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RESEARCH ARTICLE

Adipogenic Gene Expression and Regulation Pattern in Adipose Tissues Located on Mammary, Inguinal and Renal Fat Pad of Korean Black Goat (*Capra hircus*)

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ABSTRACT

Development of new fat cells appears to be a permanent event throughout the life process of both humans and animals. Though, this process appears to be similar all over the body, but whether their anatomical location keeps them distinct in their genetic makeup is quite uncertain. Therefore, to investigate the difference in the adipogenic gene expression and their regulation pattern at the molecular level, we collected adipose tissues surgically from prepubertal Korean black goat reared in stall fed system and with high fat rich diet. Perirenal fat, inguinal fat and mammary fat pad adipose tissues were collected and cyropreserved in LN2 for mRNA isolation subsequently. Reverse transcriptase PCR revealed significant ($P<0.05$) down-regulation of adipo Q in mammary fat pad and inguinal fat, but no change was observed in renal fat in the high fat rich diet fed group as compared to the control group. Significant down-regulation in LPL and resistin gene was noticed in parallel in all the tissues in the treatment group. However, PPAR-γ was observed to be significantly down-regulated only in perirenal fat and whereas CEBP-α was down-regulated in both inguinal and perirenal fat in the high fat rich diet fed goats as compared to the control. Down-regulation of specific adipogenic gene clearly shows that optimization of high fat rich diet in dairy goat management is highly essential. This further suggests that adipose tissue participates more directly than previously thought in metabolic activities and energy balance.

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INTRODUCTION

Adipose tissues in mammals have been basically divided into two types like brown adipose tissue (BAT) and white adipose tissue (WAT) (Gesta *et al.,* 2007). Both these tissues are important in energy balance, however, they differ in their functions. BAT is specialized in energy dissipation as heat during cold- and diet-induced thermogenesis, whereas WAT is mainly involved in energy storage and mobilization in the form of triacylglycerols. In addition, white adipocytes have intracrine, autocrine/paracrine and endocrine properties, such as the secretion of leptin and adiponectin. WAT is found in several depots throughout the body, and location varies between species. Adipose tissues found in the mammary fat pad and inguinal regions are directly controlled by hormonal influence (Chen *et al.*, 2013). Peri-renal fat does not have functional activity, however, it serves to protect kidney from any injury. In general, fat deposition in mammary fat pad and inguinal region respond strongly to sex hormones, whereas peri-renal fat deposition responds to corticosteroids from adrenal glands (Peckett *et al.*, 2011). Moreover, high fat rich diet intake has also been shown to increase mammary fat pad (MFP) and inguinal fat as well as adipose formation in the body (Gorres *et al.*, 2010). High fat rich diet intake in prepubertal heifers has been the regular practice in dairy industry to earn quick bucks instead it causes economic setbacks in terms of decline in the productive efficiency of the bovine mammary tissues. Moreover, genetic alteration in the developing fat under the influence of high fat rich diet has received very little attention until now. Several studies on adipose tissues from single location have been put forward and investigated. Nevertheless, some results of tissues obtained from different location have shown differences in their metabolic activity. However, literature

on adipose gene expression from different locations in ruminants, particularly goats are meager. Therefore, we decided to investigate adipose gene expressions on fat located at different anatomical location of Korean black goat (KBG) fed on a high fat rich diet.

MATERIALS AND METHODS

Eight KBGs (approximately 90 days old) were grouped into two with four goats in each. All the animals were maintained as per the animal ethics guidelines of Kangwon National University, Republic of Korea. The concentrate and timothy hay as per the standard was fed to animals (g/kg dry matter basis) as per the standards of treatment group.The BW of individual calves were recorded at start and thereafter at fortnightly intervals until 90 days of the trial in the morning before feeding, in order to assess changes in BW and average daily gain (ADG).

The goats were biopsied and all animals were sedated mildly via intramuscular injection of xylazine hydrochloride (Bayer Korea, Korea). A sterilized biopsy instrument was used to collect a sample of mammary gland tissue from midsection of left or right rear quarters using a biopsy instrument (4 mm diameter and 100 mm length; Somatex Medical Technologies GmbH, Germany). Following biopsy, postoperative care and management was done under the supervision of veterinarian for a period of one week.

