Impact of vitamin C on concentrations of thyroid stimulating hormone and thyroid hormones in lambs under short-term acute heat stress

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Abstract

The present study evaluated the effect of vitamin C on alteration in thyroid hormones induced by short-term acute heat stress. Eight male lambs were divided into two groups of 4 animals each. Both groups were placed in an environment with hyper-acute heat stress based on the temperature – humidity index (THI). Groups I and II were injected intramuscularly normal saline and vitamin C (20 mg/kg), respectively, for the first five consecutive days of the experiment. All lambs were fed *ad libitum*. Blood samples were collected from both groups on days one, two, four, six and eight. Thyroxine and free thyroxine numerically increased (91.03 *vs*. 70.78 nmol L-1, P=0.080 and 29.8 *vs*. 24.8 pmol L-1, P=0.080; respectively) in heat stressed lambs supplemented with vitamin C compared to control group. Respiration rates and heart rates were elevated until day five of the experiment and then decreased. Mechanism for increasing the levels of thyroxine and free thyroxine by vitamin C is not well known. However, it may occur in part because of vitamin C antioxidant properties. The present study revealed that vitamin C might ameliorate the adverse effect of heat stress in lambs.

Introduction

Heat stress is a main issue in the welfare of livestock that can induce profound effects on metabolism of energy in sheep. Heat-stressed animals are presumed to increase maintenance requirements because of enhanced energy consumption for heat loss via panting and sweating.[1] The thyroid hormones regulate basal metabolism in various tissues by affecting the metabolism of lipids and carbohydrates.[2] Thyroid gland is regulated by the release of thyroid stimulating hormone (TSH) from the anterior pituitary gland. TSH production and release is under the influence of thyrotropin releasing hormone (TRH) from the hypothalamus.[3] The activity of TRH cells can be influenced by the temperature regulation center that integrates the environmental temperature.[4] It has been shown that heat stress in sheep alters TSH secretion and triiodothyronine (T3) and thyroxin (T4) concentration in serum.[5] Heat stress has been implicated in promoting oxidative stress either through excessive production of reactive oxygen species (ROS) or decreased antioxidant defenses, including vitamin C.[6] Moreover, a drop in vitamin C concentration has been reported in heat-stressed lactating cows,[7] pigs[8] and chickens.[9] On the other hand, vitamin C supplementation improves feed intake and growth rates in heat-stressed birds.[10] Domestic animals like sheep are believed to meet their vitamin C requirements in normal conditions from synthesizing it in liver.[11] It has been demonstrated that heat stress induces oxidative stress and vitamin C as an important water-soluble antioxidant might reduce the adverse effects of heat stress.[12] Based on our knowledge, the vitamin C and heat stress impact on thyroid hormone levels has not been clarified. This investigation was designed to examine the possible effect of vitamin C on the level of plasma thyroid hormones and TSH in clinically healthy male Iranian lambs under short-term acute heat stress.

Materials and Methods

This study was performed in summer 2010 in the Animal Husbandry Unit of The Faculty Of Agriculture, Birjand University, Iran. Birjand as a semitropical area is located in the east of Iran, (32°52'26" north latitude, 59°12'52" east longitude, and average elevation of about 1445 meters above sea level). Birjand has a dry climate with significant difference between day and night temperatures. On average, the warmest month is July and June is the driest month.[13] The animals were placed under controlled temperature condition. This experiment was accomplished under the approval of the state committee on animal ethics, Birjand University, Birjand, Iran (10/90-2-R-A/BU). In addition, we used the recommendations of European Council Directive (86/609/EC) of November 24, 1986, regarding the standards in the protection of animals used for experimental purposes. The present experiment was carried out on eight healthy Iranian male lambs about 4-5 months of age. Lambs were fed hay, mainly alfalfa and concentrate according to NRC (1985).[14] All animals were treated against internal and external parasites by Albendazole (Damloran Company, Borujerd, Iran) 10 mg/kg, and Ivermectin (Razak Company, Tehran, Iran) 0.22 mg/kg, 30 days prior to the study. After two weeks of acclimatization to the indoor facility, the lambs were allocated randomly to heat stress conditions induced by diesel heater. The temperature was monitored with a thermocouple thermometer. Animals were divided into two equal groups: i) control group (normal saline); ii) treatment group that received vitamin C (20 mg/kg) intramuscularly in five consecutive days. Animals were subjected to heat stress (40±2°C) between 7.00 and 15.00 for 8 days and for the remaining time kept at 28°C. Relative humidity of the room was measured three times daily and the mean of the measurements was used for the calculation of the temperature-humidity index (THI). THI was calculated according to the formula reported by some researchers.[15,16] Physiological parameters [rectal temperature (RT), heart rates (HR) and respiration rates (RR)] were recorded daily and then blood samples were collected

