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## PLASMA BIOCHEMICAL VALUES FROM APPARENTLY HEALTHY FREE-RANGING POLAR BEARS FROM SVALBARD

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ABSTRACT: To establish reference values for free-ranging polar bears (Ursus maritimus) at Svalbard, Norway, plasma samples from 15 females and 20 males were analyzed for 28 blood biochemistry parameters. Animals were chemically immobilized (Zoletil®: tiletamine and zolazepam) on land at Barentsøya, Edgeøya, and the eastern coast of Spitsbergen in August 1998. All bears were apparently healthy, with ages ranging from 1-22 yr. Females had almost two times higher levels of lipase than males. Several parameters varied with age. Levels of alkaline phosphatase (ALP) and calcium (Ca) decreased with age, being significantly higher in young individuals (<6 yr) compared to middle-aged (6-13 yr) and older bears (>13 yr). Globulin was lower in animals <6 yr of age than in animals >13 yr of age, while the opposite was the case for albumin. Levels of ALP, Ca, and potassium decreased with age. We found no significant changes in total protein correlated to age, but total protein levels were higher in obese compared to lean individuals. Further, total protein levels were slightly lower and had greater variation compared to data from polar bears in captivity, which may reflect food availability for the latter group. The mean ratio between urea and creatinine was 10.9 and indicated these bears were fasting. These data provide a baseline from which to compare biochemical parameters in captive and free-ranging polar bears and will be especially valuable for future studies of polar bears at Svalbard.

Key words: Clinical plasma biochemistry, cortisol, marine mammal, polar bear, Ursus maritimus, wildlife medicine.

#### INTRODUCTION

Hematologic and blood biochemical analyses are valuable tools for evaluating health of captive and free-ranging wildlife. To interpret such data knowledge of normal individual variability is needed. For free-ranging polar bears (*Ursus maritimus*) such information is scarce, although some reports on hematologic and serum or plasma biochemical values from Canada exist (Manery et al., 1966; Lee et al., 1977; Nelson et al., 1983a; Ramsay et al., 1991; Bossart et al., 2001). Some additional information on blood biochemistry exists for captive polar bears (Kuntze and Hunsdorf, 1985; Kuntze et al., 1988; Derocher et al., 1990; Kuntze, 1995). However, captive polar bears do not experience seasonal changes in food availability and the food often consists of commercial pellets and/ or dog food, supplemented with fish. Differences in hematologic and blood biochemical parameters between free-ranging and captive polar bears may thus reflect differences in nutrition as well as in physiologic conditions related to exercise and stress (Torgerson, 1990).

Polar bears are on the top of the Arctic marine food web. They feed primarily on ringed (*Phoca hispida*) and bearded seals (*Erignathus barbatus*), but also hunt other seals like harp (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*), as well as scavenge carcasses of walrus (*Odobenus rosmarus*) and whales. At Svalbard they may also prey on Svalbard reindeer (*Rangifer tarandus platyrhynchus*), birds, and eggs (Lønø, 1970; Smith and Lydersen, 1991; Derocher et al., 2000).

The Svalbard polar bear population was placed on the protected list in Norway in 1973 (Prestrud and Stirling, 1994), but recent studies have shown that cross-border movements between the Svalbard population and the Franz Josef Land/Novaya

| Age           | Female |        |       |           | Male |        |       |           |       |
|---------------|--------|--------|-------|-----------|------|--------|-------|-----------|-------|
| group<br>(yr) | Lean   | Normal | Obese | Sub-total | Lean | Normal | Obese | Sub-total | Total |
| 1–6           | 0      | 5      | 0     | 5         | 0    | 5      | 0     | 5         | 10    |
| 7-13          | 0      | 3      | 4     | 7         | 0    | 3      | 3     | 6         | 13    |
| 14-22         | 1      | 2      | 0     | 3         | 3    | 2      | 4     | 9         | 12    |
| Total         | 1      | 10     | 4     | 15        | 3    | 10     | 7     | 20        | 35    |

TABLE 1. Sex, age and body condition distribution of 35 free-ranging polar bears from Svalbard, Norway.

