



Expression of Three Adipokines (Adiponectin, Leptin and Resistin) in Normal Canine Skin: a Pilot Study

T. Brément, C. Cossec, C. Roux, A.C. Knol, B. Dréno, A. Khammari, P. Bourdeau, V. Bruet

► To cite this version:

T. Brément, C. Cossec, C. Roux, A.C. Knol, B. Dréno, et al.. Expression of Three Adipokines (Adiponectin, Leptin and Resistin) in Normal Canine Skin: a Pilot Study. *Journal of Comparative Pathology*, 2019, 167, pp.82 - 90. <10.1016/j.jcpa.2018.10.179>. <hal-03486021>

HAL Id: hal-03486021

<https://hal.science/hal-03486021v1>

Submitted on 20 Dec 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

SPONTANEOUSLY ARISING DISEASE

Short Title: Adipokines in Normal Canine Skin

Expression of Three Adipokines (Adiponectin, Leptin and Resistin) in Normal Canine Skin: a Pilot Study

**T. Brément^{*}, C. Cossec[†], C. Roux^{*}, A. C. Knol[‡], B. Dréno^{‡, §}, A. Khammari^{‡, §}, P.
Bourdeau^{*, †} and V. Bruet^{*, †}**

^{}Dermatology/Parasitology/Mycology Unit and [†]NP3 Unit, School of Veterinary Medicine-
ONIRIS, University of Nantes, CS 40706, [‡]CRCINA, INSERM, Université d'Angers,
Université de Nantes and [§]Dermatology Service, CIC1413, CHU Nantes, Nantes, France.*

Correspondence to: V. Bruet (e-mail: vincent.bruet@oniris-nantes.fr).

Summary

Adipokines are biologically active cytokines that are mainly produced in adipose tissue. There is evidence, in man and mice, that some adipokines may be secreted in other tissues including the vascular endothelium, epithelia and sebaceous glands. Moreover, modified serum levels of adipokines have been detected in people with acne vulgaris or psoriasis; it is suspected that adipokines could contribute to local and systemic inflammatory conditions. We aimed to evaluate the expression of three adipokines (i.e. leptin, adiponectin and resistin) in normal canine skin. Formalin-fixed, paraffin wax-embedded punch biopsy samples were obtained from the sparsely-haired skin of the caudal ventral abdomen of a single clinically healthy dog with no history of skin disease. Immunohistochemistry was applied, using rabbit polyclonal primary antibodies specific for leptin, adiponectin and resistin. Adipokines were not expressed in normal canine dermis or hypodermis. In contrast, they were detected in the keratinocytes of all epidermal layers and hair follicle segments, sebocytes, apocrine gland cells and in the vascular endothelium. This is the first report on the expression of adipokines in normal canine skin, a first step in studying their role in the skin physiology and inflammatory skin diseases of dogs.

Keywords: dog; skin; adipokines; immunohistochemistry

Introduction

Adipokines (or adipocytokines) are biologically active proteins primarily synthesized by adipocytes, fibroblasts and macrophages within the adipose tissue (Chaldakov *et al.*, 2003; Curat *et al.*, 2006). They include cytokines, chemokines, growth factors, enzymes, hormones, complement factors and matrix proteins (Chaldakov *et al.*, 2003), which are either

pro-inflammatory (interleukin [IL]-6, IL-1 β , aP2, tumour necrosis factor [TNF]- α , leptin, resistin, visfatin and adiponectin) or anti-inflammatory (adiponectin, omentin, apelin, IL-10, IL-1ra, complement factor 1q/TNF-related proteins) in function (Aguilar-Valles *et al.*, 2015).

The literature reports that adipokines are also produced to a lesser extent by other cells and tissues including vascular endothelium, epithelia (including keratinocytes), hepatocytes or sebaceous glands (Poeggeler *et al.*, 2010; Akazawa *et al.*, 2011; Kovacs *et al.*, 2016; Wolk and Sabat, 2016). Therefore, adipokines are involved in numerous organs and metabolic pathways including the regulation of local and systemic inflammatory processes through autocrine/paracrine or endocrine ways (Chaldakov *et al.*, 2003; Murad *et al.*, 2003; Radin *et al.*, 2009).

In man, reported adipokine-associated diseases include obesity, type II diabetes, metabolic syndrome, cardiovascular diseases and inflammatory diseases such as atherosclerosis, Crohn's disease, atopic dermatitis, psoriasis, acne vulgaris and brain diseases (Chaldakov *et al.*, 2003; Koerner *et al.*, 2005; Aguilar-Valles *et al.*, 2015; Karadag *et al.*, 2015; Han *et al.*, 2016; Banihani *et al.*, 2018a, b).

In domestic animals, adipokines may contribute to the regulation of diverse biological processes, including haemostasis/coagulation, blood pressure, lipid metabolism, appetite and energy balance, cell proliferation, angiogenesis, insulin sensitivity, immunity and inflammation (Trayhurn *et al.*, 2006; Radin *et al.*, 2009).

Among the adipokines discovered, leptin, adiponectin and resistin are well documented in the literature (Koerner *et al.*, 2005). Leptin is a highly conserved 167 amino acid protein, encoded by the *ob* gene (Radin *et al.*, 2009). Its main function is to regulate the metabolism of adipose tissue and fat storage through the regulation of feeding behaviour (afferent satiety signal) by complex interactions between the autonomic nervous system and multiple organs. It also affects maternal, placental and fetal function, modifies insulin

sensitivity in the muscle or liver, prevents ectopic lipid deposition, and is involved in some pathophysiological cutaneous processes in man and mice, including skin repair and inflammation (Goren *et al.*, 2003; Koerner *et al.*, 2005; Johnston *et al.*, 2008; Poeggeler *et al.*, 2009; Torocsik *et al.*, 2014; Karadag *et al.*, 2015; Kovacs *et al.*, 2016; Wolk and Sabat, 2016; Zouboulis *et al.*, 2016).

