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Expression of NGF, BDNF and their receptors in subcutaneous adipose tissue of lactating cows

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ABSTRACT

Currently, there are no reports of neurotrophins in adipose tissue of cows. The distribution of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and their high-affinity tyrosine kinase receptors TrkA and TrkB, was investigated by immunohistochemical method in the subcutaneous adipose tissue of cow at mid-lactation. Results revealed the localization of NGF and BDNF along the plasma membrane and cytoplasm of adipocytes. Neurotrophin receptors TrkA and TrkB showed moderate and strong positive staining in adipocytes, respectively. The expression of NGF, BDNF, TRKB — but not of TRKA — was also confirmed at transcriptional level by RT-PCR analyses.

Considering the involvement of BDNF on fat metabolism and of NGF on activation of the sympathetic response in human and rodents, these neurotrophins could be related to lipogenesis and lipolysis occurring during lactation in cows. The local production of these neurotrophins supports their potential paracrine function for the regulation of adipocyte activity and deserve further investigations.

In the past, the white adipose tissue (WAT) was recognized only as a reservoir of high-energy substrates such as cholesterol, triglycerides, and fat-soluble vitamins. Currently, adipose tissue is also considered an endocrine organ capable of releasing a variety of bioactive substances known as adipose-derived secreted factors or adipokines (Wang et al., 2008; Colitti and Grasso, 2014). The WAT is innervated by the sympathetic nervous system (SNS), although less extensively than brown adipose tissue (BAT) (Bartness et al., 2001). The SNS is considered to be the major physiological regulator of lipolysis in WAT and has been implicated in the regulation of cell number and the synthesis of several adipokines, particularly leptin (Rayner and Trayhurn, 2001). In a number of murine tissues, the development and survival of sympathetic neurons depend upon the presence of target-derived neurotrophins the best characterized of which is nerve growth factor (NGF) in BAT (Nisoli et al., 1996) and WAT (Peeraully et al., 2004). In spite of their function as growth factors primarily for development and survival of neural cells (Skaper, 2012), Chaldakov et al. (2010) reported that many neuropeptides, neurotrophic factors and receptors are shared by the brain and adipocytes, hence, proposing adipose tissue as a member of the diffuse neuroendocrine system.

Among the neurotrophin family, NGF binds specifically TrkA, BDNF and NT-4 recognize TrkB and NT-3 activates TrkC (Bothwell, 2014) and all neurotrophins bind to and activate p75 NTR. Neurotrophin/Trk signaling is regulated by a variety of intracellular signaling cascades, transmitting positive signals to promote survival and growth. On the other hand, p75NTR transmits both positive and negative signals (Reichardt, 2006). To date, neurotrophins have been mainly studied in nervous cells, but, as multifunctional growth factors, they can exert various effects through their receptors on non-neuronal cells such as testis (Müller et al., 2006), thymus (Maroder et al., 2000), skin (Di Marco et al., 1993), salivary glands and mammary ducts (Shibayama and Koizumi, 1996; Sariola, 2001) and ovine mammary glands (Colitti, 2015). It is noteworthy that, because NGF and BDNF are secreted by WAT and BAT (Sornelli et al., 2009) and their levels in the circulation and tissue are altered in cardiometabolic diseases (Chaldakov et al., 2009), neurotrophins are considered as metabotrophic factors, implicating in metabolic diseases (Chaldakov et al., 2010).

To date, no measurements of neurotrophic factors and their receptor in adipose tissues of cattle have been reported, but it is well known that fat depot mass and biological activity changes dramatically during the lactation cycle in dairy cows (Saremi et al., 2014). Similarly, a large body of research has been devoted to the study of fat metabolism and its implication on physiological regulation during health and disease conditions in dairy cows (McNamara and Hillers, 1986; Graugnard et al., 2012 and Sgorlon et al., 2015).

Considering the relevance of neurotrophins on fat metabolism, the aim of the study was to investigate the distribution of NGF, BDNF and their high-affinity receptors TrkA and TrkB in subcutaneous adipose tissue (scAT) of lactating cows.

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Six mid lactation cows (180 ± 20 days in milking, DIM) from a commercial farm were selected. Biopsies of scAT were collected from the dorsal pelvic region as described by McNamara and Hillers (1986), using a 20 gauge biopsy needle. Tissue samples were divided in two aliquots, one immediately frozen at −80 °C and the second fixed in 10% (w/v) neutral formalin for 24 h at room temperature. Bovine cerebellum was promptly collected in a slaughterhouse fixed in 10% (w/v) neutral formalin and used as positive control. The study complied with the national legislation for animal welfare and was approved by the bioethical committee of the University of Udine.

Immunohistochemical investigation was performed according to procedures previously described (Colitti and Parillo, 2013). Primary antibodies used in this study are listed in Supplementary Table S1. Total RNA was extracted from about 5 mg of scAT using TRIzol® Plus RNA Purification System (LifeTechnologies™, Monza, Italy), following the manufacturer's instructions. Primers and product lengths for each gene are listed in Supplementary Table S2 according to the HUGO Gene Nomenclature Committee.