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from the jugular vein between 10.00 and 11.00 on days 1, 2, 4, 6, and 8 and serum or plasma was separated by centrifugation at 750 *g* for 15 min and stored at –20ºC. Determination of serum T3 was carried out by the microplate enzyme immunoassay method (Monobind Inc, Lake Forest, USA). Serum T4 concentration was measured using a competitive enzyme immunoassay kit (Monobind Inc, Lake Forest, USA). Serum free triiodothyronine (fT3) and free thyroxine (fT4) concentrations were determined by the fT3 and the fT4 ELISA kits (Monobind Inc, Lake Forest, USA). TSH was measured using a sheep TSH ELISA Kit (My Bio Source Company, San Diego, CA, USA). The intra- and inter-assay coefficient of variations of the assays for the mentioned parameters were (12.6% and 13.2%; T3), (3.0% and 3.7%; T4), (4.1% and 5.2%; fT3) and (4.5% and 3.7%; fT4) respectively. The sensitivity of the tests was (0.2 ng/mL; T3), (0.4 mg/dL; T4), (0.05 pg/mL; fT3) and (0.05 ng/dL; fT4) respectively. The data were expressed in SI units and analyzed by repeated measurements ANOVA and t-test using SPSS/PC software (version 18). P<0.05 was considered significant and all values were expressed as mean and standard error (SE).

Results

The severity of heat stress was estimated using THI formula.[15,16] Under heat stress condition the obtained value of THI was in the range of 23-26, indicating severe heat stress. RT, HR and RR are presented in Table 1. Mean (±SE) of RT was 39.6 (±0.5) and 39.6 (±0.03) °C in treatment and control lambs respectively. The HR was numerically lower in the treatment than control lambs (96.5±3.2 *vs* 101±3.3 beats/minutes respectively). RR under heat stress did not vary significantly between treat-

Table 1. Recorded vital signs during the experiment in control and treatment groups of healthy male lambs under experimental induced heat stress (mean ± standard error).

Variable	Rectal temperature, °C	Heart rate, beats/min	Respiratory rate, breaths/min
Control (n=4)			
Day 1	39.6±0.11	69±1.9	55±6.4
Day 2	39.8±0.02	94±5	61±3
Day 3	39.6±0.09	97±10.8	61±4.4
Day 4	39.5±0.2	108±3.6	65±10.8
Day 5	39.6±0.1	120±1.6	68±12.5
Day 6	39.6±0.02	114±8.4	52±4.3
Day 7	39.7±0.04	108±7.3	54±1.1
Day 8	39.6±0.02	91±5.7	51±3
Mean±SE	39.6±0.03	101±3.3	58.37±2.3
Treatment (n=4)			
Day 1	39.4±0.17	65±5.2	45±5.2
Day 2	39.6±0.19	80±7.6	59±1.9
Day 3	39.6±0.02	92±1.6	61±5.7
Day 4	39.7±0.08	109±5.7	65±2.5
Day 5	39.8±0.12	115±1.9	67±7.8
Day 6	39.7±0.13	104±4.6	60±3.2
Day 7	39.7±0.22	109±7.1	61±3.4
Day 8	39.6±0.22	98±1.1	52±2.8
Mean±SE	39.6±0.5	96.5±3.2	58.43±1.81
P-value	0.91	0.21	0.98

SE, standard error.

Table 2. Thyroid hormones concentrations and thyroid stimulating hormone in control and treatment group of lambs under experimental induced heat stress (mean ± standard error).