Adiponectin is a 244-amino acid anti-inflammatory protein with a protective role in the pathogenesis of obesity-related disorders and atherosclerosis. Adiponectin is also an important factor in skin physiology and function as it enhances the proliferation and migration of keratinocytes and sebocytes, stimulates the re-epithelialization process, sebaceous lipid production and promotes hair growth in man and mice (Jung *et al.*, 2017). This adipokine can undergo multiple post-translational modifications such as multimer formation or forming free-circulating globular domains whose biological roles are not fully understood (Kadowaki and Yamauchi, 2005).

Resistin is a cysteine-rich protein, which is poorly conserved between species and secreted as a dimer by adipocytes. It is thought to have a role as a mediator of insulin resistance and in inflammatory processes that may be involved in atherosclerosis in man, but its metabolic functions in dogs are not currently well known (Radin *et al.*, 2009).

The role of the adipokines mentioned above in inflammatory skin diseases such as psoriasis or acne vulgaris has been the subject of research in man. There is some evidence showing that adipokines can participate in the pathogenesis of psoriasis. Elevated serum levels of pro-inflammatory adipokines (i.e. leptin and resistin) and decreased serum levels of anti-inflammatory adiponectin were detected in human psoriasis (Shibata *et al.*, 2009; Aly *et al.*, 2013; Huang *et al.*, 2015). Serum leptin levels, tissue leptin and leptin receptor expression were significantly higher in non-obese human patients with severe psoriasis than in patients with mild–moderate psoriasis and controls, and expression showed a positive

correlation with disease duration (Cerman *et al.*, 2008). Moreover, serum concentrations of leptin and resistin were positively correlated with the psoriasis area severity index (PASI), while plasma concentrations of adiponectin were negatively correlated with this index (Johnston *et al.*, 2008; Rajappa *et al.*, 2015; Cerman *et al.*, 2008), and treatment of psoriasis was associated with a return to normal adipokine concentrations (Rajappa *et al.*, 2015).

Adipokines may also contribute to the pathogenesis of human acne vulgaris. In one study, significantly lower serum concentrations of leptin were detected in acne patients compared with a control healthy group (Karadag *et al.*, 2015). Discrepancies exist between studies for adiponectin, since in one of them, higher serum concentrations of adiponectin were detected in acne patients compared with a control healthy group (Karadag *et al.*, 2015), while they were significantly lower in another study (Cerman *et al.*, 2016). Treatment with the anti-acne agent isotretinoin resulted in decreased leptin and increased adiponectin concentrations (Karadag *et al.*, 2015). Leptin was shown to have a modulatory effect on the metabolism of sebaceous glands (Chen *et al.*, 2002; Torocsik *et al.*, 2014; Kovacs *et al.*, 2016) and resistin was suspected to link lipid metabolism and inflammation and to be related to clinical manifestations of acne (Kovacs *et al.*, 2016; Younis *et al.*, 2016).

As nothing is currently known about the role of adipokines in the pathogenesis of canine skin disease, the aim of this pilot study was to assess the expression of three adipokines (i.e. pro-inflammatory leptin and resistin and anti-inflammatory adiponectin) in normal canine skin.

Materials and Methods

Samples of Canine Skin

Punch biopsy samples (8 mm) from the sparsely haired skin of the caudal ventral abdomen and visceral omental adipose tissue were collected from a single clinically healthy 8-year-old neutered female English setter dog with a normal body condition score (BCS) and with no history of skin disease. The dog was anaesthetized, but local analgesia was not performed in order to avoid artefactual tissue modification secondary to injection. All procedures were approved by the institutional ethics committee (APAFIS 8427). Specimens were fixed in 10% neutral buffered formalin for 4 days before being processed routinely and embedded in paraffin wax. Sections (4 μ m) were cut in triplicate on microscope slides.

Primary Antibodies

Prior to antibody selection, the amino acid sequences of canine adiponectin, leptin and resistin were searched using the NCBI protein BLAST software (Supplementary Table 1). To optimize the specificity of binding, the primary antibodies specific for adiponectin, leptin and resistin were chosen after comparing their target sequences (Supplementary Table 2) with the canine epitopes in each adipokine (Supplementary Table 1). Homology between the target sequences and epitopes was compared using the NCBI protein software. The cut-off for choosing an antibody was a minimum of 80% sequence identity and cover, well above the 30% sequence identity, a threshold above which 90% of protein pairs can be considered as structurally homologous according a previously published study (Rost, 1999). Each primary antibody was chosen when a maximum percentage of identity and cover of the epitope by the antibody was reached (Supplementary Table 3).

Rabbit polyclonal primary antibodies were selected. A biotinylated polyvalent goat anti-mouse and rabbit IgG (heavy and light chains) was chosen as a secondary reagent (as part of a mouse- and rabbit-specific HRP/AEC [ABC] detection immunohistochemistry [IHC] Kit, Abcam, Paris, France).

Determination of the Dilution of the Antibodies

For each selected antibody, according suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories (Ramos-Vara *et al.*, 2008), three dilutions around those previously described in studies of dog, mice or man were tested (Table 1). The optimal working dilution was defined as that subjectively giving the strongest labelling with the least background and cheapest cost price.

