



## RESISTIN GENE EXPRESSION: NOVEL STUDY IN DROMEDARY CAMEL (*CAMELUS DROMEDARIUS*)

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### Summary

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Resistin, an adipocyte-specific hormone involved in insulin resistance and adipocyte differentiation, was initially identified in adipose tissue and macrophages. The physiological role of this molecule in camels remains largely unexplored. This study analysed for the first time blood and tissue levels of resistin as well as expression of resistin gene by real time PCR in adipose tissue (hump, visceral & epididymal) and different muscles (gastrocnemius, heart and caecum) in dromedary camels. The results revealed that resistin concentration was significantly ( $P < 0.01$ ) higher in epididymal adipose tissue as compared to other tissues and the lowest concentration was detected in serum. Additionally, the differential mRNA expression levels of resistin gene showed the highest expression level in epididymal adipose tissue as compared to other tissues. In conclusion, the results demonstrated for the first time that resistin was expressed in different tissues of dromedary camels. These data underscore an important facet of the physiological role of resistin as a factor involved in insulin resistance and glucose metabolism in camels.

**Key words:** adipose tissue, dromedary camel, gene expression, resistin

### INTRODUCTION

Adipose tissue is an active metabolic tissue that secretes multiple metabolically important proteins known as adipokines (e.g., leptin, adiponectin, TNF- $\alpha$ , IL-6 and resistin among others). These adipocyte-derived products are presently subject to intensive research concerning their involvement in the regulation of adipose tissue physiology (Gulcelik *et al.*, 2009). In this context, resistin is an adipocyte-

derived cytokine. Also known as found in inflammatory zone 3 (FIZZ3) or adipocyte specific secretory factor (ADSF), it belongs to the family of resistin-like molecules defined by a cysteine-rich region in the C-terminal domain (Steppan *et al.*, 2001).

Resistin gene could be expressed in several cell types. In mice, adipocytes are considered the major source of resistin

(Steppan *et al.*, 2001), whereas in humans, resistin mainly comes from monocytes and macrophages (Patel *et al.*, 2003).

Resistin plays an important role in the development of insulin resistance and obesity in rodents (Lazar, 2007). Beside its effects on glucose metabolism and insulin sensitivity and through its action on multiple cell targets in both rodents and humans, it exerts proinflammatory processes in adipose tissue (Nagaev *et al.*, 2006) and vascular endothelium (Li *et al.*, 2007), promotes vascular smooth muscle cell proliferation (Calabro *et al.*, 2004), and stimulates *in vitro* angiogenesis (Di Simone *et al.*, 2006). Furthermore, Kim *et al.* (2001) revealed that resistin was involved in the control of adipocyte differentiation. Also, it had a role in feeding behaviour (Tovar *et al.*, 2005) and energy metabolism (Zhang *et al.*, 2016; Cisternas *et al.*, 2019). Additionally it was reported that resistin could affect male and female fertility. Indeed, expression of resistin (mRNA and protein) had been reported in several reproductive tissues including hypothalamus (Morash *et al.*, 2002; Tovar *et al.*, 2005; Wilkinson *et al.*, 2005), pituitary gland (Morash *et al.*, 2002), and testis (Nogueiras *et al.*, 2004).

The ability of the dromedary to live under desert conditions and to survive in incredibly harsh environment is due to its biological and physiological particularities. Indeed, all the functions of the camel are conceived to be physiologically adapted to water and food restrictions and to an excessively hot climate (Ouajd & Kamel, 2009).

Although several studies on presence of resistin had been performed with rodents, humans, cattle, buffaloes, sheep, pigs and hamsters, to our knowledge no studies are available in camels. So, the present study explored the possible role of

resistin in the camel physiology through detection of resistin concentration in blood, different muscle types and adipose tissues with investigation on the expression and distribution pattern of resistin gene on the mRNA level in different muscle types and adipose tissues of dromedary camels.

## MATERIALS AND METHODS

### *Animals*

In the current study, three male dromedary camels (3–4 years old; weighing 400–500 kg) were used. The animals were kept under hygienic conditions in a shaded place in the farm of Faculty of Veterinary Medicine – Zagazig University. The animals care and use were performed following the rules of the Institutional Animal Care and Use Committee (IACUC) of Zagazig University (approval number: ZU IACU/2/F/34/2020). All animals were fed diet that fulfilled their nutritional recommendations. Water was available *ad libitum*.

