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Plasma leptin, glucose and non-esterified fatty acid variations in dromedary camels exposed to prolonged periods of underfeeding or dehydration

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ABSTRACT

The involvement of plasma leptin in the adaptation of dromedary camels to harsh conditions such as food or water shortages was studied through 2 experiments. In experiment 1, fourteen female camels were either fed at 68% of maintenance energy requirements (MER) during 112 d ($n = 4$) or overfed at 134% of MER during the first 56 d and then underfed at 17% of MER the next 56 d (OV-UN, $n = 5$), or underfed and then overfed for the same durations and energy intake levels (UN-OV, $n = 5$). Weekly plasma samples showed that leptin, glucose and non-esterified fatty acid (NEFA) concentrations were significantly modulated by energy intake level. NEFA increased sharply but transiently in underfed camels of the UN-OV or OV-UN groups, whereas glucose and leptin concentrations decreased with underfeeding and increased with overfeeding with more significant effects in camels that were previously overfed or underfed, respectively. In experiment 2 twelve female camels were either normally watered ($n = 6$) or dehydrated ($n = 6$) during 23 d and then rehydrated during 4 d. Dehydration specifically increased blood hematocrit, plasma NEFA and glucose whereas leptin decreased slightly. For both experiments, leptinemia was positively related to hump adipocyte volume. Taken together these results provide new data for a better understanding of lipid and energy metabolism in camels.

1. Introduction

In the camel, adipose reserves are mainly stored in the hump and perirenal adipose tissue (Faye et al., 2002). However, changes in lipid reserves and related mechanisms are not well understood in this species which is able to adapt to harsh environmental conditions. In ruminant species, leptin (a hormone mainly secreted by adipose tissue) was shown to play a key role in the regulation of energy metabolism and to be closely linked with body reserves (Chilliard et al., 2005). Therefore, the study of leptin regulation should be of particular interest in providing greater understanding of energy and lipid metabolism in the camel.

In 1994, Zhang et al. (1994) identified the *ob* gene encoding the 16 kDa protein, leptin. Cloning and sequencing of ovine (Dyer et al., 1997) and bovine (Ji et al., 1998) leptin cDNA, respectively, revealed a good level of similarity between these sequences and that of humans (86 and 87%, respectively). More recently, leptin and its receptors were characterized in adipose tissue, the mammary gland and liver of one-humped camels and the sequenced cDNA was closely related to those of cows and water buffalos (Bartha et al., 2005; Sayed-Ahmed et al., 2005). This high conservation of leptin and its receptor genes across different species suggests that the central role of leptin in regulating energy homeostasis should be similar between species. In addition, plasma leptin has been shown to be strongly and positively related to adiposity in humans (Morio et al., 1999), rodents (Houseknecht et al., 1998) and ruminant species. Several studies conducted in cows and sheep have shown that plasma leptin concentration was regulated over the long term by adiposity, the medium term by energy intake level and the short term by meal intake (Chilliard et al., 2005; Delavaud et al., 2007).

In the young camel, leptin has been positively related to back-fat thickness (Al-Azraqi, 2007) and differentially affected by season in function of gender without apparent relation to insulin or glucose

Abbreviations: Ab, antibody; BW, body weight; CTRL, control; D, day; EXP, experimental; FL, feeding level; MER, maintenance energy requirement; NEFA, non-esterified fatty acid; OV-UN, overfed then underfed; RIA, radioimmunoassay; UN-OV, underfed then overfed.

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regulation (Al-Suhaimi et al., 2009). However, the limited available data obtained using non-specific 'multispecies' commercial kits for plasma leptin analysis does not allow us to ascertain if leptin constitutes a key element in the adaptation of camels exposed to various conditions of food availability. Therefore, two experiments were conducted in the present study to address this question. The first one involved camels that were either strongly underfed to induce significant body lipid mobilization with a decrease in the adipocyte size, and weight and lipid content of the hump, or strongly overfed to induce reconstitution of body lipids (Bengoumi et al., 2005). During the second experiment, camels were deprived of water for 23 days in order to force them to lower their basal metabolism to spare water. For both experiments the aim was to study the regulation of plasma leptin concentration and its relationship to body adiposity indicators, as well as plasma glucose and non-esterified fatty acid (NEFA) concentrations as major parameters of lipid and energy metabolism.

2. Materials and methods

2.1. Animals

Both experiments were conducted at the Regional Insemination Center of Ain Jemaa (Casablanca, Morocco) and involved non-pregnant dry adult female camels (*Camelus dromedarius*). All experimental procedures involving animals were conducted according to the Moroccan recommendations for the use of experimental animals including animal welfare and appropriate conditions.

2.1.1. Experiment 1

Fourteen 10 to 15 year-old female camels were fed a pre-experimental diet (2 kg/day of wheat straw and 0.5 kg/100 kg BW/day of barley) during a one-month adaptation period. Afterwards, they were divided into 3 groups: OV-UN ($n = 5$, 345 ± 41 kg, overfed then underfed), UN-OV ($n = 5$, 354 ± 42 kg, underfed then overfed) and CTRL ($n = 4$, 384 ± 26 kg, control group). During an experimental period of 112 days the CTRL group received a basal diet providing around 68% of maintenance energy requirements (MER: 7.82 MJ net energy/100 kg BW/d as estimated by Wardeh (1997)). During the same period a cross-over design was applied to the OV-UN and UN-OV groups that were respectively overfed on a diet providing 134% of MER (basal diet with a barley supplement of 0.5 kg/100 kg BW/day) and underfed on a diet providing 17% of MER (2 kg/day of wheat straw) during the first 56 days and then were respectively underfed (17% of MER) and overfed (134% of MER) during the last 56 days of the experimental period. Water was provided *ad libitum*.

