for normal activation of insulin signaling pathway (Le Marchand-Brustel et al., 2003). Glucose-fatty acid cycle is another underlying mechanism contributing to fatty acid-induced insulin resistance in which high availability of fatty acids and their subsequent oxidation inhibit utilization of glucose as substrate for cellular metabolism. This process is mediated by different intracellular pathways including inhibition of pyruvate dehydrogenase, phosphofructokinase and hexokinase activity and decreased GLUT4 translocation in skeletal muscle (Hue and Taegtmeyer, 2009). Increased concentration of plasma NEFA through fasting or intravenous administration of tallow has been reported to hamper insulin-stimulated glucose uptake by insulin-sensitive tissues (Oikawa and Oetzel, 2006; Pires et al., 2007b; Schoenberg et al., 2012), whereas reduction of plasma NEFA by abomasal delivery of nicotinic acid improved insulin-stimulated glucose uptake (Pires et al., 2007a). Despite the higher enhancement in plasma NEFA in response to negative energy balance in thin-tailed lambs, it showed stronger association with

RQUICKI in fat-tailed lambs which demonstrates that elevation of plasma NEFA as a consequence of negative energy balance whould be more detrimental in fat-tailed compared to thin-tailed lambs. The negative correlation between plasma NEFA and insulin content in thin-tailed lambs demonstrates that plasma NEFA can hamper insulin secretion in thin-tailed but not fat-tailed lambs. Plasma NEFA and insulin were the main metabolites affecting insulin sensitivity in fat-tailed lambs, whereas in thin-tailed lambs, insulin was the main factor affecting insulin sensitivity.

Conclusion: The results of current study demonstrate that in fat-tailed lambs, plasma NEFA and insulin content are the major determinants of insulin sensitivity index, whereas in thin-tailed lambs, plasma insulin content is by far the main determinant of insulin sensitivity index. These difference might originate from different response of fat-tailed and thin-tailed lambs to negative energy balance as insulin secerotary capacity was suppressed more in thin-tailed lambs in response to negative energy balance, while insulin responsiveness was suppressed more in response to negative energy balance.

Akcnowledgements

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همبستگی بین متابولیت‌های خونی و شاخص‌های حساسیت انسولین در بره‌های دنبه‌دار و بدون دنبه در دوره‌های با تعادل منفی و مثبت انرژی

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چکیده

زمینه مطالعه: بسـیج اسـیدهای چرب و پاسخ التهابی بافت‌های چربی بسـته به محل قرارگیری آن‌ها در بدن متفاوت است و بنابراین فرض شد که پاسخ نژادهای گوسفند دنبه‌دار و بدون دنبه به تعادل انرژی‌های مختلف به دلیل نسبت‌های متفاوت و همچنین متابولیسم متفاوت بافت‌های مختلف چربی در بدن آن‌ها متفاوت باشد که این امر می‌تواند باعث اثرگذاری بر حساسیت به انسولین بدن حیوان گردد.

هدف: هدف از این مطالعه بررسی تغییرات متابولیت‌های خونی شامل اسیدهای چرب غیراستریفه، گلوکز، انسولین، تری‌گلیسرید و TNF-α در پاسخ به تعادل منفی و مثبت انرژی و ارزیابی میزان همبستگی این متابولیت‌ها با شاخص‌های حساسیت به انسولین در بره‌های دنبه‌دار نژاد خالص لری-بختیاری و بره‌های آمیخته بدون دنبه لری-بختیاری و رومانو بوده است.

روش کار: تعداد ۳۶ بره (۱۸ بره نر دنبه‌دار و ۱۸ بره بدون دنبه) به منظور تجربه کردن شرایط تعادل منفی (۲۱ روز) و مثبت (۲۱ روز) انرژی در پن‌های انفرادی قرار داده شدند. خون‌گیری از بره‌ها به صورت هفتگی به منظور اندازه‌گیری متابولیت‌های خونی انجام شد. ضرایب همبستگی پیرسون در بین متغیرهای مورد بررسی با استفاده از رویه Corr نرم‌افزار آماری SAS تعیین شد.

نتایج: در بره‌های بدون دنبه و نه بره‌های دنبه‌دار، NEFA پلاسمایی همبستگی منفی با غلظت‌های پلاسمایی گلوکز (P<۰/۰۰۳ ; R=-۰/۴۷) و انسـولین (R=-۰/۴۶ ; P<۰/۰۰۵) نشـان داد. میزان NEFA پلاسـمایی همبستگی منفی با شاخص حساسیت به انسـولین (RQUICKI) نشان داد و شدت این همبستگی در بره‌های دنبه‌دار (R=-۰/۵۸ ; P<۰/۰۰۰۱) بیشتر از بره‌های بدون دنبه (R=-۰/۴۰ ; P<۰/۰۰۳) بود. در بره‌های دنبه‌دار، NEFA و انسولین تأثیرگذارترین عوامل موثر بر RQUICKI بودند در حالی که در بره‌های بدون دنبه انسولین موثرترین عامل اثرگذار بر RQUICKI بود.

نتیجه‌گیری نهایی: نتایج مطاله حاضر نشان می‌دهد که علیرغم غلظت‌های بالاتر NEFA پایه و القاء شده توسط تعادل منفی انرژی در بره‌های بدون دنبه، اثرگذاری NEFA بر مقاومت به انسـولین در بره‌های دنبه‌دار بیشـتر بود در حالی که همبستگی منفی بین غلظت‌های پلاسمایی NEFA و انسولین در بره‌های بدون دنبه نشان‌دهنده حساسیت بیشتر ظرفیت ترشحی انسولین نسبت به NEFA پلاسمایی در این بره‌ها می‌باشد.

واژه‌های کلیدی: همبستگی، تعادل انرژی، بره‌های دنبه دار، حساسیت به انسولین، اسیدهای چرب غیراستریفه

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