Dorso-ventral incision below the umbilical cord was done under epidural analgesia obtained with xylazine and ketamine (Singh *et al.*, 2001). Renal adipose tissues were also collected after locating the right kidney. All the tissues stored in LN2 were first taken out in sterilized mortar and it was grounded to powder with pestle. Finally, mRNA was isolated from the tissue samples using TRIzol reagent (Invitrogen; Life Technologies Inc., Grand Island, NY) according to manufacturer's instructions. Various genes in the tissues were detected and quantified by reverse transcriptase-PCR with specific primers on 1% agarose gel and gel documentation system. List of primers used have been tabulated (Table 1). Results were analyzed using ANOVA with the help of SAS statistical software package.

RESULTS

Body weight changes and growth: Average daily intake of the concentrate mixture per goat was observed as expected. The BW of the goats at start and during all other measurements was not different ($P>0.05$) between the groups, but the values tended to increase ($P<0.05$) with increase in their age (Fig.1). All the animals fed with high fat rich diet gained significant ($P<0.05$) body weight compared to the control animals that were given only timothy hay on ad libitum basis (Fig.1).

Reverse transcriptase PCR: Significant ($P<0.05$) down-regulation of adipo-Q was observed particularly in mammary fat pad and inguinal fat. However, no alteration was noted in renal fat. CEBP-α and PPAR-γ was found to be significantly down-regulated in renal fat, but their expressions did not change for mammary fat pad and inguinal fat. On the contrary, significant ($P<0.05$) down-regulation of LPL and resistin gene was noticed in parallel with all the tissues in treatment group as compared to the control animals (Table 3). Contrastingly, the expressions of UCP1 and ap2 were not observed in any of the adipose tissues obtained from different locations of Korean black goat (Fig. 2).

DISCUSSION

Adipose tissue located in different parts of the body are not characteristically similar, as the fat deposition at different parts are influenced by different phenomena such as food consumption, age, breed or ethnicity, activity of sex steroids, environmental stress factors and other regulatory hormones at the particular site of fat deposition (Chen *et al.*, 2012). Furthermore, their functional properties also depend on such sites, where they categorized accordingly as white adipose tissue (WAT) and brown adipose tissue (BAT) (Gesta *et al.*, 2007). The fat accumulation and site of deposition is highly dependent on the sex, which means the sex steroids play pivotal role in the adipose tissue formation and deposition (Taylor *et al.*, 2010). However, to establish whether adipose tissue genes get influenced with their location other than their native expressions, we analyzed and discussed the expression of different genes in different adipose tissue in our experiment. Adipo-Q is a secreted protein and has been reported to be affected by obesity and was established in rodents and humans (Hu *et al.*, 1996).We observed significant ($P<0.05$) down-regulation of adipo-Q in the mammary fat pad and inguinal fat with no alteration in renal fat and that indicated that the deposition of mammary, inguinal and renal fat is not being influenced and regulated by the same factors. Sex steroids play important and pivotal roles (has been reported) in mammary development (Lamote *et al.*, 2004). Therefore, estrogen, being the female sex steroids (estrogens) in the Korean black goats, assisting the mammogenesis might have caused the down-regulation of adipo-Q in the mammary fat and inguinal fat as the estrogens have been demonstrated to protect against adipose accumulation particularly in these subcutaneous fats (Ribas *et al.*, 2010).