Immunohistochemistry

Sections were dried for 20 min at 70°C then dewaxed in two changes of xylene for 10 min each, followed by two changes of absolute ethanol (5 min each). Heat-induced antigen retrieval was performed with sections incubated in citrate buffer (pH 6.0; $\times 10$, Antigen Retriever[®], Sigma, St. Louis, Missouri, USA) in a microwavable vessel for 40 min at 150W power. Slides were then cooled for 10 min at room temperature.

Following antigen retrieval, each section was delimited with a Dako Pen (Dako, Glostrup, Denmark). Endogenous peroxidase activity was blocked by incubation in H₂O₂ 3% in methanol (included in the mouse- and rabbit-specific HRP/AEC [ABC] detection kit described above) for 10 min. Each section was saturated in protein block solution (included in the detection kit as described above) at room temperature for 10 min. Avidin–biotin endogenous activity was blocked by incubation in avidin–biotin blocking solution (Abcam) for 15 min.

Sections were incubated with primary antibodies overnight (16 h) at 4°C. Antigen detection was performed with the HRP–AEC detection kit. Briefly, sections were incubated with biotinylated secondary antibody for 10 min at room temperature. To detect the complex formed by the primary and secondary antibodies, slides were incubated with streptavidin

peroxidase for 10 min then with the chromogenic substrate (3-amino-9-ethylcarbazole [AEC]) for 10 min. The reaction was stopped by incubating the sections in distilled water for 10 min. Counterstaining was with Mayer's haematoxylin (Abcam) for 30 sec. Slides were mounted with an aqueous permanent medium (ImmunoHisto Mount™, Sigma).

Positive and Negative Controls

Blood vessel labelling was used as an internal positive control as previously described (Table 1). Additionally, the same protocol was applied on visceral omental adipose tissue as an external positive control. Negative controls were obtained by incubation of slides in pre-absorbed sera as previously described (Ivell *et al.*, 2014). Briefly, recombinant proteins were used in excess and mixed with the matching primary antibody solutions. Recombinant proteins were chosen with the maximum percentage of identity and cover with the primary antibody target sequences. Human adiponectin and leptin recombinant protein were used, as well as rat resisitn recombinant protein, due to a high percentage of homology calculated from the NCBI protein blast software (Supplementary Table 4).

Analysis

A digital image of each slide was taken at low (×40) and high (×400) magnification with an Nikon Labophot-2 microscope (Nikon, Tokyo, Japan) equipped with a digital camera (Olympus DP21; 2 megapixel). For each slide, two examiners assessed the expression pattern and evaluated the localization of the labelling for each epidermal layer and skin structure (i.e. sebaceous glands, hair follicles, sweat glands, blood vessels, dermis and hypodermis). Positive labelling was characterized by pale red to intense brown colouration. A semiquantitative scale was used to assess labelling intensity: 0, no labelling; +, weak labelling; ++, moderate labelling; +++, high labelling).

Results

Optimal Working Dilutions

The following optimal working dilutions were determined: 1 in 200 for the anti-adiponectin antibody and 1 in 500 for the anti-resistin and anti-leptin antibodies.

Expression Profiles of Adipokines in the Skin

The expression profiles of the three studied adipokines were comparable, although some differences in terms of labelling intensity between the different adipokines and structures were noticed (Table 2). Adiponectin, leptin and resistin were expressed in vascular endothelium (positive control), in keratinocytes of all layers of the epidermis except the stratum corneum, in the outer root sheath of hair follicles in all stages of hair growth, in sebocytes from the basal to the luminal part of sebaceous glands and apocrine gland cells (Fig. 1). Globally, the labelling of resistin was weaker than for adiponectin and leptin.

In contrast, labelling was not detected in the dermis and hypodermis (Fig. 2).

Expression Profiles of Adipokines in Omental Adipose Tissue

In contrast to resistin, both adiponectin and leptin were expressed in the omental adipose tissue (Fig. 3).

Discussion

This study aimed to describe the expression of leptin, adiponectin and resistin in normal canine skin. To the best of our knowledge, this is the first report on this subject.

In the present study, leptin, resistin and adiponectin were found to be expressed in the cytoplasm, sometimes also the nucleus, of keratinocytes from each layer of the epidermis, the outer root sheath of hair follicles and in the sebaceous glands. These findings are comparable with features previously described in the literature. Adipokines were reported to be expressed in various murine and human skin structures. Leptin was expressed in the cytoplasm or in both cytoplasm and nucleus of human basal and suprabasal epidermal keratinocytes (Farag *et al.*, 2016; Seleit *et al.*, 2016) and resistin expression was detected in the interfollicular epidermis (Harrison *et al.*, 2007). IHC and in-situ hybridization techniques also showed that leptin was expressed in hair follicles, from catagen to early anagen in mice (Sumikawa *et al.*, 2014) and in the hair matrix keratinocytes, the outer and inner root sheaths and dermal papilla in human skin (Poeggeler *et al.*, 2009). The outer root sheath of hair follicles was also shown to be a source of resistin in human skin (Harrison *et al.*, 2007). Moreover, it was shown that leptin, resistin and adiponectin proteins were expressed in sebaceous glands of healthy patients or patients affected by various skin diseases such as acne, rosacea, melanoma or psoriasis (Harrison *et al.*, 2007; Kovacs *et al.*, 2016). Full length leptin receptor expression was also detected in sebaceous glands, suggesting that those glands are both a source and a target of this adipokine (Torocsik *et al.*, 2014).

The role of these adipokines in cutaneous pathophysiological processes remains unexplored in dogs, but it can be suspected that adipokines may have a function in skin regeneration through keratinocyte proliferation and epithelialization, in hair biology and in the pathogenesis of inflammatory skin diseases, as in man and mice (Harrison *et al.*, 2007; Sumikawa *et al.*, 2014; Farag *et al.*, 2016; Kovacs *et al.*, 2016; Seleit *et al.*, 2016).