Variable	T4, nmol/L	T3, nmol/L	fT4, pmol/L	fT3, pmol/L	TSH, mIU/L
Control (n=4)					
Day 1	74.65±7.8	2.81±0.81	24.77±1.92	0.03±0.0	0.22±0.05
Day 2	75.08±5.9	2.60±0.54	24.93±0.96	0.03±0.0	0.17±0.08
Day 4	62.21±7.5	2.17±0.59	23.48±1.6	0.03±0.0	0.12±0.0
Day 6	68.21±11.6	2.95±0.78	25.8±2.78	0.03±0.0	0.17±0.06
Day 8	73.79±10.6	2.94±0.80	25.09±2	0.03±0.0	0.16±0.11
Mean±SE	70.78±3.53	2.69±0.28	24.81±0.79	0.03±0.001	0.17±0.02
Treatment (n=4)					
Day 1	88.15±5. 8	2.94±0.47	30.88±1.89	0.06±0.02	0.27±0.13
Day 2	91.37±11.9	2.77±1.01	29.17±2.38	0.04±0.01	0.19±0.09
Day 4	91.37±7.3	2.72±0.38	29.6±2.03	0.03±0.01	0.12±0.05
Day 6	92.02±3.5	3.12±0.26	28.95±0.64	0.04±0.01	0.19±0.07
Day 8	92.34±4.1	3.55±0.35	30.24±0.83	0.03±0.0	0.25±0.14
Mean±SE	91.03±2.6	2.99± 0.21	29.8±0.66	0.04±0.005	0.2±0.04
P-value	0.08	0.75	0.08	0.56	0.98

SE, standard error; T4, thyroxin; T3, triiodothyronine; fT4, free thyroxin; fT3, free triiodothyronine; TSH,-thyroid stimulation hormone. Treatment group received vitamin C in 20 mg/kg; control group received equal amount of normal saline.

ment and control lambs, and averaged 58.43 (±1.81) (S.E.) and 58.37 (±2.3) breaths/minute respectively. After the induction of heat stress, HR and RR started to increase to a peak (day 5 of the experiment) and then decreased, while alterations of RT were in a narrow range (Figure 1). Results of thyroid hormones and TSH concentration tests are summarized in Table 2. Thyroid hormones and TSH concentrations were numerically higher in treatment than control lambs. The higher levels of T4 and fT4 tended to be significant (P=0.08 for both of them). Average level of T4 was 91.03 (±2.6) (S.E.) and 70.78 (±3.53) (nmol/L) respectively, and average level of fT4 was 29.8 (±0.66) (S.E.) and 24.81 (±0.79) (nmol/L) in treatment and control lambs respectively.

Discussion

In the present study, average RT was the same (39.6 C) in both groups of control and treatment lambs. Sheep were able to maintain their body temperature within a narrow range, even when exposed to elevated temperatures. The increase in RR or panting is the most obvious reaction to heat stress.[17,18] During heat stress, the HR was significantly increased to a peak until day 5 of the experiment and then decreased. To combat heat stress, cardiac output and as a result blood flow to the skin increases as well as visceral organs. The same trend was observed in respiratory frequency as an indicator of heat stress by changing the rapid shallow breathing to slower deeper panting.[17] Our finding was in agreement with the findings of Hales and Webster (1976).[19] In the present study, coping with heat stress and vitamin C injection did not result in significant changes in the thyroid hormones and TSH concentrations, although T4 and fT4 tended to significantly increase with treatment by vitamin C injection (P=0.08 and 0.08 respectively). Thyroid hormone levels in serum tend to be lower during the summer months.[20] Moreover, many investigators reported a significant depression in thyroid gland activity under heat stress conditions.[5,20,21] Oxidative stress has an impact on thyroid physiologies[22] and vitamin C has an important function as an antioxidant due largely to its redox properties.[23] In addition, its role in cell respiration, synthesis of adrenocorticotropic hormone (ACTH) and metabolism of some other vitamins and amino acids has been revealed. Vitamin C is an essential vitamin for many normal physiological functions in humans and animals including immune functions, collagen formation, and sparing action of other vitamins (vitamin A, E and some B-complex) from oxidation.[24] Many peptide hormones require vitamin C in their synthesis. Vitamin C is also required for the hydroxylation reactions in the synthesis of corticosteroid hormones.[25] Thyroid hormones are of importance in the heat adaptation process.[1] Sivakumar *et al.*[26] state that the thyroid hormone (free T3 and free T4) levels were decreased in heat-stressed goats in an attempt to reduce metabolic rate and heat production.