Zemlya population is much more common than previously thought (Paetkau et al., 1999). Currently, information on health and diseases among polar bears from Svalbard is scarce (Tryland et al., 2001), and no information on blood biochemistry is available from these animals. Possible immunotoxic effects of exposure to organochlorines have been suggested (Bernhoft et al., 2000; Skaare et al., 2001) but limited information is available on the effects of such exposure on marine mammals (O'Hara and O'Shea, 2001).

The aim of this study was to establish reference values for selected plasma biochemistry parameters for apparently healthy free-ranging polar bears from Svalbard and to determine variation in such parameters with sex, age, and physical condition.

#### **MATERIAL AND METHODS**

Polar bears (n=35) were captured on land at Barentsøya, Edgeøya, and the eastern coast of Spitsbergen, Svalbard, Norway (77°45′–  $78^{\circ}66' \text{N}, 17^{\circ}62' - 23^{\circ}56' \text{E})$ , during 4 days in the middle of August 1998. Capture and handling methods are described elsewhere (Tryland et al., 2001). Tiletamine-zolazepam (Zoletil®; Virbac International, Carros Cedex, France) was administered in a solution of 200 mg/ml at a dosage of 5-10 mg/kg of body mass (Stirling et al., 1989). Chase time prior to capture was not recorded for each individual but was estimated to be less than 10 min for all bears >1 yr. Cubs were immobilized after their mothers. Animal handling methods were approved by the National Animal Research Authority (NARA; Norwegian Animal Health Authority, Oslo, Norway). Blood samples were collected from the femoral vein into evacuated heparin containers. Samples were kept cool and dark until transferred to the laboratory where plasma was prepared by centrifugation and frozen within 8 hr

of sampling. Samples were stored at  $-20~\mathrm{C}$  until analysis. A first premolar tooth was extracted from all bears  $>1~\mathrm{yr}$  for age determination (Calvert and Ramsay, 1998). Each bear was characterized as lean, normal, or obese on the basis of palpation of subcutaneous lipid stores. Animals were grouped by age to compare plasma biochemistry parameters in sexually immature individuals (young,  $<6~\mathrm{yr}$ ), bears in their prime reproductive period (middle-aged,  $6-13~\mathrm{yr}$ ), and older bears ( $>13~\mathrm{yr}$ ) (Table 1).

Plasma samples were analyzed 5 mo after sampling for 24 different parameters at the Central Laboratory, The Norwegian School of Veterinary Science (Oslo, Norway) in a Technicon AXON System (Miles Inc., Tarrytown, New York, USA). The methods used for each parameter are described elsewhere (Tryland and Brun, 2001). The lipemic status of the samples was evaluated by the Technicon AXON System (Anonymous, 1994). Cortisol was measured using a competitive immunoassay (Whitehead et al., 1983) with standards (Amerlite Cortisol Assay; Ortho-Clinical Diagnostics, Nycomed Amersham plc, Buckinghamshire, UK).

Additionally, plasma total protein was estimated with Biuret technique adapted to an automatic high throughput analyzer (Cobas IN-TEGRA 700; Hoffman La Roche, Basel, Switzerland). Plasma electrophoresis was performed on precast agarose gels (REP HI-Res-15, Helena Rett analyzer; Helena Labs, Belamont, Texas, USA) to reveal amounts of albumin and the globulins alpha 1, alpha 2, beta 1, and gamma. Series of 13 samples and two controls were run on each gel, and separated fractions were automatically scanned by the instrument. The degree of agreement between the two methods (Technicon AXON and electrophoresis) measuring the same parameter on a continuous scale was estimated by using the mean difference between the respective test results and the standard deviation (SD) of this difference (Bland and Altman, 1986).

