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Research Note: Do broiler breeder hens use hypothermia to cope with cold environments and feed restriction?

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Abstract

The deep body temperature (T_b) of broiler breeder hens was measured in a pilot trial conducted as part of an experiment in which the response of the birds to dietary protein was evaluated at different cyclical air temperatures (T_a). Temperature data-loggers were implanted in the abdominal cavity of the birds. Most birds presented night T_b (T_{bn}) compatible with nocturnal, facultative hypothermia, a phenomenon not previously reported in broiler breeders. Hypothermia could not be related to level of dietary protein, feed intake, body weight, or change in body weight. It appeared in birds subjected to nocturnal T_a (T_{an}) ~10 °C but not at T_{an} of 20 °C, except in poor egg producers, which were normothermic when exposed to T_{an}= 10 °C. Sensors were calibrated and data corrected with calibration factors, overruling the possibility of sensor failure. Some of the sensors became embedded in the abdominal fat pad; considering that the area of surgical incision was defeathered, a possible reduction of the temperature of the abdominal wall could be the reason for the unusual T_b measurements. Nevertheless, this would not explain the occurrence of normothermia in birds kept at T_a = 20 °C, a value much lower than T_b. Unfortunately, no significant conclusions could be drawn from this experiment due to the reduced number of replications. Currently, additional experiments are being conducted to verify the occurrence of nocturnal, facultative hypothermia. Should the occurrence of nocturnal, facultative hypothermia be real, it will affect the way in which the circadian rhythms of T_b in broiler breeders are modelled.

Keywords: body temperature, circadian rhythms, rhythm amplitude, rhythm acrophase, rhythm MESOR, rhythm robustness
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Introduction

Broiler breeders are individuals of the species *Gallus gallus domesticus* selected to generate meat type chickens (or broilers). After years of genetic selection, their progeny can achieve market weight (2500 g) in 5–6 w, yielding carcases with high proportions of lean meat. The achievement of such body masses has been associated with an increase in osteoarticular problems in limbs. Additionally, as breeders mature, the mass accretion contains a progressively higher proportion of fat, a phenomenon that has been related to a reduction in reproductive performance (Robinson & Wilson, 1996). This intense selection for body weight gain has therefore resulted in the need to restrict the daily feed intake of broiler breeders to prevent them from reaching their potential mature body weights. Consequently their body weight at the start of the laying period is constrained to 2975 g for females and 3620 g for males at 25 w, and when culled at 60 w, their target weights are 3985 g for females and 4945 g for males (Aviagen, 2007).

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In many bird species, a suboptimal supply of feed in combination with low ambient temperatures (T_a) induces facultative hypothermic responses (McKechnie & Lovegrove, 2002). The reduction of T_b and metabolic rate below the normal physiological values over a certain period of time allows birds to decrease their energy requirements to cope better with inhospitable environments. Among galliform species, these responses have been studied only in Japanese quail (*Coturnix japonica*). According to Ben Amo *et al.* (2010), feed withdrawal determined the occurrence of hypothermia in Japanese quail during the rest phase (ρ), whilst the depth of the T_b drop depended on the value of T_a to which the birds were exposed. Those bouts of hypothermia were relatively shallow and more compatible with rest-phase hypothermia rather than with heterotermia (*personal appreciation*). These findings lead us to suspect that broiler breeders, due to the fact that they are feed-restricted, may present some form of nocturnal, facultative hypothermia. If this is true, the way in which the circadian rhythm of T_b of these birds is modelled will be affected.

To the knowledge of these authors, no research with Japanese quail has been conducted to investigate how nocturnal hypothermia is affected by feed composition. It has been documented in poultry that the thermogenic effect of dietary protein affects the ability of birds to withstand hot weather conditions. Additionally, chickens eating feed with high levels of protein reduce feed intake when exposed to high T_a. However, the effect of dietary protein on T_b profiles has not been measured in broiler breeders.

This paper discusses the results of a pilot trial in which the T_b of broiler breeders exposed to constant and cyclic thermal treatments while eating restricted amounts of feed with different protein levels was measured. Although no definitive conclusions could be achieved due to the limited number of replications used in this trial, we report interesting tendencies that need to be studied further.