Few data are available with regard to dermal expression of adipokines. Our results are in accordance with data from the literature for resistin, which did not show any labelling in human dermis (Harrison *et al.*, 2007). However, unlike the findings of the present study,

leptin was found to be expressed in human dermal fibroblasts and appeared to promote their proliferation as well as collagen synthesis, resulting in accelerated wound healing (Farag *et al.*, 2016). Further studies should be performed to confirm this difference between dogs and man.

Leptin, adiponectin and resistin were previously found to be expressed in canine and rat white adipose tissues, including visceral and subcutaneous adipose tissue (Steppan *et al.*, 2001; Nogueiras *et al.*, 2003; Eisele *et al.*, 2005; Green *et al.*, 2013; Farag *et al.*, 2016). However, in the present study, none of these adipokines were detected in subcutaneous adipocytes and both adiponectin and leptin expression were detected, in contrast to resistin, in omental adipose tissue. This could support the hypothesis of a different metabolism according the localization of adipose tissues and between the different adipokines.

In man, there are some evidence that different fat depots, in particular subcutaneous and visceral adipose tissues, have distinct biological functions and metabolism (Wajchenberg, 2000; Atzmon *et al.*, 2002; Tran *et al.*, 2008; Ibrahim, 2009). Subcutaneous and visceral adipose tissues are different in terms of anatomy, physiology, metabolism (including response to glucocorticoids, androgens and adrenergic stimulation, insulin sensitivity and glucose metabolism, lipolysis and free fatty acids and triglycerides absorption) and clinical impact (Ibrahim, 2009).

Differences between subcutaneous and visceral adipose tissues regarding the capacity to synthesize and release adipokines were also reported and vary according to the adipokine. One study showed that resistin mRNA was significantly more expressed in visceral adipose tissue than in peripheral fat depots (Atzmon *et al.*, 2002). In contrast, leptin mRNA expression was higher in subcutaneous adipose tissue than in visceral fat (Montague *et al.*, 1997; Atzmon *et al.*, 2002). Data about adiponectin were more controversial as mRNA expression of this adipokine as found to be higher in visceral than in subcutaneous adipose

tissue in one study (Atzmon *et al.*, 2002), while the opposite was reported in another (Samaras *et al.*, 2010). Mature adipocytes and stromovascular cells of subcutaneous origin secreted higher concentrations of adiponectin compared with cells of visceral origin in healthy dogs (Mazaki-Tovi *et al.*, 2016). Discrepancies between these results could be explained by species metabolic specificities or sensitivity differences between the techniques used to study the expression profile of the adipokines. We used IHC with specific antibodies that could reveal both secreted or transiently-fixed adipokines, while quantitative reverse transcriptase polymerase chain reaction (PCR), used in all the studies mentioned above, only focuses on secreted adipokines.

The main limitation of the present study was the number of animals studied. All samples were obtained from a single healthy dog. A larger study would be useful to estimate the individual variability in the expression of the adipokines considered in this pilot study and whether the results reported above are consistent among the canine population. Moreover, the use of a single technique (IHC) was another limitation of this study and this technique alone may not be reliable enough to fully evaluate the expression profile of adipokines in the skin. The use of other complementary techniques such as western blotting should be considered to provide confirmation of the specificity of the chosen antibodies (Kurien *et al.*, 2011). One of the major concerns in veterinary IHC is the poor availability of high-quality antibodies. The use of polyclonal antibodies might be responsible for a greater non-specific background labelling and therefore polyclonal antibodies may appear less specific than monoclonal antibodies but, on the other hand, they may be more sensitive (Ramos-Vara, 2008). In human studies, the use of a full-length sequence for proteomic analysis was reported to have a better sensitivity than incomplete sequences since it takes into account the possible variants of the protein secondary to mutations or post-translational modifications (Borges *et al.*, 2010). Therefore, we chose to use polyclonal antibodies based on previous papers and the research

of the best matching profile between the sequence of the suspected epitope inside the full-length amino acid sequence of each adipokine and the targeted sequence of each commercially acquired antibody, to increase both specificity and sensitivity. Further work is needed and should include more sampling sites and animals without skin disease (obese and non-obese dogs) as a control group to compare with animals having various skin diseases such as atopic dermatitis or primary seborrhoeic disorders. This could help to better understand the involvement and the role of adiponectin, leptin and resistin in the pathogenesis of such diseases in dogs. Combining IHC with other techniques such as western blotting, enzyme-linked immunosorbent assay, PCR and in-situ hybridization could bring more qualitative and quantitative data and strengthen the reliability of the results from the present study.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The results of the study were presented at the 29th ESVD–ECVD Annual Congress, 2017, Lausanne, Switzerland.

Conflict of Interest Statement

The authors declare no conflict of interest with respect to the publication of this manuscript.