All statistical analyses were performed in the SAS-PC System® Version 6.12 for Windows (SAS Institute Inc., Cary, North Carolina,

TABLE 2. Clinical plasma biochemistry parameters for 35 (20 male and 15 female) free-ranging polar bears from Svalbard presented as mean (median)  $\pm$  one standard deviation (SD), and 90% central range.

|                                     |                     | 0                 |
|-------------------------------------|---------------------|-------------------|
| Parameter                           | Mean (median) ± SD  | 90% central range |
| AST <sup>a</sup> (U/l)              | 67 (53)±37          | 37–132            |
| ALTa (U/l)                          | 24 (24)±9           | 10-42             |
| ALPa (U/l)                          | 51 (46)±36          | 17–141            |
| CKa (U/l)                           | $251\ (130)\pm395$  | 55–1519           |
| LDH <sup>a</sup> (U/l)              | $832(764)\pm300$    | 523-1178          |
| Amylase (U/l)                       | $2(2)\pm1.4$        | 0–5               |
| Lipase (U/l)                        | $47 (40) \pm 37$    | 10–107            |
| Protein, total (g/l)                | $76 (77) \pm 6$     | 67–85             |
| Albumin (g/l)                       | $46 (47) \pm 4.4$   | 43–51             |
| Globulin (g/l)                      | $29(29)\pm 4$       | 23–38             |
| Alpha 1 (g/l) <sup>b</sup>          | $4.6(4.7)\pm1.1$    | 3.2-6.4           |
| Alpha 2 (g/l) <sup>b</sup>          | $7.4(7.9)\pm1.9$    | 3.5–10.2          |
| Beta 1 (g/l) <sup>b</sup>           | $6.6(6.3)\pm1.4$    | 4.7-9.2           |
| Gamma-globulin (g/l) <sup>b</sup>   | $10.8(11.2)\pm3.1$  | 6–15.6            |
| Urea (mmol/l)                       | $4.7(4.1)\pm3$      | 2.2–12.3          |
| Creatinine (µmol/l)                 | $107(103)\pm22.4$   | 79–143            |
| Bilirubin, total (µmol/l)           | $1(1)\pm0.6$        | 0–2               |
| Cholesterol (mmol/l)                | $8.3(8.3)\pm1.2$    | 5.8–10.3          |
| Triglycerides (mmol/l)              | $2.7(2.4)\pm0.9$    | 1.6-4.2           |
| Non-esterified fatty acids (mmol/l) | $1.7(1.7)\pm0.8$    | 0.7 - 3.3         |
| Glucose (mmol/l)                    | $6.2(6.1)\pm1.5$    | 4.3–9             |
| Calcium (mmol/l)                    | $2.5(2.5)\pm0.2$    | 2.3–2.8           |
| Phosphorus (mmol/l)                 | $2(2)\pm0.3$        | 1.6-2.7           |
| Calcium/phosphorus                  | $1.2(1.2)\pm0.2$    | 1.0-1.6           |
| Magnesium (mmol/l)                  | $0.8(0.9)\pm0.1$    | 0.6-1.0           |
| Sodium (mmol/l)                     | $138(137)\pm5.4$    | 130–147           |
| Potassium (mmol/l)                  | $3.7(3.6)\pm0.4$    | 3-4.5             |
| Sodium/potassium                    | $38(38)\pm3.6$      | 31.2-44.6         |
| Chloride (mmol/l)                   | $100(100)\pm3.6$    | 94–105            |
| Cortisol (nmol/l)                   | $557 (590) \pm 200$ | 151–883           |

 $<sup>^{</sup>a}$  AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; CK = creatine kinase; LDH = lactate dehydrogenase.

USA). The modules Proc freq and Proc univariate were used for the descriptive analyses including frequencies, means, median, and test for normality. Proc GLM and Ismeans were used for variance analyses between groups defined by sex, age, and body condition. A linear regression model was run for age and different response variables. The effect of the explanatory variable was adjusted for confounding as indicated in the tables. Unless otherwise stated, the variables were shown to have an approximated normal distribution.