Materials and Methods

This pilot trial was run in conjunction with an experiment that evaluated the productive performance of broiler breeders subjected to different environmental conditions when fed decreasing concentrations of protein. Two hundred and eighty 36-w-old broiler breeder hens were randomly distributed into six environmentally-controlled chambers for the abovementioned nutritional experiment. Six birds from each chamber were randomly selected and temperature data loggers (iButton®, Maxim Integrated Products, Inc., Sunnyvale, USA) were surgically implanted in the abdominal cavity. Surgery was performed one week before the beginning of the trial to avoid recording the changes in the circadian pattern of T_b usually verified in the days following general anaesthesia (Refinetti, 2006). The surgical procedure and anaesthetic protocol were approved by the Ethics Committee of the University of KwaZulu-Natal (Ref: 059/10/Animal) and executed by a registered veterinary surgeon. The loggers were programmed to start measuring T_b 1 d before the beginning of the experiment, every 15 min, over a 42-d period.

A 13L:11D light program was followed, the photophase starting at 04:00 in all chambers. Five biphasic thermal treatments were synchronised with the light cycle. The thermal difference between day and night was reduced by 4 °C per thermal treatment: treatment 1 (T_1), with a day temperature (T_{ad}) of 30 °C and a night temperature (T_{an}) of 10 °C; T_2: T_{ad} = 28 °C, T_{an} = 12 °C; T_3: T_{ad} = 26 °C, T_{an} = 14 °C; T_{a4}: T_{ad} = 24 °C, T_{an} = 16 °C; T_{a5}: T_{ad} = 22 °C, T_{an} = 18 °C. In T_6, T_a was kept at 20 °C throughout the experiment. Relative humidity (RH) was maintained at 50–60%.

In each chamber, the implanted birds received 160 g/day of one of six isoenergetic diets that decreased in protein content from diet 1 to 6. Diet 1 was a balanced diet containing 166 g crude protein (CP)/kg. Diets 2 to 5 were made by progressively diluting the balanced feed with a nitrogen-free feed, achieving 159, 132, 114, 94, and 74 g CP/kg for the respective diets in the series.

As part of the nutritional experiment, egg production and FI were recorded daily, eggs were weighed on Mondays, Tuesdays and Wednesdays, and chickens were weighed every second Monday. The results of these productive parameters will be published separately.

In order to facilitate the analysis, the T_b data were smoothed by applying moving averages ($T_{b\text{-}MAV}$) using an average window of 20 values (program 'Moving Average', Refinetti, 2006). The periodicity of the $T_{b\text{-}MAV}$ was analysed using the program 'Cosinor periodogram' (Refinetti, 2006). This program (based on the Cosinor method) was used to identify, among a range of periods of different lengths, the one that best suited the dataset under analysis. The program calculated the significance of the chosen period (P) (Fig. 1), as well as the MESOR (*m*idline-*e*stimating *s*tatistic *o*f *r*hythm, an indicator of central tendency more reliable than arithmetic mean when dealing with horizontally asymmetric oscillation, as in the case in T_b) (Fig. 1), the average amplitude (A) (Fig. 1), the acrophase (Acro, the time to reach the maximum T_b) (Fig. 1), and the minimum and maximum T_b achieved during the experimental period. The program also estimated the robustness of the circadian pattern throughout time. The average maximum

T_b reached during the photophase (T_{bd}), the minimum value reached during the scotophase (T_{bn}), and the full range of excursion (FRE) (Fig. 1) were calculated using the estimated MESOR and A.

Results

Failure in the control mechanisms of chambers 2, 3, 4, and 5 rendered undesired T_a profiles. Chamber 2 averaged T_{ad} = 27.5 °C and T_{an} = 11 °C and became erratic from day 21 onwards. In chamber 3, T_a cycled (on average) between T_{ad} = 22.5 °C and T_{an} = 11.5 °C. Chambers 4 and 5 showed a completely erratic pattern and their T_b data were discarded together with those collected in chamber 2 for the last 3 w. The T_{ad} values reached in chambers 1, 2, and 3 were close to or above that leading to least thermoregulatory effort (assumed to be 20 °C). The values of T_{an}, relevant to the occurrence of night hypothermia, were quite similar between those chambers. The day temperature controller of chamber 6 maintained the T_{ad} at 19 °C during the first 3 w and at 18 °C during the last 3 w, whilst T_{an} was kept at 20 °C except for the last week, in which T_{an} was 19 °C. However, the inaccuracies verified in chamber 6 fell within the expected range.