Jugular blood samples were drawn at 0900 h (1 h before food distribution in the morning) every week from day −7 to 112 of the experimental period and plasma was stored at −20 °C prior to leptin, glucose and NEFA (non-esterified fatty acid) measurements.

2.1.2. Experiment 2

Twelve 12 to 17 year-old female camels were separated into 2 groups of 6 animals that were adapted during a 40 d pre-experimental period in order to obtain two homogeneous groups in terms of nutritional and hydration status. The camels were fed a diet providing 100% of MER (Wardeh, 1997) that was distributed twice daily (0900 h–1000 h in the morning and 1500 h–1600 h in the afternoon) and composed of wheat straw (2.84 MJ/kg DM), sunflower meal (4.26 MJ/kg DM), barley (7.11 MJ/kg DM) and supplemented with a mineral and vitamin premix.

Water was provided *ad libitum*. At the beginning of the experimental period the camels' BW was: 384 ± 44 kg for the control group (CTRL) and 412 ± 31 kg for the experimental (EXP) camels. Afterwards, the EXP group received no water during 23 d, whereas the CTRL group was normally watered (ca. 6 L/100 kg BW as determined during the pre-experimental period) every day at 1100 h (*i.e.* after morning food distribution). Water refusals were individually measured and camels were maintained on the same ration as defined during the pre-experimental period. Food refusals were globally measured by group and the two groups were pair-fed to take into account the lower intake of the EXP group.

During the final rehydration period which lasted 4 days (d 24 to d 28), EXP camels had free access to water the first 2 days and were thereafter watered as defined during the pre-experimental adaptation period. All camels received the pre-experimental diet which provided 100% of MER and was completely consumed.

Body condition score (BCS) was determined on a scale of 0 to 5 as previously described by Faye et al. (2002) before and after dehydration (d 0 and d 23, respectively) and on day 28, four days after rehydration. Body weight (BW) was recorded at days −7, 0, 7, 14, 23, 25 and 27 of the experimental period. The 68 d experimental period was organized during spring (March to May) and outside temperatures ranged between 13.6 ± 2.6 °C in the morning and 23.2 ± 4.8 °C in the afternoon.

Jugular blood samples were drawn at 0900 h (one hour before food distribution in the morning) at days −7,−3, 0, 5, 10, 15, 21, 23 of the dehydration experimental period and at days 24 (at 1500 h, 6 h after rehydration), 25 (+24 h) and 28 (+96 h) of rehydration. Blood drawn on heparinized tubes was immediately centrifuged for hematocrit determination (volume of red blood cells/volume of total blood, expressed as percentages). The other blood samples drawn on EDTA were centrifuged and plasma was stored at −20 °C prior to leptin, glucose, NEFA, albumin, and total protein measurements.

Two hump biopsies consisting of about 1 cm^3 of adipose tissue were performed 7 d before the end of the pre-experimental period and by the end of dehydration (d 23), as described by Bengoumi et al. (2005). The first one was immediately stored at +39 °C and fixed in osmium tetroxide (Carlo Erba, Nanterre, France) prior to determining adipose cell size with optical microscopy (Robelin, 1981) and the second one was used to determine lipid content *via* petroleum ether extraction in a Soxhlet apparatus (AOAC, 1989).

2.2. Plasma assays

Plasma leptin concentration was determined in duplicate on 100 μL aliquots of plasma samples collected according to a disequilibrium, double-antibody, ovine-specific radioimmunoassay (RIA) (Delavaud et al., 2000). The intra- and inter-assay coefficients of variation were 3 and 9% respectively. The validation for camels was performed using a representative samples, which was a mix of 3 plasmas obtained from camels that were different in terms of body composition. Six anti-ovine leptin polyclonal antibody (Ab) fractions obtained from the immunization of 6 rabbits (Delavaud et al., 2000) were tested in duplicate against 4 volumes (50, 75, 100, 150 μL) of camel plasma in order to determine which Ab fraction had the best affinity with endogenous camel leptin. This Ab was selected considering 1) parallels with the standard curve composed of 4 levels (0.0833, 0.25, 2, 5 ng/tube) of recombinant ovine leptin and 2) the linearity of responses of plasma dilutions. The results obtained with the selected Ab fraction are illustrated in Fig. 1. The value of the slope obtained for the regression of camel plasma was slightly lower than that of the ovine standard curve, but not statistically different as shown by a Student's t-test comparison (calculated t (1.35) < theoretical t (2.447), dof = 6, α = 0.05). This illustrates a high cross-reactivity between the two species.

Plasma glucose was determined enzymatically by the glucose oxidase method (Boehringer and Manheim®, Germany) using a VITROS 950 (Johnson & Johnson, USA) for experiment 1 or an Ektachem-Kodak 700® auto-analyzer (Eastman Kodak Company, USA) for experiment 2. NEFA concentrations were determined enzymatically by the acyl-CoA synthase method (Wako Chemicals GmB R, Germany) using an auto-analyzer COBAS MIRA PLUS® (Roche, USA) for both experiments.

Total soluble protein concentration in plasma was determined in triplicate using the Bio-Rad Protein Assay kit (Bio-Rad, France)