Mechanism for increasing the levels of T4 and fT4 by vitamin C is not well known. However, it may occur in part because of vitamin C antioxidant properties. Endogenous vitamin C production may be insufficient under some conditions such as heat stress. The present study indicates the likely beneficial effect of injection of vitamin C on adaptation mechanisms against heat-stressed lambs. A limitation of this study was the small num-

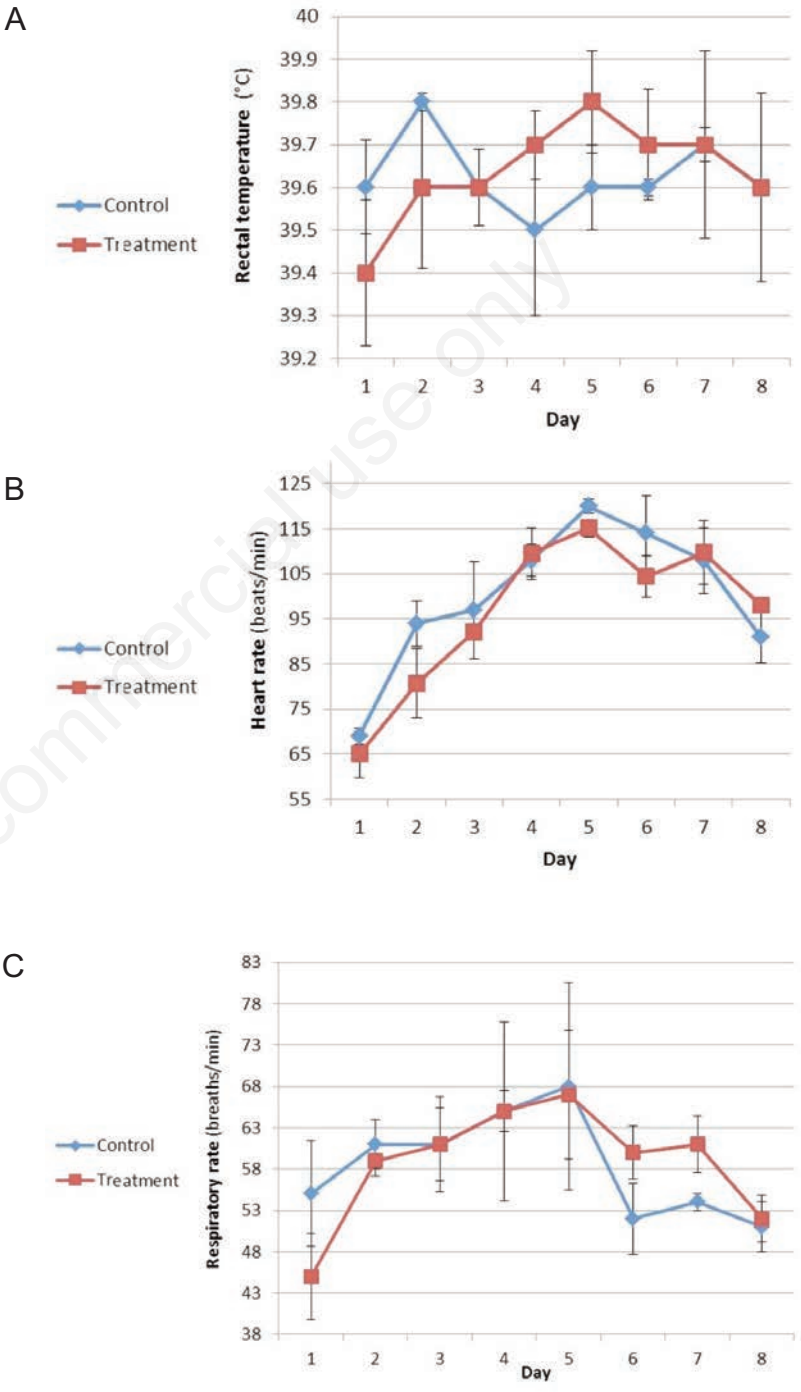

Figure 1. Vital signs of healthy male lambs under heat stress are shown (mean ± standard error). A) rectal temperature (RT); B) heart rate (HR); C) respiration rate (RR).

bers of lambs used as control and treatment group. However, more research is needed to investigate the efficacy of vitamin C in cases of heat stress.