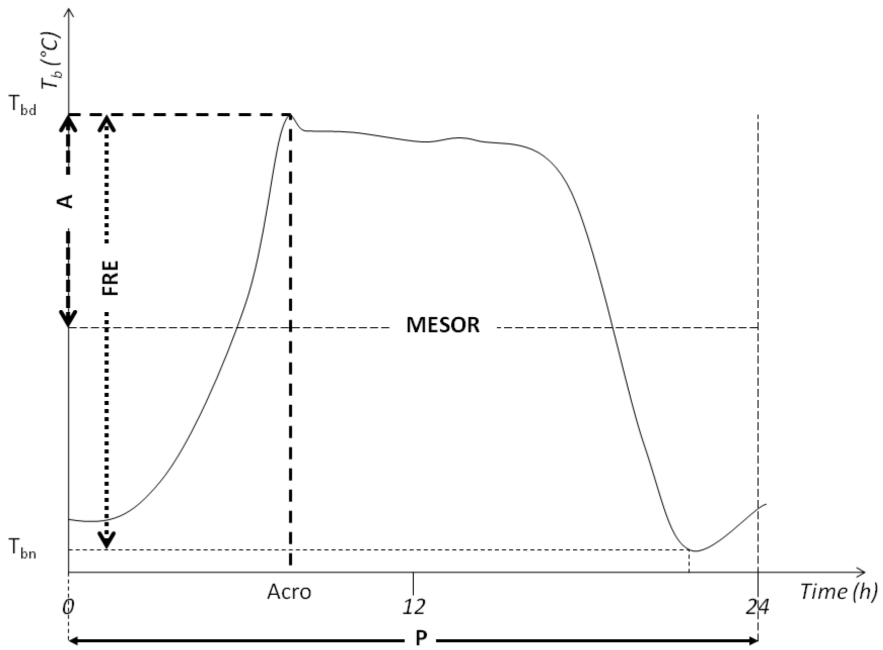

Figure 1 Hypothetical circadian variation in body temperature (T_b), showing the parameters measured in the experiment. P: period; A: amplitude; FRE: full range excursion; Acro: acrophase; T_{bn}: maximum photophase T_b; T_{bn}: minimum scotophase T_b

The birds receiving diet 6 in chamber 2 and diets 3, 5, and 6 in chamber 6 died in the early stages of the experiment. Therefore no data for those combinations of T_a and protein levels are available.

The Cosinor periodogram showed that T_b had a 24 h periodicity (circadian) in all birds, with highest temperatures reached during the photophase and the lowest during the scotophase. The value of A varied between chickens. Although such changes could not be related to any nutritional or thermal treatment, A seemed to be shallower in chamber 6. Since T_{bd} was in most cases constant between 40 and 41 °C, the increase in full range of excursion (FRE) was mainly given by a drop in T_{bn}. In most cases, T_{bn} was below 40 °C (Table 1; Fig. 2) and the values were very variable, dropping in some chickens down to 30 °C. In chambers 1, 2, and 3, where T_{an} was between 10 and 11.5 °C, T_{bn} reached the lowest values compared to chamber 6 (average T_{an} = 20 °C). Consequently, wider FREs were accompanied by lower MESOR values (Table 1). This observation coincides with that reported in Japanese quail (Ben Amo *et al.*, 2010). Due to an insufficient number of replications to obtain statistical significance, these observations are not conclusive.

The robustness of the circadian patterns was between 80 and 90% in most cases. A lower robustness in six cases was due to day-to-day inconsistencies or to progressive changes in the MESOR and A throughout the experiment, the latter resulting in an increasing or decreasing trend as the experiment progressed. For example, in the case of chicken 2 in chamber 2, between days 1 and 8, there was a gradual decrease in amplitude and increase in MESOR as a result of the progressive increase in T_{bn}, rendering a robustness of 31.2%. From days 9–21, the oscillations were quite regular, increasing the robustness to 85.3% if only the data recorded in that period were considered.