### *Blood and tissue sampling*

Jugular vein blood samples were collected from camels in clean tubes without anticoagulant and allowed to clot at room temperature, then centrifuged at 3000 rpm for 15 minutes for sera separation. The obtained sera were stored at –80 °C until used for hormonal analysis.

Immediately after slaughtering the animals, tissue samples from skeletal muscles (gastrocnemius), cardiac muscles (heart), smooth muscle (caecum) and adipose tissues (hump, visceral & epididymal) were collected. The tissues were washed in ice-cold 0.9 % (w/v) sodium chloride and quickly frozen in liquid nitrogen then stored at –80 °C for RT-qPCR

analysis to evaluate the mRNA expression profile of resistin gene.

Tissue segments from skeletal muscles (gastrocnemius), cardiac muscles (heart), smooth muscle (caecum) and adipose tissues (hump, epididymal and visceral fat) were carefully homogenized in phosphate buffer saline (PBS, pH 7.4, 10 mg tissue to 100 µL PBS) and then centrifuged at 5000 rpm for 15 min. After that the supernatants were stored at -20 °C until analyzed.

#### *Resistin hormone assay*

Resistin levels were measured in serum, tissue supernatants of different muscle types, and adipose tissue using commercial rat resistin (RSN) Sandwich ELISA Kit (Cat.No: MBS013451; MyBiosource, Inc., San Diego, CA 92195- 3308, USA).

#### *Quantitative real-time polymerase chain reaction for resistin gene*

RNA was isolated from the camel tissues, muscles (gastrocnemius, heart, caecum) and adipose tissues (hump, epididymal and visceral fat) using easy-REDTM total RNA extraction kit (cat No 17063; iNtRON Biotechnology, Inc), following the manufacturer's guidelines. Quantitative real-time PCR was conducted on Applied Biosystems™ 7400 real-time PCR system using TOPreal™ qPCR 2× premix (SYBR Green with low ROX) (cat No RT500M; Enzynomics). The specific primers for the different genes of interest are

presented in Table 1. The target genes expression level was normalised to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

After the reaction, the threshold cycle (Ct) was obtained for each sample. ΔCt values of each specimen were calculated by subtracting the Ct values of specimen's GAPDH from Ct value of sample's resistin. Relative resistin mRNA expression levels were calculated as  $2^{-\Delta Ct}$ .

#### *Statistical analysis*

Data analysis was performed by one-way ANOVA followed by Dunnett's multiple comparisons test (GraphPad Prism v. 7.00 for Windows, GraphPad Software, La Jolla California USA). Data were expressed as means±standard deviation (SD).

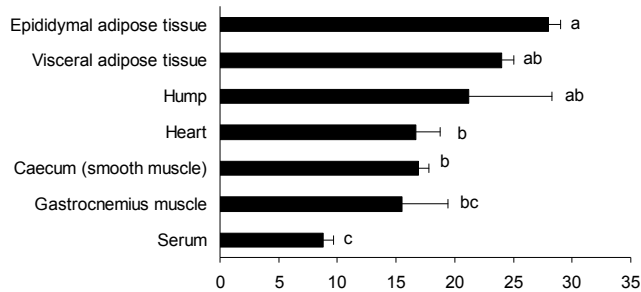
## RESULTS

#### *Resistin hormone levels*

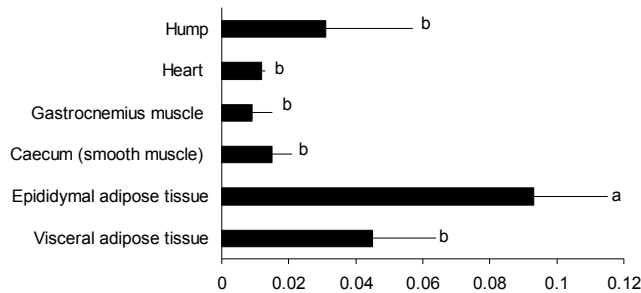
The values of resistin hormone detected in serum & tissue supernatants of skeletal muscle (gastrocnemius), cardiac muscle (heart), smooth muscle (caecum) and adipose tissues (hump, visceral epididymal) are shown on Fig. 1. The serum resistin value was  $8.75 \pm 0.93$  ng/mL. Concerning tissue values of resistin hormone, the concentrations in different muscles (gastrocnemius, heart, caecum) were  $15.5 \pm 3.90$ ;  $16.67 \pm 2.12$  and  $16.92 \pm 0.87$  ng/mg

