

# Thermoregulation in hibernating mammals: the role of the “thyroid hormones system”.

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**SI Table 1: Total thyroid hormones in Arctic ground squirrels.**

Reference	TT4 (ng/mL)				TT3 (ng/mL)					
	a	b <sup>1</sup>		c <sup>2</sup>	d	a	b <sup>1</sup>		c	d
Winter Season:										
<i>T<sub>a</sub></i>	2°C				<i>n/a (NP)</i>	2°C				<i>n/a (NP)</i>
<i>Photoperiod</i>	4L:20D				<i>n/a (NP)</i>	4L:20D				<i>n/a (NP)</i>
Torpor	<b>86.5 ±7.2</b>				<b>80.6 ±7.2</b>	<b>5.17 ±0.7</b>				<b>7.07 ±0.7</b>
Early Torpor (24-28h from onset)										
Late Torpor (24h before arousal)										
Early Aroused (2-3 hr)					<b>145 ±5<sup>4</sup></b>					<b>7.2 ±0.6<sup>4</sup></b>
Late Aroused (12-48 hr)	<b>62.1 ±8.9</b>				<b>73.4 ±8.9</b>	<b>2.06 ±0.4</b>				<b>5.2 ±8.9</b>
Spring Season:										
<i>T<sub>a</sub></i>		<i>n/a (field)</i>		<i>n/a (field)</i>			<i>n/a (field)</i>		<i>n/a (field)</i>	
<i>Photoperiod</i>		<i>n/a (field)</i>		<i>n/a (field)</i>			<i>n/a (field)</i>		<i>n/a (field)</i>	
		<b>65 ±3.8</b>	<b>44.5 ±3.5</b>	<b>44.5 ±5</b>			<b>1.8 ±0.1</b>	<b>1.25 ±0.1</b>	<b>2.2 ±0.2</b>	
Summer Season:										
<i>T<sub>a</sub></i>	2°C	<i>n/a (field)</i>		<i>n/a (field)</i>		2°C	<i>n/a (field)</i>		<i>n/a (field)</i>	
<i>Photoperiod</i>	4L:20D	<i>n/a (field)</i>		<i>n/a (field)</i>		4L:20D	<i>n/a (field)</i>		<i>n/a (field)</i>	
	<b>57.2 ±9.7</b>	<b>33.3 ±7.3</b>	<b>34.5 ±7.5</b>	<b>27 ±2.5</b>		<b>1.43 ±0.9</b>	<b>1.5 ±0.3</b>	<b>1.03 ±0.3</b>	<b>2.6 ±0.2</b>	
Fall Season:										
<i>T<sub>a</sub></i>	2°C	<i>n/a (field)</i>		<i>n/a (field)</i>	<i>n/a (NP)</i>	2°C	<i>n/a (field)</i>		<i>n/a (field)</i>	<i>n/a (NP)</i>
<i>Photoperiod</i>	4L:20D	<i>n/a (field)</i>		<i>n/a (field)</i>	<i>n/a (NP)</i>	4L:20D	<i>n/a (field)</i>		<i>n/a (field)</i>	<i>n/a (NP)</i>
	<b>53.2 ±8.1</b>	<b>37.5 ±7</b>	<b>16 ±5.5</b>	<b>23 ±3</b>	<b>48.6 ±4.7<sup>3</sup></b>	<b>2.45 ±0.3</b>	<b>1.45 ±0.2</b>	<b>1.13 ±0.4</b>	<b>1.65 ±0.2</b>	<b>2.0 ±0.3<sup>3</sup></b>
Assay	LC/MS	EIA		EIA	RIA	LC/MS	RIA		RIA	RIA

Data are reported as mean ± SEM. Values from literature (a (Frare et al., 2018), b(Williams et al., 2019), c(Wilsterman et al., 2015), d(Nevretdinova and Shvareva, 1987)) are converted to equivalent units for comparison. *T<sub>a</sub>*= ambient temperature, NP= natural photoperiod: animals were maintained under conditions similar to natural light and temperature, filed= the animals were capture in the field under natural light and temperature.

<sup>1</sup> the original paper have a collection of data from 3 different years, we choose to report the data from 2015 and 2016, since in these two years there are not significant interactions between sexes and sampling intervals; therefore, we report the data in table collapsed by sex. Active field animals collected in April during the mating season (Spring), in July in the post-lactation season (Summer) and in August in the fattening season (Fall).

<sup>2</sup> The authors did not report any significant interactions between sampling location and sampling intervals, therefore we report the data in table collapsed by sampling locations. Active field animals collected in April during the mating season (Spring), in July in the post-lactation season (Summer) and in August in the fattening season (Fall).