Table I: List of primers used in RT-PCR gene expression

Gene	Accession number	5′sequence	3′ sequence
Adipo Q	BC140488	5'-GATCCAGGTCTTGTTGGTCCTAA-3'	5'-GAGCGGTATACATAGGCACTTTCTC-3'
LPL	M16966	5'-TACCCTGCCTGAAGTTTCCAC-3'	5'-CCCAGTTTCAGCCAGACTTTC-3'
UCP1	NM_012682.2	5'-AACACTGTGGAAAGGGACGAC-3'	5'-CATGGTCATTGCACAGCTG -3'
Resistin	NM_183362.1	5'-AGTCCACAGAGAGGCACCTG-3'	5'-TGGTGACCTCCTGGATCTTC-3'
PPARγ	NM_001100921.1	5'-ACGGGAAAGACGACAGACAAA-3'	5'-GACGGAGCGAAACTGACACC-3'
C/EBPα	BC149006.1	5'-AGTCCGTGGACAAGAACAGC-3'	5'-GGTCATTGTCACTGGTCAGC-3'
ap2	NM 024406	5'-CCGCAGACGACAGGA-3'	5'-CTCATGCCCTTTCATAAACT-3'

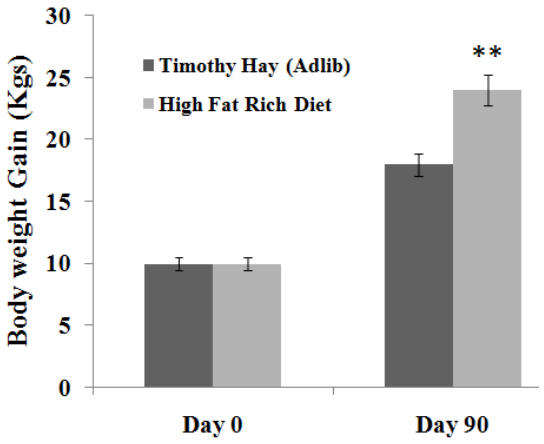

Fig. 1: Body weight (kg) gain by Korean black goats fed with timothy hay and high fat rich diet.

Moreover, pathology due to obesity (high fat rich diet animals) induces important signaling molecules such *ob* gene product and TNF-α have been reported to get expressed and produced and which finally down-regulates adipo-Q expression (Hu *et al.*, 1996). However, renal fatty tissue development has always been under the influence of glucorticoids and adipo-Q expression and has been reported to be down-regulated by glucocorticoids (Oliveira *et al.*, 2011). High fat rich diet fed goats showed consistent expression in adipo-Q group, however, difference was non-significant as compared with control animals. An increase in the expression of adipo-Q during obesity phenomenon in goats compelled us to assume and understand that HFD fed goats did not realize any stressors during the course of experiment. It is a knownfact that secretion of glucorticoids occurs only under stressful conditions and down-regulation of adipo-Q in control animals apparently revealed that animals fed without high fat rich diet were certainly under stressful conditions during the course of experiment.

PPARγ has been shown to mediate the expression of specific genes to activate adipocyte differentiation programs in vitro (Tontonoz *et al.*, 1994). Moreover, overfeeding causes adipogenesis by activating PPARγ, which could be a vital part of molecular mechanisms in adipocyte deposition (Spiegelman *et al.*, 1997). In this present research, the PPARγ gene showed consistent expression in both mammary and inguinal fats of both HFD fed and control goats. But, the PPARγ gene got down-regulated in renal fat of HFD fed goat compared to the control animals. We assume that down-regulation of the PPARγ gene in the renal fat of HFD fed goats might have been due to the less activity of the glucocorticoids as a result of its meager supply or no production. Glucocorti-coid is one of the major stress hormones that influences the adipogenesis in the renal area and has been well known as a factor which intensifies PPARγ expression, in fact the direct involvement of glucocorticoids is required for the initiation of PPARγ expression (Wu *et al.*, 1996). Glucocorticoids are released resultant to stressful conditions (Hamm *et al.*, 1999) within the biological system and due to this fact, we believe that HFD must have reduced or combated any such stressors and have reduced the stress level absolutely which might have restricted the stimulus for glucocorticoids release and therefore the PPARγ gene did not maintain its expression within the renal tissues and subsequently their expression levels got down-regulated (Hamm *et al.*, 1999).