**Table 1.** Oligonucleotide primers sequences used for real time PCR

Genes	Primers	Accession number
Glyceraldehyde 3-phosphatedehydrogenase (GAPDH)	Sense: 5'ATGGTGAAGGTCGGAGTGAACGG-3' Antisense: 5'GCAGAGATGATGACCCTCTTGGC-3'	XM_010990867.1
Resistin gene	Sense: 5'-GCACCTGCAGGATGAAGGCTCTC-3' Antisense: 5'-TCCATGCCTGCGCACTGGCAGT-3'	XM_010978247.1



**Fig. 1.** Mean±SD resistin hormone levels (ng/mL) in serum, different muscles (gastrocnemius, heart, caecum) and adipose tissues (hump, visceral, epididymal) in dromedary camels. Values with different letters are significantly different for similar tissue types.



**Fig. 2.** Mean±SD resistin gene expression in different muscles (gastrocnemius, heart, caecum) and adipose tissues (hump, visceral, epididymal) in dromedary camels. Gene expression data are derived from normalisation of resistin gene expression by the expression of the internal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) control gene. Values with different letters are significantly different for similar tissue types.

respectively, without statistical significant ( $P>0.05$ ) differences among different muscle types. The resistin levels in hump, visceral, epididymal adipose tissues were  $21.17\pm 7.11$ ;  $24\pm 1$ ; and  $28\pm 1$  ng/mg, respectively ( $P>0.05$ ). Collectively, the highest ( $P<0.01$ ) resistin concentration was observed in epididymal adipose tissue as compared with other tissues.

*Resistin gene expression*

The expression and normal distribution patterns of resistin mRNA were examined in muscles and adipose tissues of drome-

dary camel (Fig. 2). The differential mRNA expression level in gastrocnemius, heart, caecum muscles was  $0.009\pm 0.006$ ;  $0.012\pm 0.001$ ;  $0.015\pm 0.006$  respectively but differences were insignificant ( $P>0.05$ ). Regarding adipose tissue, respective expression levels were  $0.031\pm 0.026$ ;  $0.045\pm 0.019$  and  $0.093\pm 0.022$  for hump, visceral, and epididymal adipose tissues respectively. The highest ( $P<0.01$ ) expression level was observed in epididymal adipose tissue as compared with other tissues.

## DISCUSSION

This study is the first demonstration of distribution and expression of resistin mRNA in different types of muscles and adipose tissues of dromedary camels and also, provides quantification of resistin protein contents in camel blood and tissues.

Till now, resistin transcript has been detected in mammals including humans (Patel *et al.*, 2003) where it was firstly expressed in peripheral blood mononuclear cells (PBMCs) with increased expression during differentiation into macrophage (Kaser *et al.*, 2003), in mice (Steppan *et al.*, 2001), pigs (Dai *et al.*, 2006), yaks (Mengdian *et al.*, 2019), goats (Zhang *et al.*, 2019), cattle (Kang *et al.*, 2006) and sheep (Zhao *et al.*, 2015). In rodents, resistin mRNA was expressed only in the adipose tissue (Steppan *et al.*, 2001), but several studies had shown that resistin was also expressed in other tissues such as lung and liver in sheep (Zhao *et al.*, 2015), goat (Zhang *et al.*, 2019), yak (Mengdian *et al.*, 2019) and the liver of fish (Siberian sturgeon) (Tang *et al.*, 2020).

Beside adipose tissue and macrophages, resistin is secreted by epithelial cells from the gastrointestinal tract (mainly the colonic epithelium), goblet cells, skeletal muscle, adrenal glands, spleen, pancreas, trophoblastic cells of placenta, and synovial tissue (Steppan & Lazar, 2002; Rajala *et al.*, 2003; Banerjee *et al.*, 2004; Gravelleau *et al.*, 2005; Filková *et al.*, 2009; Jamaluddin *et al.*, 2012; Codoner-Franch & Alonso-Iglesias, 2015), testis (Nogueiras *et al.*, 2004) and astrocytes (Morash *et al.*, 2002). It is also detected in hypothalamus and pituitary gland, revealing its role in reproduction in humans and rodents (Wilkinson *et al.*, 2007). Furthermore, resistin is not only

present in human serum, but also in amniotic fluid (Yura *et al.*, 2003; Di Simone *et al.*, 2006), cerebrospinal fluid (Kos *et al.*, 2007), saliva (Yin *et al.*, 2012; Mamali *et al.*, 2012) and breast milk (Savino *et al.*, 2012). Overall, these observations indicate that the mRNA expression level of resistin in tissues is species-specific; however, to our knowledge no information regarding the resistin gene is available in dromedary camels.