The raw data showed that the values of acrophase differed between chickens, but in most cases the maximum T_b was reached in the late morning. The Cosinor method showed that the average acrophase appeared at least 1 h after feeding time (08:00) and it shifted towards noon for thermal treatments with lower T_{an}. However, statistical significance could not be reached in this respect with these data.

Discussion

Although the small number of birds utilised in the experiment limited us from drawing significant conclusions from this dataset, some inferences can be made based on these preliminary results.

Except for birds held at 20 °C, T_{bn} in most cases dropped below 40 °C, considered to be the lower physiological limit of T_b for chickens (Refinetti, 2006), whilst the T_{bd} remained in the normothermic range or very close to it. Values of T_{bn} close to 30 °C were verified (Fig. 2), even after applying the MAV procedure. The peak of T_b of variable intensity verified during the late morning in the raw data was ameliorated (or disappeared) and shifted to earlier parts of the morning after applying the MAV procedure. A day-by-day analysis of the circadian rhythms in the raw data revealed an important variability of Acro in the same bird, which affected all the estimations obtained using the Cosinor method. Such a peak could have been related to metabolism of dietary protein or to anticipatory behaviour due to feed entrainment of circadian clocks. The limited number of birds per treatment, the smaller-than-planned difference between thermal treatments, the loss of data from three chambers due to mechanical failure, and the death of some birds reduces the validity of these results. Nevertheless, to the best of our knowledge, T_b profiles of this nature have never been reported for broiler breeders, and it is therefore prudent to theorise on the possible causes leading to the unusual readings obtained.

Sensor failure is the first possible reason for the atypical T_b profiles, but this does not account for the normothermia (or quasi-nomothermia) or for the consistency of the shape and magnitude of T_{bn} verified in all birds during the light-phase and throughout the experiment. Furthermore, the sensors were calibrated before the experiment and the offsets were minimal. The T_b database was corrected using calibration regressions before beginning the analysis.

In a series of experiments reported by Paul *et al.* (2009), T_b in broiler breeder pullets of between 10 and 18 weeks of age was measured while exposing them to 15, 19, 23, and 27 °C in four consecutive periods of 2 w. Even for the lowest T_a, the value of T_b was maintained within normal limits. Those birds differed from ours in that they were still growing and hence not producing eggs. Regarding the effect of oviposition, our data do not show any relationship between the production of an egg and the minimum T_b on the day of oviposition or the preceding day. However, it is interesting to note that two very low producing birds (bird 5 in chamber 3, which produced only one egg throughout the experiment, and bird 6 in chamber 3, which did not produce any eggs) did not exhibit nocturnal hypothermia even when subjected to T_b as low as 11 °C. Conversely, only two high-producing hens (laying 8 and 22 eggs, respectively) were normothermic. It is tempting to hypothesise that broiler breeders develop facultative, nocturnal hypothermia only after the onset of sexual maturity, but further experimental evidence is required to test this hypothesis.

After culling the birds to retrieve the sensors, many of these were found to be embedded in the abdominal fat pad. Bearing in mind that the feathers covering the lower abdomen were removed before surgery, it could be speculated that the temperature of the abdominal wall in that area may have dropped to levels below the rest of the body, perhaps leading to the inconsistencies in occurrence and depth of hypothermia. Furthermore, in the experiments performed by Paul *et al.* (2009), the sensors were implanted through the flank, reducing the possibility of them contacting the defeathered surgical area and/or being embedded by the fat tissue during the cicatrisation process. Nevertheless, in defence of the facultative hypothermia hypothesis, one should ask why the birds in chamber 6, where the temperature was held at 20 °C, which is much lower than T_b, maintained T_{bn} in the normothermic range.

Table 1 Average MESOR[1], amplitude, T_{bn}, and T_{bd} for broiler breeders exposed to three different thermal treatments and six levels of dietary protein