<sup>3</sup> Active captive animals collected in August and October (Fall), <sup>4</sup> Early arousal defined by brain temperature between 17.5-21° C

SI Table 2: Free thyroid hormones in Richardson's and Arctic ground squirrels.

	FT4 (pg/mL)				FT3 (pg/mL)				
	Richardson's GS		Arctic GS		Richardson's GS		Arctic GS		
Reference	a <sup>1</sup>		b <sup>2</sup>		c	b <sup>2</sup>		c	d <sup>3</sup>
Winter Season:									
<i>T<sub>a</sub></i>	6°C		6°C		2°C	6°C		2°C	
<i>Photoperiod</i>	2L:22D		2L:22D		4L:20D	2L:22D		4L:20D	
Torpor	<b>43.8 ±5</b>	<b><u>14.7 ±2.5</u></b>	<b>17.5 ±3.4</b>	<b><u>4.17 ±1.04</u></b>	<b>8.6 ±1</b>	<b>13.3 ±2.5</b>	<b><u>1.07 ±0.15</u></b>	<b>6.6 ±0.4</b>	
Late Aroused (12-48 hr)			<b><u>12.8 ±0.1</u></b>	<b>2.45 ±0.4</b>	<b>15.5 ±5.5</b>	<b><u>5.51 ±0.52</u></b>	<b>0.44 ±0.04</b>	<b>5.2 ±1.1</b>	
Spring Season:									
<i>T<sub>a</sub></i>									<i>n/a (field)</i>
<i>Photoperiod</i>									<i>n/a (field)</i>
									<b>6.5 ±0.3</b>
Summer Season:									
<i>T<sub>a</sub></i>	<i>n/a (field)</i>		<i>n/a (field)</i>		2°C	<i>n/a (field)</i>		2°C	<i>n/a (field)</i>
<i>Photoperiod</i>	<i>n/a (field)</i>		<i>n/a (field)</i>		4L:20D	<i>n/a (field)</i>		4L:20D	<i>n/a (field)</i>
	<b><u>6.7 ±1</u></b>		<b><u>11.9 ±0.7</u></b>	<b>3.3 ±0.25</b>	<b>10.8 ±1.8</b>	<b><u>2.51 ±0.44</u></b>	<b>0.57 ±0.12</b>	<b>6.5 ±0.9</b>	<b>4.6 ±0.8</b>
Fall Season:									
<i>T<sub>a</sub></i>	<i>n/a (field)</i>				2°C			2°C	<i>n/a (field)</i>
<i>Photoperiod</i>	<i>n/a (field)</i>				4L:20D			4L:20D	<i>n/a (field)</i>
	<b><u>6.3 ±1</u></b>	<b>2.1 ±0.3</b>			<b>8.7 ±1.7</b>			<b>5.7 ±0.7</b>	<b>4.4 ±0.7</b>
Methods of separation of FTH from TTH	ED	ED	ED	ED	Ultrafiltration	ED	ED	Ultrafiltration	None
Temperature during separation	37°C	6°C	37°C	6°C	37°C	37°C	6°C	37°C	<i>n/a</i>
Assay	Calculation %FT4*TT4		Calculation %FT4*TT4		LC/MS	Calculation %FT3*TT3		LC/MS	RIA

Data are reported as mean± SEM. Values from literature ((a (Demeneix and Henderson, 1978c), b (Magnus and Henderson, 1988), c (Frare et al., 2018), d(Williams et al., 2019)) are converted to equivalent units for comparison. *T<sub>a</sub>*= ambient temperature, field= the animal were capture in the field under natural light and temperature, *ED*= *equilibrium dialysis which was used to measure the %FT4 and %FT3*, underline values correspond to the values used to assess FTH pattern in ED studies.

<sup>1</sup>In the original paper (a) the samples collected from June- October are grouped together as the preparative phase; here, we decided to separate them in a summer group ( Active field animals collected in July and August) and fall group ( Active field animals collected in September and October). Torpor includes hibernating animals collected from November to February.

<sup>2</sup> We reported the summer group as the active field animals collected between May and September.

<sup>3</sup>Values are available only for the 2015 year. We reported the group as seasonal groups: active field animals collected in April during the mating season (Spring), in July in the post-lactation season (Summer) and in August in the fattening season (Fall).

SI Table 3: Total thyroid hormones in Richardson's ground squirrels.