CCAAT/Enhancer binding protein (C/EBP) plays a key role in adipocyte differentiation (Im *et al.*, 2007), thereby it is possible to play a central role in the regulation of adipose tissue metabolism. Among the C/EBPs, C/EBPα has a predominant role in the adipogenesis in a "feed forward" manner, where the C/EBPα expression is induced by PPARγ, further the C/EBPα enhances the fatty acid binding protein ap2 expression (Rosen, 2005; Chu *et al.*, 2012). The present finding showed that C/EBPα has been down-regulated in the inguinal and renal fat of HFD fed goat compare to the control goat. However, the mammary fatty tissue had an almost similar expression for C/EBPα in both control and treatment goat that might indicate lack of availability of glucocorticoid release in HFD fed goats due to combated stressors in this group of animals.

Lipoprotein lipase (LPL) is the enzyme that regulates deposition and metabolism of fatty acids in adipose tissue (Frayn *et al.*, 1995). Down-regulation of LPL gene in the mammary fat pad collected from all three sites of HFD fed Korean black goats have been observed compared to the control animals in our experiment established that there was limited secretion and activity of stress combating hormone (glucocorticoids and sex steroids) in the HFD fed goat compared to the control goat. However, in certain studies, higher expression of LPL was reported in obese animals (Berman *et al.*, 1999), which indicates the up-regulation of LPL expression in obesity, however we observed down-regulation of this gene. Nevertheless, high fat rich diet down-regulated LPL in our study in KBG defines that either LPL has different signaling factors or pathways which needs to be discovered to establish why higher expression were not evident in these species. However, some other studies have also been reported weakened LPL activation in obese subjects (Reynisdottir *et al.*, 1997).

Resistin, also known as FIZZ3 (found in inflammatory zone 3) and ADSF (adipocyte-secreted factor), belongs to a novel family of cysteine-rich proteins, was initially identified in a screen for adipocyte specific transcripts down-regulated by the treatment with TZDs and it was initially suggested as a link between diabetes and obesity (Holcomb *et al.*, 2000). Glucocorti-coidsare known to be an inducer for resistin expression (Shojima *et al.*, 2002). Since resistin gene was downer-gulated, we assume that the stimulus for glucocorticoid release was absent and therefore the said gene expression was not up-regulated in treatment animal group.

Table 3: Adipogenic gene expression in fat pads of mammary, inguinal and renal tissues of high fat rich diet fed KBG

	Fat Depots	Adipo-Q	PPARγ	C/EBPα	LPL	Resistin
Expression in the HFD group compared to the control	Mammary	↓	NSCE	NSCE	↓	↓
	Renal	NSCE	↓	↓	↓	↓
	Inguinal	↓	NSCE	↓	↓	↓

