Cyclic changes in carbohydrate concentrations during hibernation in the arctic ground squirrel

WILLIAM A. GALSTER AND PETER MORRISON
Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99701

GALSTER, WILLIAM A., AND PETER MORRISON. Cyclic changes in carbohydrate concentrations during hibernation in the arctic ground squirrel. Am. J. Physiol. 218(4): 1228-1232. 1970.—Blood samples from right ventricular cannulas and tissues were collected from arctic ground squirrels during summer and winter. Average plasma glucose (mg/ml) and tissue glycogen (mg/g) values from “unexcited” fasted ground squirrels in summer (plasma, 0.85; liver, 17.8; thigh muscle, 4.3; kidney, 0.2; heart, 0.8; and brown fat, 0.1) were not significantly different from values in nonhibernating squirrels during winter. Results in hibernating squirrels were correlated with the time in hibernation since the previous arousal. In the first one-fifth of each hibernation period, average values for liver glycogen did not change, but values in the other tissues and plasma glucose were doubled. Plasma glucose, and glycogen in liver and thigh muscle declined steadily during each hibernation period, but glycogen concentrations in kidney, brown fat, and heart did not change. Values at the end of the hibernation period approached 0.5 mg/ml (plasma), 5.0 mg/g (liver) and 4.0 mg/g (thigh muscle). These tissues appear to represent a glucose “pool,” one-half of which is utilized during each of the hibernation periods and restored during each active period. It is suggested that these declines, and particularly that in plasma glucose, may directly initiate arousal in this species.

DURING HIBERNATION, mammals can reduce their overall metabolic activity through hypothermia to less than one-tenth and sometimes to as little as one hundredth of the ordinary resting level. By this means they can extend their nutritional reserves through an unfavorable season which may exceed half of the year (11). However, the hibernation season does not represent an uninterrupted period of hypothermia but rather a series of hibernation cycles—consisting of entry, torpor, and arousal phases—interspersed with short periods of activity. Although in time, these periodic arousals roughly double the energy requirement during the hibernation season. An explanation to account for this seeming extravagance is needed. The depletion of an essential constituent is an obvious candidate, i.e., some substance which cannot be maintained under hypothermia. Although clearly a large range of essential functions are maintained in balance, despite differences in temperature characteristics of the component systems, this successful adjustment to hypothermia is an essential adaptation for hibernation.

One important function is the maintenance of blood glucose concentration which has been compared during the active and hibernation seasons in a number of species (18). Although results reveal extreme variation, there is general agreement that average glucose levels are lower during the hibernation season. Some authors report that the blood glucose concentration is maintained during hibernation (1, 5, 18) and others (2, 4, 14) have observed declines in blood glucose as hibernation continued. Although glycogenic capacity during hibernation appeared sufficient to maintain blood glucose (3) and to supply the special metabolic requirements of the CNS (6, 9, 15), it seemed unlikely that adequate glucose precursors were available to support these levels of gluconeogenesis. Apparent inhibition of glycogenolysis during hibernation (5) would also reduce this contribution to the maintenance of blood glucose. If gluconeogenesis was unavailable or insufficient for the glucose requirements of the hibernator, a decline in blood glucose levels would be expected as hibernation continued.

Since blood glucose represents about 1% of the carbohydrate reserve, muscle and liver glycogen must also be considered in assessing the ability of the hibernator to maintain blood glucose. Although several workers have measured liver and muscle glycogen concentrations during hibernation, the results have been contradictory (16). This study combines sequential measurements of plasma glucose and single measurements of tissue glycogen to identify interrelations in the arctic ground squirrel during the hibernation season.

METHODS

Arctic ground squirrels (Spermophilus undulatus plesius) provide exceptionally favorable material for studies on hibernation, since they are the largest of this numerous and widely distributed genus, maintain the longest period of hibernation, and have a favorable temperament for experimental studies. Our animals were collected during the summer months in the Thompson Pass and Paxson Lake areas of Alaska and were individually caged in the laboratory with a constant supply of rat pellets and water. In the summer, the active ground squirrels were kept at 18 C with a seasonal light cycle. In the fall and winter the squirrels were kept at 5 C with no light. Hibernation was monitored by temperature changes detected with a thermocouple positioned in the nest under the squirrel.
and recorded hourly. Results from hibernating squirrels were correlated with the duration of hibernation period expressed as the ratio of time in hibernation to the average of three or more hibernation periods.