#### **RESULTS**

All bears were apparently healthy at capture and showed no obvious signs of disease or injury. Of the 35 sampled polar bears, 20 were males and 15 were females with ages ranging from 1–22 yr (mean 10.5

yr, SD=6.2 yr). Twenty animals were characterized as being in normal condition, four animals as lean, and 11 as obese (Table 1). None of the samples were hemolyzed as assessed by visual inspection. All samples were classified as non-lipemic having a lipid concentration <10 mg/dl. Mean, median, SD, as well as the 90% central range for the plasma biochemistry parameters of the 35 free-ranging polar bears are presented in Table 2.

A few sex variations were noted. Male polar bears had a significantly (P=0.05) higher mean level of phosphorus (P) than females, 2.1 (P=0.36) and 1.9 (P=0.29) mmol/l, respectively. Additionally, females had an almost two fold great-

b Based on serum electrophoresis.

TABLE 3. Mean plasma values (standard deviation) for some biochemical parameters in three different age groups of 35 free-ranging polar bears from Svalbard. Statistical differences between groups are shown at three different levels (*P*-values), where groups with mutually significant differences are designed by their respective letters.

|                                     | Group A          | Group B           | Group C          | P-value |               |       |
|-------------------------------------|------------------|-------------------|------------------|---------|---------------|-------|
| Parameter                           | <6  yr<br>(n=10) | 6–13 yr<br>(n=13) | >13 yr<br>(n=12) | ≤0.08   | ≤0.05         | ≤0.01 |
| ALPa (U/l)b                         | 85 (38)          | 50 (32)           | 30 (14)          |         | AB, BC        | AC    |
| CK <sup>a</sup> (U/l) <sup>b</sup>  | 398 (576)        | 262 (390)         | 115 (68)         | AB, BC  |               |       |
| LDH <sup>a</sup> (U/l) <sup>b</sup> | 1043 (431)       | 737 (144)         | 760 (217)        |         | $\mathbf{AC}$ | AB    |
| Albumin (g/l)                       | 48 (3)           | 48 (2)            | 44 (6)           |         | AC, BC        |       |
| GLobulin (g/l)                      | 27 (3)           | 29 (2)            | 31 (5)           |         | $\mathbf{AC}$ |       |
| Gamma-globulin (g/l)                | 8.8 (2)          | 11.2(2)           | 12.2(4)          |         | AB            | AC    |
| Ca (mmol/l)                         | 2.6(0.1)         | 2.5(0.2)          | 2.4(0.2)         |         | $\mathbf{AC}$ |       |
| K (mmol/l)                          | 4.1(0.4)         | 3.6 (0.3)         | 3.4(0.2)         |         | AB            | AC    |

a ALP = alkaline phosphatase; CK = creatine kinase; LDH = lactate dehydrogenase.

er mean level of lipase (64.5 U/l, SD=45.6) than males (34.0 U/l, SD=21.7) (P=0.004), however, using the likelihood ratio test, there was some evidence that sex and age interacted (P=0.08), indicating that the sex effect on lipase was not equal for all age groups. Finally, males had a slightly higher mean gamma-globulin level than females, but this difference was not statistically significant.

The parameters that varied significantly between the different age groups are presented in Table 3. Table 4 shows the relationship between age and response variables with a linear association significantly different from zero. The regression-coefficients ( $\beta$ -coefficient), indicating the

strength of the relationships, showed an average annual increase of 0.3 and 0.25 g/ l for globulin and gamma-globulin, respectively, and an annual decrease of 0.06 U/l for alkaline phosphatase (ALP) and 0.02 mmol/l for calcium (Ca). The log-transformed values of ALP, creatine kinase (CK), and lactate dehydrogenase (LDH) decreased annually by 0.96, 0.96, and 0.98 U/l, respectively. The adjusted  $R^2$  values, which indicate how well the regression models fit the data, showed that age explained more than 40% of the annual variation of the parameters ALP, Ca, and K. Running the regression excluding these values decreased the β-coefficient and the width of the 95% confidence interval, but