Parameter	Diet	T_a			
		D:30/N:10	D:28/N:12	D:23/N:11	D:20/N:18/19
MESOR	1	38.7	41.0	36.8	39.1
	2	37.3	39.5	35.1	40.2
	3	38.2	39.1	37.7	*
	4	35.4	39.2	37.3	39.7
	5	38.5	38.4	40.5	*
	6	39.5	*	40.6	*
Amplitude	1	1.6	0.7	2.7	0.7
	2	3.3	0.9	4.1	0.6
	3	2.5	1.3	0.8	*
	4	5.2	1.3	1.8	0.6
	5	2.4	2.4	0.6	*
	6	0.9	*	0.5	*
T_{bn}	1	37.1	40.3	34.1	38.4
	2	34	38.6	31	39.6
	3	35.6	37.8	36.9	*
	4	30.2	37.9	35.5	39
	5	36.1	35.9	40	*
	6	38.6	*	40.1	*
T_{bd}	1	40.3	41.7	39.5	39.8
	2	40.6	40.4	39.2	40.8
	3	40.7	40.4	38.6	*
	4	40.6	40.5	39.1	40.3
	5	40.9	40.8	41.1	*
	6	40.5	*	41.1	*
Acrophase	1	11:18	09:44	11:03	10:12
	2	11:40	11:18	10:56	08:51
	3	11:43	10:17	10:11	*
	4	11:11	10:26	11:22	09:14
	5	11:29	09:41	10:14	*
	6	13:18	*	11.53	*

[1] *m*idline-*e*stimating *s*tatistic *o*f *r*hythm

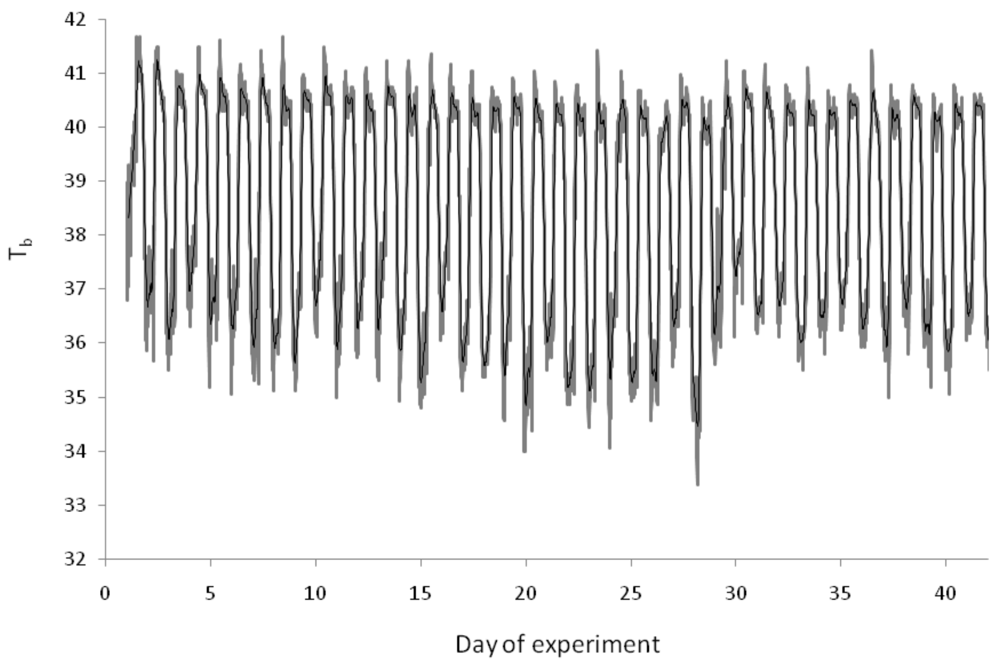

Figure 2 T_b profile of chicken 5 from chamber 1 (T_{ad} = 30 °C; T_{an} = 10 °C). Observe the square shaped profile. The grey line represents the raw data and the black line corresponds to the data after the application of the MAV procedure ($T_{b\text{-}MAV}$).

The data also show no relationship between the depth of the FRE and thermal or nutritional treatments. Data obtained in quail indicate that FI and T_a have a decisive role in the development of nocturnal, facultative hypothermia. Ben Amo *et al.* (2010) found that feed withdrawal induced the occurrence of facultative hypothermia and the magnitude of T_a determined its depth. In our case, due to mechanical failure in the chambers, the range of night temperatures was not sufficiently varied to allow inferences on the effect of T_a, but in chambers 1, 2, and 3, where T_{an} was close to 10 °C, the depth of hypothermia was greater than at the constant 20 °C of chamber 6. Regarding the effect of FI, no apparent relationships with protein intake were found in our data, but all birds received a limited amount of feed, with no *ad libitum* treatments being included. Therefore, it is not clear whether the lack of relationship between the factors studied and the T_b profiles is the result of the bias introduced by the low number of replications, or it actually constitutes the reflection of a true inconsistency of T_b responses between birds. In terms of the effect of protein levels, it should be borne in mind that, although diets differed in protein to energy ratio, the thermogenic effect of dietary protein metabolism may not affect the occurrence of nocturnal hypothermia simply because the birds were fed in the morning and no major differences in heat production should be expected during the night.