Right ventricular cannulations were performed following the procedure of Popovic et al. (13). In summer, cannula patency of active squirrels was maintained with biweekly flushings with heparin-saline solution. In winter, squirrels were cannulated during active periods between hibernation. A minimum of 3 days was allowed for surgical recovery. After discarding the contents of the cannula, blood was withdrawn and mixed with 0.05 mg of powdered anticoagulant (heparin:NaF = 1:3). The plasma was then separated and stored at −120°C. Glucose analysis was completed within 1 month, using the micromodification of the Hoffman method (7) for the Technicon AutoAnalyzer system. A comparison of six duplicate analyses (no NaF) using the glucose oxidase method (Worthington) gave similar results (97.6 ± 2.1% of Hoffman results).

Liver, thigh muscles, kidney, heart, diaphragm, and brown and white fat were removed in that order from decapitated squirrels and dropped into liquid nitrogen within 4 min. The liver was divided into five samples; and all other tissues, except the heart, into duplicates. The frozen tissues were weighed on a torsion balance and then quickly divided and stored at −120°C. Glucose analysis was completed within 1 month, using the micromodification of the Hoffman method (7) for the Technicon AutoAnalyzer system. A comparison of six duplicate analyses (no NaF) using the glucose oxidase method (Worthington) gave similar results (97.6 ± 2.1% of Hoffman results).

Liver, thigh muscles, kidney, heart, diaphragm, and brown and white fat were removed in that order from decapitated squirrels and dropped into liquid nitrogen within 4 min. The liver was divided into five samples; and all other tissues, except the heart, into duplicates. The frozen tissues were weighed on a torsion balance and then quickly digested in hot 30% KOI. Glycogen was precipitated with ethyl alcohol, hydrolyzed with HCl and quantitated with the Hoffman glucose method (7) in the Technicon AutoAnalyzer system. In experiments using reference rabbit glycogen (Sigma), recovery levels of 99.5 ± 2.4% were achieved.

Serial samples of blood in summer (July and August) were collected after 15 hr of fasting. Precautions were taken to ensure that the ground squirrels were not disturbed prior to the collection. A stopwatch was started and a squirrel was quickly caught and placed on ECG electrodes while the blood was being collected. After removal of heparin from the cannulas, serial blood samples were collected between 1 and 17 min. In hibernating ground squirrels (January and February) blood samples were collected via right ventricular cannulas. According to activity records, none of the squirrels aroused within 5 hr after blood collection.

Tissue samples for glycogen analysis were taken in September during the prehibernation period following a 15-hr fast. During the hibernating season (January–March) samples were taken from both active and torpid animals. All of the 33 squirrels sacrificed for tissue glycogen analysis had full ceca but less than 5 g of fill in the large intestine. All squirrels had empty small intestines with one exception. This individual (February) had tissue glycogen concentrations 30–82% higher than other values for hibernating squirrels and these results were excluded. Significance was tested at the 95% confidence limits with the standard t test.

**RESULTS**

The mean plasma concentration of nonhibernating ground squirrels in summer (1.29 ± 0.36, n = 41) was significantly higher (P < 0.01) than that of hibernating ground squirrels in winter (1.12 ± 0.43, n = 38). Both groups showed considerable variability. One reason for variability of plasma glucose in active squirrels was excitement during or prior to blood collection (Fig. 1). An increase in average plasma glucose levels from 0.85 to 1.23 mg/ml was seen between 1 and 3 min after disturbance. With the excitement associated with blood withdrawal through the ventricular cannulas, plasma glucose in active ground squirrels was increased by 0.74 mg/ml after 13 min (almost twofold) but no such effect was seen in hibernating ground squirrels (Fig. 1). In hibernating ground squirrels, differences in plasma glucose reflected a general decline during the hibernation periods.

It was further noted that the level of plasma glucose was related to the duration of the hibernation period. Thus, values from individuals with shorter- or longer-than-

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**FIG. 1.** Comparison of effect of excitement on plasma glucose concentrations during serial sampling of active and hibernating arctic ground squirrels in summer and winter. Serial plasma glucose samples from right ventricular cannulas were taken from 11 active squirrels in July and August (mean, ±SD) and 2 hibernating squirrels in January (after 2 and 12 days of torpor). Average summer squirrel heart rates were taken from ECG recordings.

