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Age-related changes of serum mitochondrial uncoupling 1, rumen and rectal temperature in goats

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ABSTRACT

Thermoregulatory processes are induced not only by exposure to cold or heat but also by a variety of physiological situations including age, fasting and food intake that result in changes in body temperature. The aim of the present study was to evaluate the differences in serum mitochondrial uncoupling protein 1 (UCP1), rumen temperature (T_{RUMEN}) and rectal temperature (T_{RECTAL}) values between adult and kids goats. Ten adult male Maltese goats aged 3–5 years old (Group A) and 30 male kids, raised for meat, were enrolled in this study. The kids were equally divided into 3 groups according to their age: Group B included kids aged 3 months, Group C included kids aged 4 months and Group D included kids aged 5 months. Blood samples and measurements of T_{RUMEN} and T_{RECTAL} were obtained from each animal. One-way repeated measures analysis of variance (ANOVA) was applied to evaluate the effect of age on the studied parameters. Statistically significant higher serum UCP1 levels ($P < 0.001$) were found in Group A as compared to Groups B, C and D. Higher T_{RUMEN} values ($P < 0.001$) were found in Group A than in Groups B, C and D, and in Group B than in Groups C and D. Group A showed lower T_{RECTAL} values ($P < 0.001$) than Groups B, C and D. The Pearson's Correlation test was applied to assess significant relationship among studied parameters showing a statistically significant negative correlation between the values of T_{RECTAL} and serum UCP1 in all studied Groups ($P < 0.001$). These results indicate that goats have good control of body temperature suggesting that further details about the thermogenic capacity and the function of UCP1 in kids and adult goats are worth exploring.

1. Introduction

The regulation of body temperature in homeotherms is ensured by mechanisms of thermolysis and thermogenesis. Thermoregulatory adjustments can be induced not only by changes in environmental temperature but also by a variety of physiological situations including age, fasting and food intake inducing changes in internal temperature (Ricquier and Bouillaud, 2000; Plush et al., 2016). When mammals are exposed to the temperature of thermal neutrality the heat produced corresponds to basal metabolism and the 60–70% of the body's metabolic heat production takes place in the heart, liver, kidneys and brain (Sjaastad et al., 2003). In ruminants, heat is also generated during bacterial fermentation in the rumen accounting for as much as 8% of total heat production (Beatty et al., 2008a). An excessive increase in the rumen may impact on microbial retention and proliferation resulting in effects on rumen function (Beatty et al., 2008a). To avoid this, the heat produced in this visceral organ must be dissipated to the body surface by blood flow (Ricquier and Bouillaud, 2000; Maloney et al., 2001; Beatty et al., 2008a, 2008b).

In animals free energy is derived from the oxidation of foodstuffs including fats and proteins. This oxidation is coupled to the reduction of NAD to NADH, and the oxidation of NADH by the mitochondrial electron transport chain is in turn coupled to the setting up of a proton gradient across the inner mitochondrial membrane (Mitchell's chemiosmotic theory). A regulated proton carrier, known as uncoupling protein-1 (UCP1), dissipates the mitochondrial membrane potential generated by the respiratory chain uncoupling ATP synthesis from respiration and releasing heat from oxidation of substrates (Ricquier and Bouillaud, 2000; Brondani et al., 2012). The UCP1 plays important roles in metabolic and energy balance and regulation, in cold- and diet-induced thermogenesis and in control of body temperature (McManus

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et al., 2000; Brondani et al., 2012). The UCP1 is enriched in brown adipose tissue (BAT) that is the principal site of thermoregulatory heat production in the young of many mammalian species. In precocious animals including cows, sheep and goats, BAT starts developing in the foetal life and it has the maximum activity in the first days after birth. In adults, BAT has a significant role by regulating energy metabolism (Lee et al., 2013). The presence of BAT in adult mammals is a recent discovery and has been demonstrated in cow (Asano et al., 2013) and sheep (Henry et al., 2010; Yuan et al., 2012); only one study demonstrated the presence of UCP1 in one-month old goat kids (Magistrelli et al., 2013).

In view of such considerations, the aim of the present study was to evaluate the differences in serum UCP1, rumen and rectal temperature values between adult goats and pre- and post-weaned kids ranging between 3 and 5 months of age in order to assess their thermoregulatory capacity. In addition, the possible correlation between these parameters was evaluated in order to evaluate a relationship among serum UCP1, rumen and rectal temperature values in kids and adults of this species.