Present immunohistochemical analyses revealed that the NGF and TrkA protein expression presents strong membranous and cytoplasmic immunostaining (Fig. 1 A–B; A′–B′), but it was negative when primary antibody was omitted (Fig. 1A and A′, inserts). It is now known in non-ruminants that WAT not only releases fatty acids and cholesterol but also a variety of cytokines and growth factors, namely adipokines. Among the secreted compounds are NGF and BDNF, which improve glucose and lipid metabolism and control energy balance and feeding behavior (Chaldakov et al., 2003). The NGF is secreted by rat, mice and human BAT (Nisoli et al., 1996) and also by WAT in mice (Peeraully et al., 2004), where its expression and secretion were related to the role of the neurotrophin in the inflammatory response. It is known that accumulation of fat could lead to a state of chronic mild inflammation (Ouchi et al., 2011), as demonstrated by granulocytic infiltration and cytokine secretion in AT (Osborn and Olefsky, 2012). An infiltration of macrophages into different AT depots was not observed after calving (Akter et al., 2012), but later in lactation, an infiltration of immune cells could occur.

However, the mRNA for NGF was detected, but it was not for TRKA, suggesting that NGF is synthesized in adipocytes but TRKA is not (Fig. 2). Although NGF mRNA was clearly expressed in human, an examination of the Expression Atlas database (Petryszak et al., 2013) revealed no indication of TRKA mRNA expression in human and bovine adipose tissue. A possible explanation would be that the TrkA protein enters the adipose cells through the signaling endosomes produced by peripheral axons of neurons. This mechanism could support adipocyte survival, similar to that reported in the retrograde signal which controls sensory neuron development (Harrington and Ginty, 2013). This possible explanation deserves further investigation.

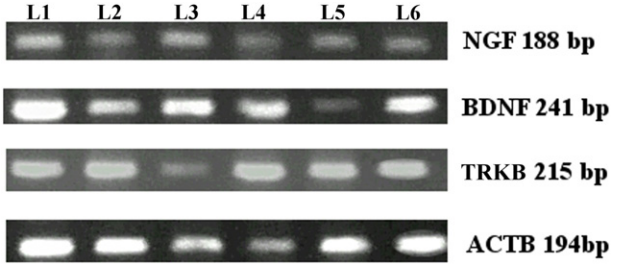

Fig. 2. Expression of mRNA in bovine subcutaneous adipose tissue. NGF, nerve growth factor; BDNF, brain derived neurotrophic factor; TRKB, neurotrophic tyrosine kinase, receptor, type 2; ACTB actin, beta. bp = base pairs. Lanes (L1–L6) are the amplifications of the mRNA extracted from adipose tissue of the six cows.

The changes in lipogenesis and lipolysis during lactation in cows are part of the control of body reserves and are supported by an adaptation of SNS in adipose tissue in rats (McNamara and Murray, 2001). In fact, adipocytes have greater sensitivity to lipolytic stimulation during lactation and this is associated with more norepinephrine stimulation of lipolysis in visceral adipose tissue (McNamara, 1995). Therefore, NGF could support the function of sympathetic innervation of adipose tissue for lipolysis.

In scAT of lactating cows, immunolocalization of BDNF (Fig. 1C–D) and TrkB (Fig. 1C′–D′) revealed a positive staining. The BDNF immunoreactivity was cytoplasmic, localized at the periphery of the adipocyte cytoplasm consistent with membranous and extracellular staining. The staining of TrkB was observed in the cytosol and along the plasma membrane of adipocytes. No immunostaining was detected in the control when antibody was omitted (Fig. 1C and C′ inserts). Of note, the immunohistochemical localization was also confirmed at the mRNA level (Fig. 2). The BDNF can modulate the hypothalamic–pituitary–adrenal axis (HPA) activity, leading to an alteration of energy metabolism,