References

- Aguilar-Valles A, Inoue W, Rummel C, Luheshi GN (2015) Obesity, adipokines and neuroinflammation. *Neuropharmacology*, **96**, 124-134.
- Akazawa Y, Sayo T, Sugiyama Y, Sato T, Akimoto N *et al.* (2011) Adiponectin resides in mouse skin and upregulates hyaluronan synthesis in dermal fibroblasts. *Connective Tissue Research*, **52**, 322–328.
- Aly DG, Abdallah IY, Hanafy NS, Elsaie ML, Hafiz NA (2013) Elevated serum leptin levels in non-obese patients with psoriasis. *Journal of Drugs in Dermatology*, **12**, e25-e29.
- Atzmon G, Yang XM, Muzumdar R, Ma XH, Gabriely I *et al.* (2002) Differential gene expression between visceral and subcutaneous fat depots. *Hormone and Metabolic Research*, **34**, 622–628.
- Banihani SA, Abu-Alia KF, Khabour OF, Alzoubi KH (2018a) Association between resistin gene polymorphisms and atopic dermatitis. *Biomolecules*, **8**, 17-28.
- Banihani SA, Elmadhoun RA, Khabour OF, Alzoubi KH (2018b) The rs2167270 polymorphism of leptin gene is associated with atopic dermatitis. *Dermato-Endocrinology*, **10**, e1454191.
- Borges CR, Rehder DS, Jarvis JW, Schaab MR, Oran PE *et al.* (2010) Full-length characterization of proteins in human populations. *Clinical Chemistry*, **56**, 202-211.

- Çerman AA, Aktaş E, Altunay İK, Arıcı JE, Tulunay A *et al.* (2016) Dietary glycemic factors, insulin resistance, and adiponectin levels in acne vulgaris. *Journal of the American Academy of Dermatology*, **75**, 155-162.
- Cerman AA, Bozkurt S, Sav A, Tulunay A, Elbaşı MO *et al.* (2008) Serum leptin levels, skin leptin and leptin receptor expression in psoriasis. *British Journal of Dermatology*, **159**, 820–826.
- Chaldakov GN, Stankulov IS, Hristova M, Ghenev PI (2003) Adipobiology of disease: adipokines and adipokine-targeted pharmacology. *Current Pharmaceutical Design*, **9**, 1023-1031.
- Chen HC, Smith SJ, Tow B, Elias PM, Farese RV Jr (2002) Leptin modulates the effects of acyl CoA:diacylglycerol acyltransferase deficiency on murine fur and sebaceous glands. *Journal of Clinical Investigation*, **109**, 175–181.
- Curat CA, Wegner V, Sengenès C, Miranville A, Tonus C *et al.* (2006) Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. *Diabetologia*, **49**, 744–747.
- Eisele I, Wood IS, German AJ, Hunter L, Trayhurn P (2005) Adipokine gene expression in dog adipose tissues and dog white adipocytes differentiated in primary culture. *Hormone and Metabolic Research*, **37**, 474-481.
- Farag AG, Elnaidany NF, El-Dien MM (2016) Immunohistochemical expression of leptin in non melanoma skin cancer. *Journal of Clinical and Diagnostic Research*, **10**, WC08-WC12.

- Fujimoto N, Matsuo N, Sumiyoshi H, Yamaguchi K, Saikawa T *et al.* (2005) Adiponectin is expressed in the brown adipose tissue and surrounding immature tissues in mouse embryos. *Biochimica et Biophysica Acta*, **1731**, 1-12.
- Goren I, Kampfer H, Podda M, Pfeilschifter J, Frank S (2003) Leptin and wound inflammation in diabetic ob/ob mice: differential regulation of neutrophil and macrophage influx and a potential role for the scab as a sink for inflammatory cells and mediators. *Diabetes*, **52**, 2821–2832.
- Greene VR, Wilson H, Pfent C, Roethele J, Carwile J *et al.* (2013) Expression of leptin and iNOS in oral melanomas in dogs. *Journal of Veterinary Internal Medicine*, **27**, 1278–1282.
- Han B, Wu WH, Bae JM, Son SJ, Lee JH *et al.* (2016) Serum leptin and adiponectin levels in atopic dermatitis (AD) and their relation to disease severity. *Journal of the American Academy of Dermatology*, **75**, 629-631.
- Harrison WJ, Bull JJ, Selmann H, Zouboulis CC, Philpott MP (2007) Expression of lipogenic factors galectin-12, resistin, SREBP-1, and SCD in human sebaceous glands and cultured sebocytes. *Journal of Investigative Dermatology*, **127**, 1309–1317.
- Huang H, Shen E, Tang S, Tan X, Guo X *et al.* (2015) Increased serum resistin levels correlate with psoriasis: a meta-analysis. *Lipids in Health and Disease*, **14**, 44.
- Ibrahim MM (2010) Subcutaneous and visceral adipose tissue: structural and functional differences. *Obesity Reviews*, **11**, 11–18.

- Ivell R, Teerds K, Hoffman GE (2014) Proper application of antibodies for immunohistochemical detection: antibody crimes and how to prevent them. *Endocrinology*, **155**, 676-687.
- Jin L, Zhang S, Burguera BG, Couce ME, Osamura RY *et al.* (2000) Leptin and leptin receptor expression in rat and mouse pituitary cells. *Endocrinology*, **141**, 333-339.
- Johnston A, Arnadottir S, Gudjonsson JE, Aphale A, Sigmarsson AA *et al.* (2008) Obesity in psoriasis: leptin and resistin as mediators of cutaneous inflammation. *British Journal of Dermatology*, **159**, 342–350.
- Jung YR, Lee JH, Sohn KC, Lee Y, Seo YJ *et al.* (2017) Adiponectin signaling regulates lipid production in human sebocytes. *PLoS One*, **12**, e0169824.
- Kadowaki T, Yamauchi T (2005) Adiponectin and adiponectin receptors. *Endocrine Reviews*, **26**, 439–451.
- Karadag AS, Ertugrul DT, Takci Z, Bilgili SG, Namuslu M *et al.* (2015) The effect of isotretinoin on retinol-binding protein 4, leptin, adiponectin and insulin resistance in acne vulgaris patients. *Dermatology*, **230**, 70–74.
- Koerner A, Kratzsch J, Kiess W (2005) Adipocytokines: leptin – the classical, resistin – the controversial, adiponectin – the promising, and more to come. *Clinical Endocrinology and Metabolism*, **19**, 525–546.