2. Materials and methods

2.1. Animals

Ten adult male Maltese goats aged 3–5 years old with a body weight of 64 ± 2 kg (Group A) and 30 male kids ranging between 3 and 5 months of age raised for meat were procured from a farm in Sicily, Italy (38°00′49″N 15°25′18″E, 80 m above sea level). The kids were divided into 3 equal groups according to their age: 3 month old kids with a body weight of 13 ± 1 kg (Group B), 4 month old kids with a body weight of 18 ± 2 kg (group C), and 5 month old kids with a body weight of 23 ± 2 kg (Group D). All the animals enrolled in the study were clinically healthy with no evidence of disease and free from internal and external parasites. Their health status was evaluated based on rectal temperature, heart rate, respiratory rate, appetite, fecal consistency and hematologic profile. Fresh fecal samples were examined according to the MC MASTER Method based on protocols previously described by Maffa (1989). All the animals were kept in two indoor pens under natural photoperiod (sunrise at 5:05 h, sunset at 20:56 h over the study period) and natural environmental temperature. Thermal and hygrometric records were carried out inside the box for the whole study by means of a data logger (Gemini, UK), and they followed the normal seasonal pattern for the location (mean ambient temperature and mean relative humidity of 22 °C and 30%, respectively). The temperature-humidity index (THI) was 66.36 °C. The THI value, an indicator of thermal comfort for goat, was calculated using the U.S. Weather Bureau's Temperature Humidity Index Formula for ruminant species (Potter and Jacobsen, 2000):

THI (°C)= T°ambient +(0.36* point of steam condensation)+ 41.5.

All goats were fed with a diet composed of a concentrate mixture which consisted of the following ingredients: oats 12%, faba bean 15%, barley 25%, pea 10%, sugar beet pulp 20%, molasses 5% and, mineral and vitamins supplements 3%. Forage-based diets were alfalfa (Medicago sativa L.) hay. About 250 g/animal of concentrate was distributed twice a day. Each day all adult goats and kids were herded out and grazed on an improved natural pasture from 11:00 to 18:00 h. At pasture all goat kids suckled freely. Water was available *ad libitum*.

2.2. UCP1 measurement

On the same day and at the same time of day (8:00 h), blood samples were collected by jugular venepuncture into 10 mL tubes containing clot activators (Terumo Corporation, Tokyo, Japan), from each animal. Blood samples were allowed to clot for two hours at room temperature, thereafter, they were centrifuged at 1000*g* for 20 min and the obtained sera were stored at −20 °C until analysis. Only the not hemolysed obtained sera were analysed to evaluate the concentration of mitochondrial uncoupling protein 1 (UCP1) using an ELISA kit (Goat Uncoupling Protein 1, Mitochondrial (UCP1) ELISA Kit, MyBioSource, Inc. San Diego, California, USA) by means of a microtiter plate reader (EZ Read 400 ELISA, Biochrom, Cambridge, United Kingdom). All calibrators and samples were run in duplicate and samples exhibited parallel displacement to the standard curve for the ELISA analysis. Both the intra- and the inter-assay coefficients of variation for UCP1 were of < 15%.

2.3. Rumen temperature measurement

To measure continuous changes in rumen temperature (T_{RUMEN}), prior to the study, temperature data loggers (Thermochron iButton® DS1921G-F5, Dallas Instrument, USA; resolution 0.500 °C; accuracy ± 1 °C from −40 °C to +85 °C; diameter 17 mm, weight 3.13 g) were implanted according to Piccione et al., (2014). The initial settings, including measurement intervals and data acquisition, are set by software (One Wire Viewer® version 03.15.50, Dallas Instrument, USA) on a computer. In our experiments iButtons® were programmed to sample every 10 min The devices were covered by a finger section from a thin latex glove (acceptable method of waterproofing) and orally inserted into the rumen, where they remained for the duration of the experiment. This was confirmed at slaughter as all data loggers were located within the rumen. At the end of the experiment, animals were sacrificed, the temperature sensors were removed, and the data were downloaded from the loggers by iButton One Wire Viewer® (Dallas Instrument, USA). When the rumen telemeters were removed in the present experiment, all were located within the actively fermenting material in the dorsal sac of the rumen.

2.4. Body temperature measurement

Measurements of rectal temperature (T_{RECTAL}), taken as representative of body temperature, were performed in each animal immediately after the blood sampling by the same operator by means of a digital thermometer whose probe was inserted into the rectum to a depth of 4 cm in kids and 6 cm in goats.

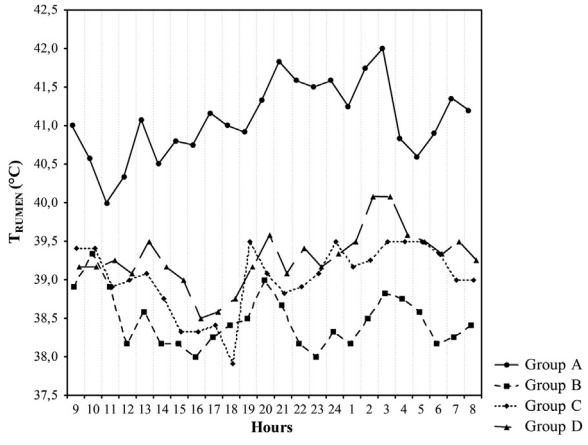

Fig. 1. Representative trend of rumen temperature (T_{RUMEN}) values recorded from adult goats (Group A) and kids aged 3 (Group B), 4 (Group C) and 5 (Group D) months during the 24 h.