This study characterised the changes of resistin mRNA expression and protein concentration in blood, different muscles and adipose tissues, and results showed that the profile of resistin mRNA consistently matched the profile of resistin protein level in examined camel tissues, out of which adipose tissue had high resistin content and also showed markedly high levels of resistin expression. Our results are in accordance with those of Steppan *et al.* (2001) who revealed that resistin mRNA expression was abundant in adipose tissue but not in other tissues of mice. Similarly, Komaatsu *et al.* (2003) found that resistin mRNA was expressed in bovine adipose tissue and mammary gland and the pattern of expression changed in association with milk production. Moreover, Tiwari *et al.* (2012a) revealed a significant positive correlation of subcutaneous adipose tissue (SAT) resistin mRNA expression with serum resistin and insulin resistance in obese postmenopausal women. Also, Terra *et al.* (2010) found higher resistin mRNA expression of both visceral and subcutaneous adipose tissues in obese vs non-obese women.

Our finding is in contrast with those of Rajala *et al.* (2004) revealing that the resistin mRNA expression was suppressed in the obese. Moreover, porcine adipose tissue showed low level of resistin mRNA

assessed by semi-quantitative RT-PCR (Dai *et al.*, 2006). Furthermore, Ikeda *et al.* (2011) detected reduction in resistin mRNA levels in obese (Zucker) rats than in non-obese (wild-type) rats. However, some reports showed down regulation in resistin mRNA expression and protein in isolated subcutaneous and omental adipocyte. In addition, Jackson *et al.* (2005) observed a significant decrease in resistin mRNA levels in adipose tissue in different obese mouse models with insulin resistance. Savage *et al.*, (2001) found no correlation between insulin resistance and resistin gene expression in whole abdominal adipose tissue.

Resistin level in camel blood serum was low, in agreement with data of Maebuchi *et al.* (2003) who found a decrease in both resistin mRNA expression and protein levels in obese mice. On the contrary, Stepan *et al.* (2001) demonstrated higher serum resistin concentrations in high-fat-induced obese and obese mice models. Additionally, Tiwari *et al.* (2012b) found that higher serum resistin levels in obese postmenopausal women. Resistin protein was significantly elevated in the serum of obese than non-obese humans (Degawa-Yamauchi *et al.*, 2003). Silha *et al.* (2003) revealed that serum resistin level did not correlate well with markers of adiposity. In the same respect, a significant negative correlation of relative visceral adipose tissue (VAT) resistin mRNA expression with serum resistin and insulin resistance (IR) in postmenopausal obese women was detected (Tiwari *et al.*, 2012b).

We must consider the specific features of camel species at various levels of the experimentation in explaining our results concerning low circulating resistin level in camels as it was reported that the diurnal resistin level was lower than the nocturnal level and that this pattern was parallel to

the changes in insulin and glucose and that resistin mRNA and protein levels were suppressed by fasting and increased by refeeding (Rajala *et al.*, 2004).

These findings strengthen the hypothesis of low circulating resistin level in dromedary camels. Finally, regarding dromedary camels, our results proved that resistin was expressed in different camel tissues with highest expression in epididymal adipose tissue as compared with hump, visceral adipose tissue, skeletal muscle, cardiac muscle and smooth muscle. The mRNA expression of resistin in the adipose tissue was higher than in other tissues, suggesting resistin might be mainly secreted from the adipose tissue of dromedary camels; however the expression differs among tissues.

## CONCLUSION

The findings of this study confirmed for the first time that resistin was present and expressed in different tissues of dromedary camels. These data underscore an important facet of the physiological role of resistin as a factor involved in insulin resistance and glucose metabolism in camel species which is characterised by high basal glucose level and low insulin sensitivity. Our data suggest that resistin is synthesised and acts locally or stored and secreted in blood in response to changes in physiological conditions in dromedary camel. Additional researches will be needed to explore the regulation and biological functions of resistin in dromedary camels.

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