	TT4 (ng/mL)					TT3 (ng/mL)				
	a	b	c	d <sup>5</sup>	e <sup>7</sup>	a	b	c	d <sup>5</sup>	e <sup>7</sup>
Winter Season:										
<i>T<sub>a</sub></i>	6°C	6°C	6°C	6°C	6°C	6°C	6°C	6°C	6°C	6°C
<i>Photoperiod</i>	2L:22D	2L:22D	2L:22D	2L:22D	2L:22D	2L:22D	2L:22D	2L:22D	2L:22D	2L:22D
Torpor	<b>55.9 ±8.4*</b>		<b>65.8 ±13.5</b>	<b>63.5 ±10.8</b>	<b>60.2 ±7.0</b>	<b>5.45 ±0.6</b>	<b>5.8 ±0.5</b>	<b>8.83 ±1.02</b>	<b>7.19 ±1.85</b>	<b>5.7 ±0.6±</b>
Early Torpor (24-28h from onset)		<b>60 ±14</b>					<b>5.5 ±0.3</b>			
Late Torpor (24h before arousal)		<b>33 ±5</b>					<b>5.6 ±0.3</b>			
Early Aroused (2-3 hr)		<b>32 ±6</b>					<b>3.5 ±0.5</b>			
Late Aroused (12-48 hr)		<b>36 ±3</b>	<b>48.4 ±5.5</b>				<b>6.1 ±0.5</b>	<b>4.25 ±0.36</b>		
Spring Season:										
<i>T<sub>a</sub></i>		<i>n/a (field)</i>			<i>n/a (field)</i>		<i>n/a (field)</i>			<i>n/a (field)</i>
<i>Photoperiod</i>		<i>n/a (field)</i>			<i>n/a (field)</i>		<i>n/a (field)</i>			<i>n/a (field)</i>
		<b>23.5 ±3<sup>3</sup></b>			<b>15.7 ±3.8</b>		<b>2.05 ±0.1<sup>4</sup></b>			<b>0.6 ±0</b>
Summer Season:										
<i>T<sub>a</sub></i>	<i>n/a (field)</i>		<i>n/a (field)</i>		<i>n/a (field)</i>	<i>n/a (field)</i>	<i>n/a (field)</i>	<i>n/a (field)</i>		<i>n/a (field)</i>
<i>Photoperiod</i>	<i>n/a (field)</i>		<i>n/a (field)</i>		<i>n/a (field)</i>	<i>n/a (field)</i>	<i>n/a (field)</i>	<i>n/a (field)</i>		<i>n/a (field)</i>
	<b>10 ±1.55<sup>1*</sup></b>		<b>25.6 ±1.2<sup>4</sup></b>		<b>20.5 ±0.8</b>	<b>1.1 ±0.2<sup>1</sup></b>	<b>1.2 ±0.15<sup>1*</sup></b>	<b>0.95 ±0.12<sup>4</sup></b>		<b>1.1 ±0.2</b>
Fall Season:										
<i>T<sub>a</sub></i>	<i>n/a (field)</i>			6°C	6°C	<i>n/a (field)</i>	<i>n/a (field)</i>		6°C	6°C
<i>Photoperiod</i>	<i>n/a (field)</i>			2L:22D	2L:22D	<i>n/a (field)</i>	<i>n/a (field)</i>		2L:22D	2L:22D
	<b>7.5 ±2.4<sup>2*</sup></b>			<b>32.1 ±6.4<sup>6</sup></b>	<b>7.75 ±2.1</b>	<b>1.2 ±0.3<sup>2</sup></b>	<b>1.65 ±0.3<sup>2*</sup></b>		<b>2.80 ±0.09<sup>6</sup></b>	<b>1.35 ±0.2</b>
Assay	RIA	RIA	RIA	RIA	RIA	RIA	RIA	RIA	RIA	RIA

Data are reported as mean ± SEM. Values from literature (a (Demeneix and Henderson, 1978c), b (Demeneix and Henderson, 1978a), c (Magnus and Henderson, 1988), d (Henderson et al., 1981), e (Winston and Henderson, 1981)) are converted to equivalent units for comparison; T<sub>a</sub>= ambient temperature.

Study a and b have the same experimental design, and some data overlap between the two papers (\*). In the papers, the samples collected from June- October are grouped together as the preparative phase; here, we decided to separate them in a summer group (<sup>1</sup> Active field animals collected in July and August) and fall group (<sup>2</sup> Active field animals collected in September and October). Torpor includes hibernating animals collected from November to February. <sup>3</sup>Active field animals collected in March and April as emergence and reproductive phase group.

<sup>4</sup> Active field animals collected between May and September

<sup>5</sup> Values from intact animals <sup>6</sup> Active laboratory animals collected in September

<sup>7</sup> Each value is an average of two animals, the author define the data collected in May as the reproductive phase and we reported them in the Spring group.