- Kovacs D, Lovaszi M, Poliska S, Oláh A, Bíró T *et al.* (2016) Sebocytes differentially express and secrete adipokines. *Experimental Dermatology*, **25**, 194–199.
- Kurien BT, Dorry Y, Dillon S, Dsouza A, Scofield RH (2011) An overview of western blotting for determining antibody specificities for immunohistochemistry. *Methods in Molecular Biology*, **717**, 55-67.
- Lim HY, Im KS, Kim NH, Kim HW, Shin J *et al.* (2015) Obesity, expression of adipocytokines, and macrophage infiltration in canine mammary tumors. *Veterinary Journal*, **203**, 326-331.
- Mazaki-Tovi M, Bolin SR, Schenck PA (2016) Differential secretion of adipokines from subcutaneous and visceral adipose tissue in healthy dogs: association with body condition and response to troglitazone. *Veterinary Journal*, **216**, 136–141.
- Montague CT, Prins JB, Sanders L, Digby JE, O'Rahilly S (1997) Depot- and sex-specific differences in human leptin mRNA expression: implications for the control of regional fat distribution. *Diabetes*, **46**, 342–347.
- Murad A, Nath AK, Cha ST, Demir E, Flores-Riveros J *et al.* (2003) Leptin is an autocrine/paracrine regulator of wound healing. *FASEB Journal*, **17**, 1895–1897.
- Nogueiras R, Gallego R, Gualillo O, Caminos JE, García-Caballero T *et al.* (2003) Resistin is expressed in different rat tissues and is regulated in a tissue- and gender-specific manner. *FEBS Journal*, **548**, 21-27.

- Poeggeler B, Schulz C, Pappolla MA, Bodó E, Tiede S *et al.* (2010) Leptin and the skin: a new frontier. *Experimental Dermatology*, **19**, 12–18.
- Radin MJ, Sharkey LC, Holycross BJ (2009) Adipokines: a review of biological and analytical principles and an update in dogs, cats, and horses. *Veterinary Clinical Pathology*, **38**, 136–156.
- Rajappa M, Rathika S, Munisamy M, Chandrashekar L, Thappa DM (2015) Effect of treatment with methotrexate and coal tar on adipokine levels and indices of insulin resistance and sensitivity in patients with psoriasis vulgaris. *Journal of the European Academy of Dermatology and Venereology*, **29**, 69–76.
- Ramos-Vara JA, Kiupel M, Baszler T, Bliven L, Brodersen B *et al.* (2008) Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories. *Journal of Veterinary Diagnostic Investigation*, **20**, 393–413.
- Ressel L, Finotello R, Innocenti VM, Vannozzi I, Poli A (2012) Preliminary report on the expression of leptin and leptin receptor (ObR) in normal, hyperplastic and neoplastic canine mammary tissues. *Research in Veterinary Science*, **93**, 343–349.
- Rost B (1999) Twilight zone of protein sequence alignments. *Protein Engineering*, **12**, 85–94.
- Samaras K, Botelho NK, Chisholm DJ, Lord RV (2010) Subcutaneous and visceral adipose tissue gene expression of serum adipokines that predict type 2 diabetes. *Obesity*, **18**, 884–889.

- Seleit I, Bakry OA, Samaka RM, Tawfik AS (2016) Immunohistochemical evaluation of leptin expression in wound healing: a clue to exuberant scar formation. *Applied Immunohistochemistry and Molecular Morphology*, **24**, 296-306.
- Shibata S, Saeki H, Tada Y, Karakawa M, Komine M *et al.* (2009) Serum high molecular weight adiponectin levels are decreased in psoriasis patients. *Journal of Dermatological Science*, **55**, 62–63.
- Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR *et al.* (2001) The hormone resistin links obesity to diabetes. *Nature*, **409**, 307-312.
- Sumikawa Y, Inui S, Nakajima T, Itami S (2014) Hair cycle control by leptin as a new anagen inducer. *Experimental Dermatology*, **23**, 27–32.
- Szalowska E, Elferink MGL, Hoek A, Groothuis GM, Vonk RJ (2009) Resistin is more abundant in liver than adipose tissue and is not upregulated by lipopolysaccharide. *Journal of Clinical Endocrinology and Metabolism*, **94**, 3051-3057.
- Töröcsik D, Kovács D, Camera E, Lovászi M, Cseri K *et al.* (2014) Leptin promotes a pro-inflammatory lipid profile and induces inflammatory pathways in human SZ95 sebocytes. *British Journal of Dermatology*, **171**, 1326–1335.
- Tran TT, Yamamoto Y, Gesta S, Kahn CR (2008) Beneficial effects of subcutaneous fat transplantation on metabolism. *Cell Metabolism*, **7**, 410–420.

- Trayhurn P, Bing C, Wood IS (2006) Adipose tissue and adipokines – energy regulation from the human perspective. *Journal of Nutrition*, **136**, 1935S–1939S.
- Wajchenberg BL (2000) Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocrine Reviews*, **21**, 697–738.
- Wolk K, Sabat R (2016) Adipokines in psoriasis: an important link between skin inflammation and metabolic alterations. *Reviews in Endocrine and Metabolic Disorders*, **17**, 305-317.
- Younis S, Blumenberg M, Javed Q (2016) Resistin gene polymorphisms are associated with acne and serum lipid levels, providing a potential nexus between lipid metabolism and inflammation. *Archives of Dermatological Research*, **308**, 229-237.
- Zouboulis CC, Picardo M, Ju Q, Kurokawa I4, Töröcsik D *et al.* (2016) Beyond acne: current aspects of sebaceous gland biology and function. *Reviews in Endocrine and Metabolic Disorders*, **17**, 319-334.