↓: Down regulation, NSCE: Non-significant change in expression

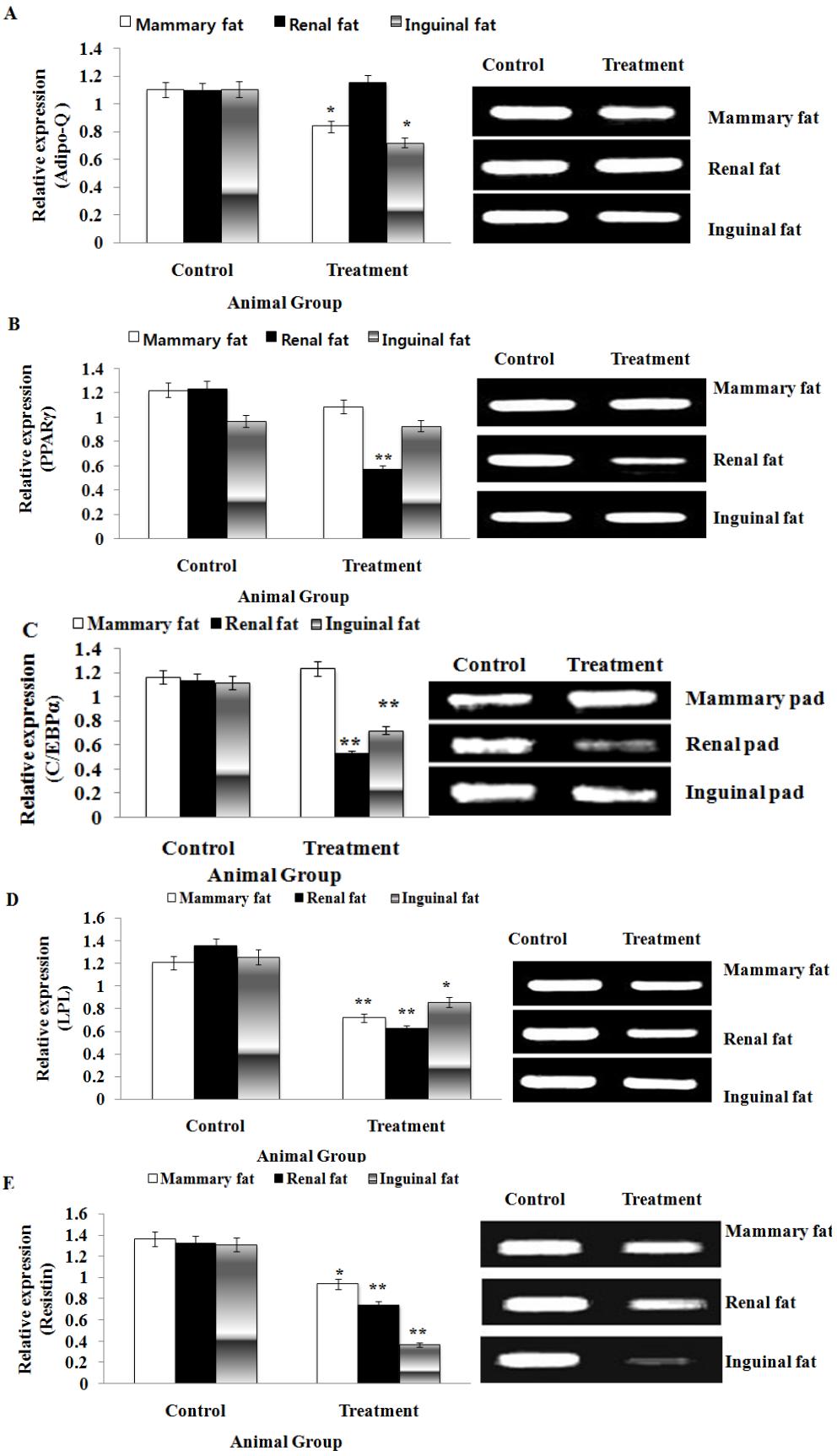

Fig 2: RT-PCR verification showing the expression percentage for different adipocyte-specific genes in adipose tissues collected from different body sites of Korean black goat (mammary gland, inguinal region and renal region). The bars represent the mean±SE. A) Adipo-Q, B) PPAR-□, C) CEBP-α, D) LPL and E) Resistin.

Mitochondrial uncoupling proteins (UCP) are members of the family of mitochondrial anion carrier proteins (MACP). UCPs separate oxidative phosphorylation from ATP synthesis with energy dissipated as heat, also referred to as the mitochondrial proton leak. Tissue specificity occurs for the different UCPs and the exact methods of how UCPs transfer H+/OH- are not known. UCPs contain the three homologous protein domains of MACPs expressed only in brown adipose tissue, which is a specialized tissue functions to produce heat (Lim *et al.*, 2012). Since we analyzed UCP in adipose tissues from mammary, inguinal, renal fat pads, where all of these fat pads are white fat, and thus the absence of this gene in these samples could be justified with above mentioned fact.

Adiponectin (aP2) is a differentiation dependent adipocyte marker expressed in both white and brown adipose tissues. aP2 is a downstream target for PPARγ and C/EBPα, which get activated when PPARγ and C/EBPα bind to the promoter enhancer of aP2 gene (Rosen, 2005). Moreover, the up-regulated expression of aP2 was detected in the glucocorticoid induced adipogenesis (Ito *et al.*, 2007). However in our study, the expression of aP2 gene was undetectable, suggestively because of the lesser secretion of glucocorti-coids and declined expressions of both PPARγ and C/EBPα.

Conclusion: Renal fat development differs from mammary and inguinal fat pad development as both of these regions are primarily influenced by different hormones.

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