**FIG. 2.** Decline in plasma glucose concentration of arctic ground squirrels having long (15–18 days), average (12–14 days), and short (8–11 days) hibernation periods. Rates of decline are extrapolated to mean hibernation period of each group and statistical treatment (n, M, ± SD) excludes one long-period value (0.42 mg/ml). Curve for 12- to 14-day group represents 15 values.
FIG. 3. Decrease in arctic ground squirrel plasma glucose concentrations during hibernation periods in January and February. Time scale represents ratio between days since last arousal and mean number of days from 3 previous hibernation periods. Serial samples during same hibernation period are connected. Statistical treatment (M, se, sd) includes all plasma glucose values taken during the first 13% (n = 11), 2nd 20% (n = 7), 3rd 20% (n = 11), and final 30% (n = 12) of the hibernation periods.

TABLE 1. Comparison of tissue glycogen concentrations before and during the hibernation season

<table>
<thead>
<tr>
<th></th>
<th>Heart</th>
<th>Thigh Muscle</th>
<th>Abdominal Wall Muscle</th>
<th>Diaphragm</th>
<th>Kidney</th>
<th>Brown Fat</th>
<th>Liver</th>
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</thead>
<tbody>
<tr>
<td>1) Before hibernation, September (fasted 15 hr)</td>
<td>M 0.8</td>
<td>4.5</td>
<td>3.0</td>
<td>1.5</td>
<td>0.2</td>
<td>0.1</td>
<td>17.8</td>
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<tr>
<td></td>
<td>se 0.2</td>
<td>1.4</td>
<td>1.5</td>
<td>0.5</td>
<td>0.1</td>
<td>0</td>
<td>10.4</td>
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<td></td>
<td>n 4</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2) During active periods, January and February</td>
<td>M 1.3</td>
<td>3.8</td>
<td>2.5</td>
<td>2.0</td>
<td>0.3</td>
<td>0.2</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>se 0.8</td>
<td>2.4</td>
<td>1.3</td>
<td>0.9</td>
<td>0.1</td>
<td>0.1</td>
<td>9.7</td>
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<tr>
<td></td>
<td>n 12</td>
<td>12</td>
<td>7</td>
<td>6</td>
<td>12</td>
<td>8</td>
<td>12</td>
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<tr>
<td>1 vs. 2 P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3) Early hibernation period (short-term cycle) January and February</td>
<td>M 3.0</td>
<td>7.3</td>
<td>6.2</td>
<td>5.0</td>
<td>0.8</td>
<td>0.5</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>se 1.7</td>
<td>0.8</td>
<td>1.0</td>
<td>0.9</td>
<td>0.2</td>
<td>0.1</td>
<td>0.6</td>
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<td></td>
<td>n 5</td>
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<td>4</td>
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<td>4</td>
<td>5</td>
<td></td>
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<td>2 vs. 3 P</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
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<tr>
<td>4) Late hibernation period (short term cycle)</td>
<td>M 3.2</td>
<td>4.0</td>
<td>0.5</td>
<td>0.3</td>
<td>0.3</td>
<td>6.1</td>
<td></td>
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<tr>
<td></td>
<td>se 1.8</td>
<td>2.8</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>2.6</td>
<td></td>
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<tr>
<td></td>
<td>n 5</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 vs. 4 P</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
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Values are expressed in mg/g. Significance (P) tested at 95% level.

average hibernation cycles lay, respectively, below or above the average curve (Fig. 2). Accordingly, we have employed a relative time scale representing the fraction of the hibernation period that has elapsed. (Fig. 3) The value after three-fourths of the predicted hibernation periods was about half of the initial value. The regularity of this decrease in plasma glucose concentration was further substantiated by results of serial samples from individual squirrels taken within the same hibernation period (Fig. 3).

Tissue samples of heart, thigh muscle, diaphragm, kidney, brown fat, and liver taken during prehibernation did not differ significantly in glycogen concentrations from those taken from spontaneously aroused arctic ground squirrels (Table 1). Immediately after the initiation of hibernation, glycogen levels in heart, diaphragm, thigh, and abdominal muscle, as well as in kidney and brown fat, were observed to be 2 times or more those in active animals, but no change was observed in liver glycogen levels (Table 1). Average liver and thigh muscle glycogen concentrations declined through the hibernation period (Fig. 4) to less than two-thirds of the levels observed in early hibernation (Table 1). Glycogen concentration in kidney, heart, and brown fat did not change significantly during hibernation periods (Fig. 5). No glycogen was detected in white fat.

During the short active periods between cycles of hib-
Carbohydrate in Arctic Ground Squirrels

REFERENCES