Received, September 5th, 2018

Accepted, October 26th, 2018

Figure legends

Fig. 1. Global expression profile of resistin, adiponectin and leptin in normal canine skin. IHC. $\times 400$. (a), (e), (i), (m) and (q) show the negative controls (normal canine skin incubated with immunoadsorbed sera). (b), (f), (j), (n) and (r), respectively, show canine vascular endothelium (internal positive control), epidermis, hair follicles, apocrine sweat glands and sebaceous glands labelled for resistin. (c), (g), (k), (o) and (s), respectively, show canine vascular endothelium, epidermis, hair follicles, apocrine sweat glands and sebaceous glands labelled for adiponectin. (d), (h), (l), (p) and (t), respectively, show canine vascular endothelium, epidermis, hair follicles, apocrine sweat glands and sebaceous glands labelled for leptin. The box at the lower right of each picture shows the evaluated labelling intensity when present (+, weak; ++, moderate; +++, high). Nuclear labelling is showed with a black arrow, while cytoplasmic labelling is demonstrated with a black asterisk.

Fig. 2. Example of negative labelling of dermis and subcutaneous adipose tissue for adiponectin. IHC. Magnification for (a) and (b), $\times 100$. Magnification for (c) and (d), $\times 400$. (a) and (c) show the absence of labelling of dermis and subcutaneous adipose tissue, respectively. (b) and (d) show the negative controls. These observations were identical for leptin and resistin.

Fig. 3. Labelling of omental adipose tissue. IHC. $\times 400$. (a) and (b) show positive labelling of the omental adipose tissue for adiponectin and leptin, respectively, and (c) shows the absence of labelling for resistin. The box at the lower right of the two first pictures shows the evaluated labelling intensity when present (+, weak; ++, moderate; +++, high). Cytoplasmic labelling is demonstrated with a black asterisk.

Table 1
Tested dilutions for the chosen primary antibodies

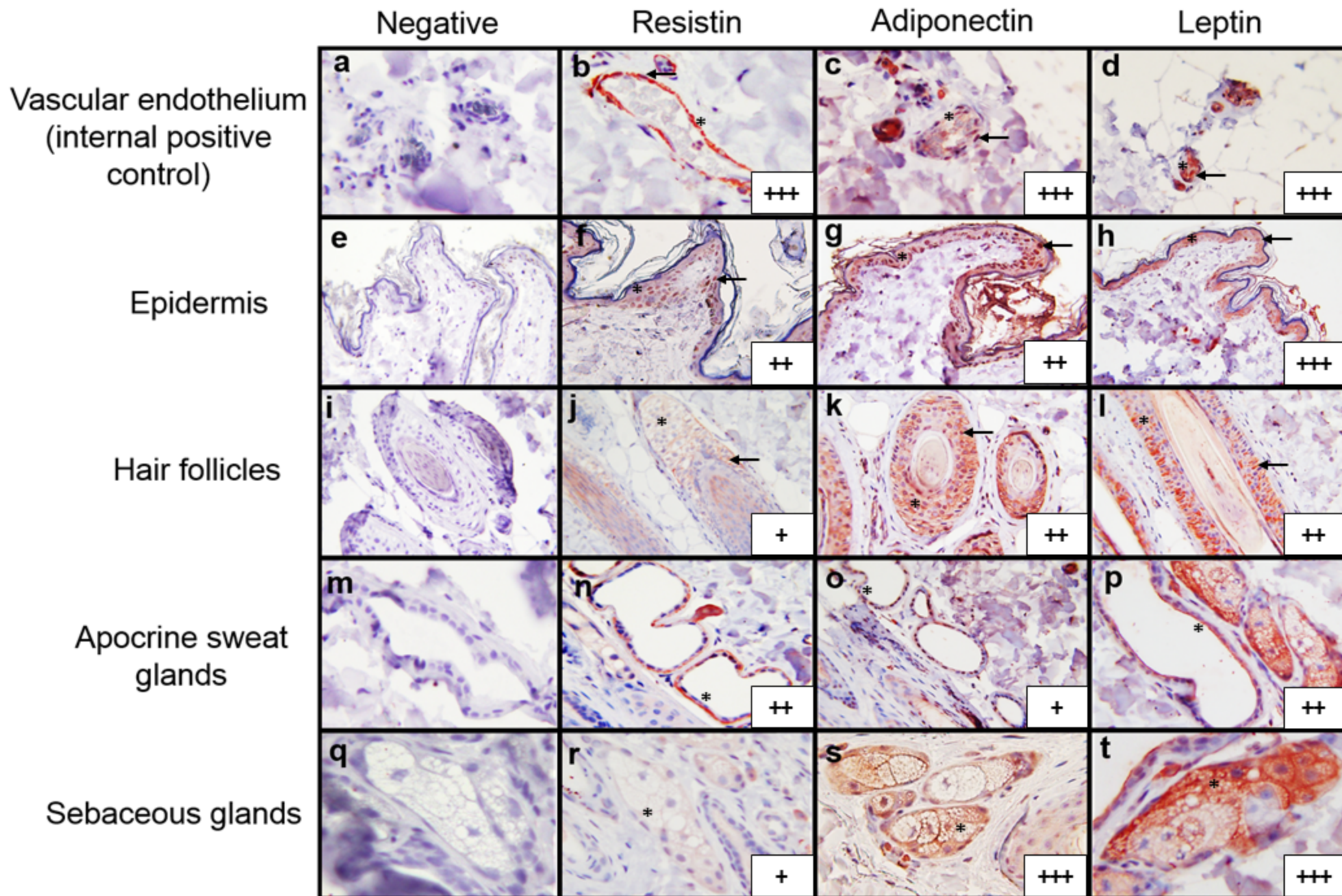
<i>Adipokine</i>	<i>Tested primary antibody dilution</i>	<i>Reference (Study species)</i>
Adiponectin	1 in 200	Seleit <i>et al.</i> , 2016 (mouse)
	1 in 400	Lim <i>et al.</i> , 2015 (dog)
	1 in 10,000	Fujimoto <i>et al.</i> , 2005 (mouse)
Leptin	1 in 50	Jin <i>et al.</i> , 2000 (mouse)
		Ressel <i>et al.</i> , 2012 (dog)
	1 in 200	Farag <i>et al.</i> , 2016 (man)
	1 in 500	Jin <i>et al.</i> , 2000 (mouse) Lim <i>et al.</i> , 2015 (dog)
Resistin	1 in 20	Szalowska <i>et al.</i> , 2009 (man)
	1 in 500	Steppan <i>et al.</i> , 2001(mouse)
	1 in 2,000	Nogueiras <i>et al.</i> , 2003 (mouse)