In pigeons, the occurrence of nocturnal, facultative hypothermia was related to weight loss secondary to feed restriction/feed deprivation (Underwood *et al*., 1999), but in our data, neither body weight nor change in body weight (data not shown) seem to relate to the depth of T_{bn}. The low frequency of weighing certainly hindered the interpretation of results, and perhaps daily weighing should be applied in the forthcoming experiments.

The reduced robustness of the circadian rhythm is another aspect that deserves further analysis. As mentioned above, loss of robustness may be the consequence of either erratic patterns in T_b or due to a variation in A and/or MESOR. Regarding the latter, issues with the control of T_a may not explain such inconsistencies, since in the same environment, only some birds presented this type of pattern. The gradual changes in A and MESOR resemble those verified in pigeons (*Columbia livia*) and Japanese quail (*Coturnix japonica*) that underwent weight loss and recovery after limited feed intake (feed restriction or fasting) and subsequent *ad libitum* feeding (Rashotte *et al.*, 1995; Laurila *et al*., 2005; Ben Amo *et al.*, 2010). Perhaps gradual changes in body mass may explain the trends observed in the T_b profiles of chickens but, as mentioned above, the frequency of weighing in this pilot trial was too low to determine the dynamics followed by body weight. In a subsequent experiment, we could observe apparent changes in body weight related to the frequency of oviposition. Hens do not lay an egg every day, but in cycles, separated by an interval characteristic of each bird. Since the birds consumed the same amount of feed each day to fulfil the requirements for maintenance and for the production of one egg, their body weight seemed to have increased on the days on which eggs were not produced. To the knowledge of these authors, the relationship between these subtle changes in body weight and the T_b profile has not been studied and, as stated above, our body weight database is insufficient to draw any valid conclusion in this respect.

Another possible source of variation in the robustness of the circadian pattern of T_b could be a change in body composition. As stated above, broiler breeders have a high tendency to deposit lipid as they mature, but the protein:lipid ratio in the body appears not to be related to body weight (Gous, *personal communication*). In an unpublished trial performed at our experimental farm, it was found that birds belonging to the same flock, with a homogeneous hatching body composition, and having been reared under the same environmental and management conditions, did not have the same carcass composition at the beginning of the laying period, in spite of being of similar body weight. This implies that birds of a same body weight may have different proportions of muscle and adipose tissue, which may affect their metabolic rate. The pattern of lipid deposition in a bird cannot be easily determined, simply because the bird needs to be sacrificed to analyse body composition but based on the observed effect of oviposition on weight gain, one may speculate that stepwise changes occur. Furthermore, it should not be forgotten that our broiler breeders, before being housed in individual pens in the experimental chambers, were part of a large flock kept in a commercial breeder unit. The position that each bird occupies in the hierarchy of the group may determine its feeding habits, particularly when fed a restricted amount of feed each day, as is the case with broiler breeders. This perhaps may have affected the body composition at the beginning of the trial and, once the competition factor was withdrawn, may have affected the rate at which lipid was deposited during the experiment.

Conclusions

In spite of some inconsistencies, the T_b records obtained in this pilot trial show the possible existence of a phenomenon not yet described in broiler breeders. Should the occurrence of nocturnal facultative hypothermia be real, it will affect the prediction of T_b.

There are some issues to be addressed in future research: firstly, the occurrence of some form of heterothermia (or any other form of facultative hypothermia) should be verified, specifying the environmental and management conditions leading to it. Secondly, similarly to the work by Ben Amo *et al.* (2010), the pattern of variation of T_b needs to be characterised, specifying in which way the circadian parameters are affected by different variables. Finally, the physiological processes involved in the development of hypothermia, as well as those determining the inconsistencies between individuals, should be identified.

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