References

1. Bernabucci U, Lacetera N, Baumgard LH, et al. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal 2010;4:1167-83.
2. Kaneko JJ. Clinical biochemistry of domestic animals. 4th ed. New York: Academic Press; 1989. pp 814-816.
3. Fliers E, Kalsbeek A, Boelen A. Mechanisms in endocrinology: beyond the fixed setpoint of the hypothalamus-pituitary-thyroid axis. Eur J Endocrinol 2014;171: R197-208.
4. Zeisberger E, Roth J. Central regulation of adaptive responses to heat and cold. In: Fregly MJ, Blatteis CM, eds. Handbook of physiology. Section 4: environmental physiology. New York, Oxford: Oxford University Press; 1996. pp 579-595.
5. Todini L. Thyroid hormones in small ruminants: effects of endogenous, environmental and nutritional factors. Animal 2007;1:997-1008.
6. Chauhan SS, Celi P, Leury BJ, et al. Dietary antioxidants at supranutritional doses improve oxidative status and reduce the negative effects of heat stress in sheep. J Anim Sci 2014;92:3364-74.
7. Padilla L, Matsui T, Kamiya Y, et al. Heat stress decreases plasma vitamin C concentration in lactating cows. Livestock Sci 2006;101:300-4.
8. Riker JT, Perry TW, Pickett RA, Heidenreich CJ. Influence of controlled temperatures on growth rate and plasma ascorbic acid values in swine. J Nutr 1967;92:99-103.
9. Kutlu HR, Forbes JM. Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. Livestock Prod Sci 1993;36:335-50.
10. Sahin K, Onderci M, Sahin N, et al. Dietary vitamin C and folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail. J Nutr 2003;133: 1882-6.
11. Weiss WP, Hogan JS, Smith KL. Changes in Vitamin C concentrations in plasma and milk from dairy cows after an intramammary infusion of Escherichia coli. J Dairy Sci 2004;87:32-7.
12. Yun SH, Moon YS, Sohn SH, Jang IS. Effects of cyclic heat stress or vitamin C supplementation during cyclic heat stress on HSP70, inflammatory cytokines, and the antioxidant defense system in Sprague Dawley rats. Exp Anim 2012;61:543-53.
13. Ashraf B, Yazdani R, Mousavi-Baygi M, Bannayan M. Investigation of temporal and spatial climate variability and aridity of Iran. Theor Appl Climatol 2014;118:35-46.
14. NRC. Nutrient requirements of sheep. 6th ed. Washington DC: National Academy Press; 1985.
15. Marai IFM, Ayyat MS, El-Monem UA. Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. Trop Anim Health Prod 2001;33:451-62.
16. Marai IFM, Habeeb AAM, Gad AE. Rabbits' productive, reproductive and physiological performance traits as affected by heat stress: a review. Livestock Prod Sci 2002;78:71-90.
17. Marai IFM, El-Darawany AA, Fadiel A, Abdel-Hafez MAM. Physiological traits as affected by heat stress in sheep: a review. Small Ruminant Res 2007;71:1-12.
18. Marai IFM, Haeeb AAM. Buffalo's biological functions as affected by heat stress: a review. Livestock Sci 2010;127:89-109.
19. Hales JRS, Webster MED. Respiratory function during thermal tachypnoea in sheep. J Physiol 1967;190:241-60.
20. Nazifi S, Saeb M, Rowghani E, Kaveh K. The influences of thermal stress on serum biochemical parameters of Iranian fat-tailed sheep and their correlation with triiodothyronine (T3), thyroxine (T4) and cortisol concentrations. Comp Clin Pathol 2003;12:135-9.
21. Ross TT, Goode L, Linnerud AC. Effects of high ambient temperature on respiration rate, rectal temperature, fetal development, and thyroid gland activity in tropical and temperature breeds of sheep. Theriogenol 1985;24:259-69.
22. Villanueva I, Alva-Sánchez C, Pacheco-Rosado J. The role of thyroid hormones as inductors of oxidative stress and neurodegeneration. Oxid Med Cell Longev 2013; 2013:1-15.
23. Schulz E, Wenzel P, Münzel T, Daiber A. Mitochondrial redox signaling: interaction of mitochondrial reactive oxygen species with other sources of oxidative stress. Antioxid Redox Signal 2014;20:308-24.
24. Grosso G, Bei R, Mistretta A, et al. Effects of vitamin C on health: a review of evidence. Front Biosci 2013;18:1017-29.
25. Khan RU, Naz S, Nikousefat Z, et al. Effect of ascorbic acid in heat-stressed poultry. Poult Sci 2012;68:477-90.
26. Sivakumar AVN, Singh G, Varshney VP. Antioxidants supplementation on acid base balance during heat stress in goats. Asian-Austral J Anim Sci 2010;23:1462-8.