Table 2

Labelling intensity and immunolocalization of adiponectin, leptin and resistin in the normal canine skin

		<i>Epidermis</i>		<i>HF</i>	<i>VE</i>	<i>AGC</i>	<i>SG</i>	<i>Dermis</i>	<i>SubC</i>
		<i>Stratum</i>	<i>Living</i>						
		<i>corneum</i>	<i>epidermis</i>						
Adiponectin	Nuclear	0	++	+	+++	0	0	0	0
	Cytoplasmic	0	++	++	+++	+	+++	0	0
Leptin	Nuclear	0	+++	+	+++	0	0	0	0
	Cytoplasmic	0	+++	++	+++	++	+++	0	0
Resistin	Nuclear	0	++	+	++	0	0	0	0
	Cytoplasmic	0	++	+	++	++	+	0	0

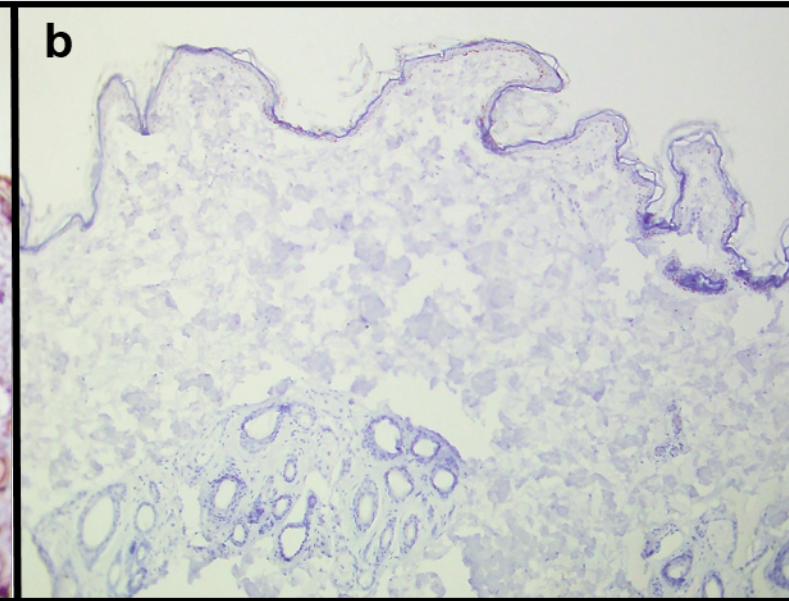
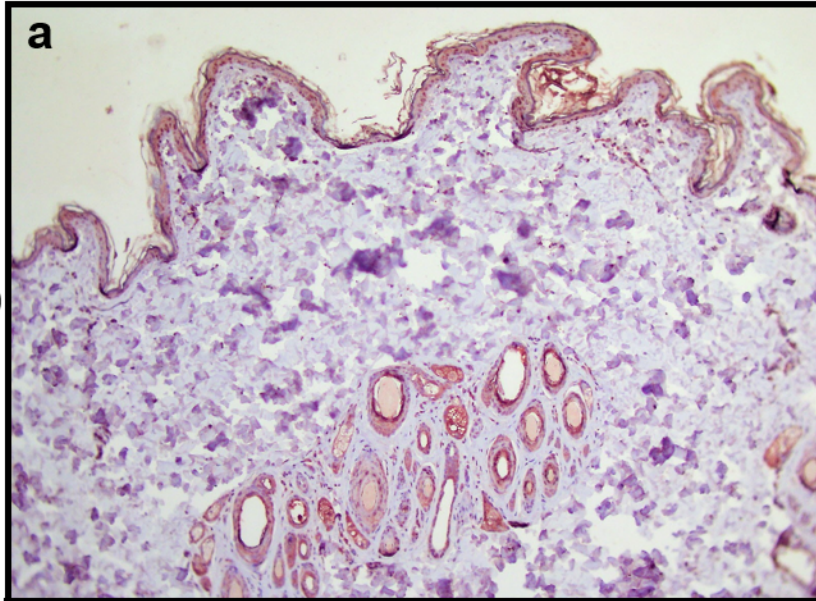
HF, hair follicles; VE, vascular endothelium; AGC, apocrine gland cells; SG, sebaceous glands; SubC, subcutis.



Labelling

Negative control

Dermis
(magnification x 100)



Subcutaneous
adipose tissue
(magnification x 400)