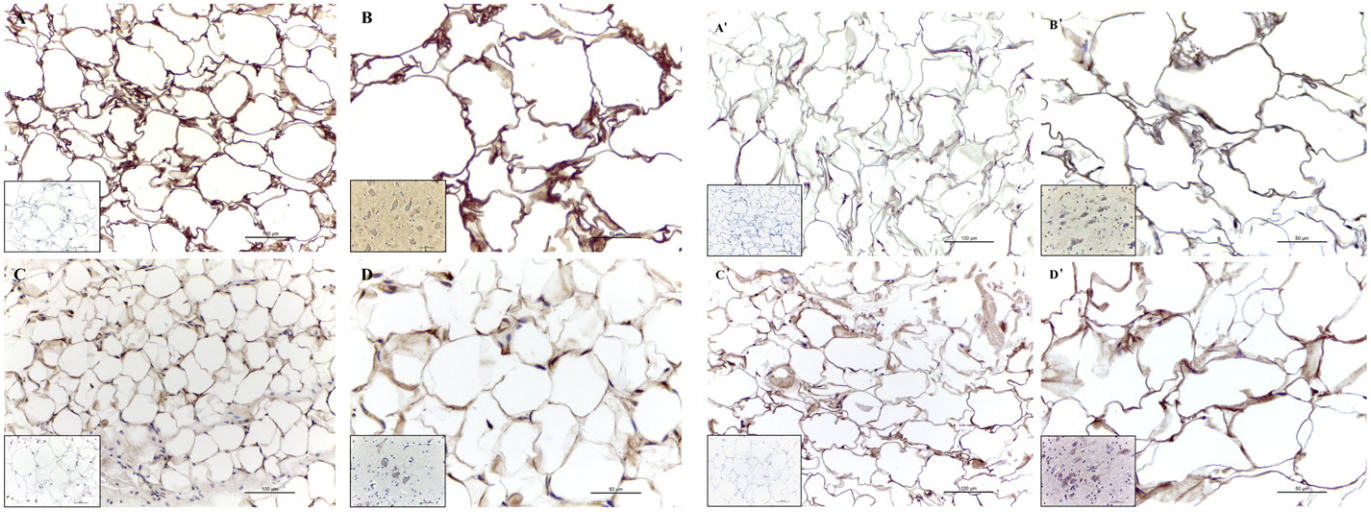

Fig. 1. Representative immunolocalisation of NGF (A–B) and BDNF (C–D) in bovine subcutaneous adipose tissue. A. Overview of NGF positive immunostaining that defines adipocyte cell borders. Insert: negative control. B. NGF immunoreaction showing cytoplasmic staining. Insert: nervous tissue, cerebellum used as positive control. C. Overview of BDNF immunostaining. Insert: negative control. D. Strong reactivity in cytoplasm of adipocytes. Insert: nervous tissue, cerebellum used as positive control. BDNF in Purkinje cells of cerebellum displayed good reaction Gill's hematoxylin counterstain. Representative immunolocalisation of TrkA (A′–B′) and TrkB (C′–D′) in bovine subcutaneous adipose tissue. A′. Overview of TrkA moderate positive immunostaining showing positivity in cytoplasm of adipocytes. Insert: negative control. B′. TrkA immunoreaction showing cytoplasmic staining. Insert: nervous tissue, cerebellum used as positive control. TrkA immunoreaction in Purkinje cells of cerebellum displayed a strong cytoplasmic positivity. C′. Overview of TrkB immunostaining. Insert: negative control. D′. Strong TrkB reactivity in cytoplasm of adipocytes. Insert: nervous tissue, cerebellum used as positive control. Gill's hematoxylin counterstain.

eating behavior and obesity (Jeanneteau et al., 2012). Furthermore, the corticotrophin-releasing hormone (CRH) is positively regulated by BDNF through its receptor TrkB via cAMP response element-binding protein (CREB) signaling (Jeanneteau et al., 2012; Fargali et al., 2012). These actions are possible because BDNF and its receptor are expressed in the hypothalamic paraventricular nucleus (PVN) as well as in other hypothalamic areas such as ventromedial hypothalamic nucleus (VMN), the dorsomedial hypothalamic nucleus (DMN), and the lateral hypothalamic area (LH) (Conner et al., 1997). From this point of view, the analogy and the relationship with leptin, secreted by adipose tissue and targeting to hypothalamus, and BDNF, secreted by both adipose tissue and hypothalamus, is noticeable. In fact, leptin increases the BDNF mRNA expression in VMN, DMN and in other brain areas where BDNF is widely expressed (Komori et al., 2006). According to Pardridge et al. (1994) in the rat the mature form of BDNF does not cross the blood–brain barrier, suggesting a paracrine/autocrine activity of this protein in scAT, although other studies reported that about 70% of the BDNF collected from the jugular vein of humans is of brain origin (Rasmussen et al., 2009). It is known that central BDNF enhances energy expenditure through the activation of the sympathetic nervous in rodents (Pelleymounter et al., 1995), however, the central and peripheral activations of the TrkB receptor in reducing the food intake and obesity observed in rodents is not maintained across all species, as monkeys and human (Noble et al., 2011). In cattle, no information is available, but two mutations in the BDNF region strongly associated with milk fat yield have been identified in *Bos taurus* (Zielke et al., 2011), supporting an active role of BDNF on energy metabolism. It should be considered that catecholamine responsiveness of bovine adipose tissue increases prior to parturition and remains elevated during lactation (McNamara, 1988). In fact, in dairy cows, the size of adipocytes decreases until about 60 DIM and then increases until late lactation (McNamara, 1995) and this is consistent with the ratio between lipogenesis and lipolysis. According to Sumner-Thomson et al. (2011) lipogenesis is observed still in the presence of lipolysis also in mid-lactation. Therefore, the peripheral production of BDNF and TrkB observed in the present study could be considered an interaction between the local control of adipocyte metabolism with the whole animal energy balance.

A large body of evidence indicated that adipose tissue has endocrine and paracrine functions and more recently it has been proposed as member of diffuse neuroendocrine system. This paper provided indications for the presence and distribution of neurotrophins factor and their receptors in scAT of dairy cow. These growth factors, being involved in fat metabolism and in the activation of the sympathetic response, could be key molecules in the physiology of lactation and deserve further investigation.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.rvsc.2015.08.016.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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