TABLE 4. Linear association between age and plasma biochemistry parameters for 35 free-ranging polar bears from Svalbard.

| Parameter             | β-coefficient | Standard<br>error | F-value | P-value | 95%<br>confidence<br>interval | $R^2$ (adjusted) |
|-----------------------|---------------|-------------------|---------|---------|-------------------------------|------------------|
| ALPa,b                | -0.06         | 0.01              | 12.9    | < 0.001 | -0.09, -0.04                  | 0.41             |
| $CK^{a,b}$            | -0.06         | 0.02              | 8.7     | 0.006   | -0.1, -0.02                   | 0.18             |
| $LDH^{a,b,c}$         | -0.02         | 0.01              | 6.0     | 0.01    | -0.03, -0.005                 | 0.23             |
| Globulin <sup>c</sup> | 0.30          | 0.10              | 6.2     | 0.005   | 0.16, -0.51                   | 0.23             |
| Gamma-globulin        | 0.25          | 0.75              | 10.9    | 0.002   | 0.1, 0.4                      | 0.23             |
| Ca <sup>a</sup>       | -0.02         | 0.005             | 10.7    | < 0.001 | -0.03, -0.01                  | 0.46             |
| $K^{a,c}$             | -0.03         | 0.01              | 10.4    | 0.003   | -0.05, -0.01                  | 0.45             |

<sup>&</sup>lt;sup>a</sup> ALP = alkaline phosphatase; CK = creatine kinase; LDH = lactate dehydrogenase; Ca = calcium; K = potassium.

<sup>&</sup>lt;sup>b</sup> To ensure an approximate normal distribution, parameters were transformed logarithmically.

<sup>&</sup>lt;sup>b</sup> eLog-transformed values.

 $<sup>^{\</sup>rm c}$  Adjusted for condition (changing the  $\beta\text{-value}$  with more than 10%).

TABLE 5. Mean plasma values (standard deviation) for some biochemical parameters for 35 free-ranging polar bears from Svalbard grouped according to body condition. Statistical differences between groups are shown at three different levels (*P*-values), where groups with mutually significant differences are designed by their respective letters.

| Parameter              | Group A<br>Lean<br>(n=4) | Group B<br>Normal<br>(n=20) | Group C<br>Obese $(n=11)$ | ≤0.14 | ≤0.09 | ≤0.05 |
|------------------------|--------------------------|-----------------------------|---------------------------|-------|-------|-------|
| LDH (U/l)a             | 868 (244)                | 913 (350)                   | 674 (120)                 | AC    |       |       |
| Total-protein (g/l)    | 70 (12)                  | 76 (5)                      | 78 (4)                    |       | AB    | AC    |
| Triglycerides (mmol/l) | 3.4(1.2)                 | 2.8(0.9)                    | 2.3(0.6)                  |       | AC    |       |
| Creatinine (µmol/l)    | 113 (12)                 | 96 (17)                     | 121 (27)                  |       | BC    |       |
| Ca (mmol/l)            | 2.3 (0.2)                | 2.5(0.2)                    | 2.5(0.1)                  | AB    | BC    |       |
| K (mmol/l)             | 3.4(0.3)                 | 3.9(0.4)                    | 3.4(0.2)                  |       | BC    |       |

<sup>&</sup>lt;sup>a</sup> To ensure an approximate normal distribution, parameters were transformed logarithmically. LDH = lactate dehydrogenase.

did not weaken the above relationships to non-significance (P<0.05). Plasma biochemical parameters that varied between the different condition groups are presented in Table 5.

A few general biochemical findings were noted. The ratio between urea and creatinine (U/C ratio; urea mg/dl:creatinine mg/dl) varied from 4.5 to 44.9 (mean 10.9, SD=7.8) for individuals. Additionally, no significant differences in cortisol levels were found related to sex or age.

Plasma electrophoresis demonstrated the usual pattern as observed in other mammal species, including humans, with albumin, alpha1, alpha 2, beta 1, and gamma-globulin fractions (Table 2). The absolute amounts (g/l) of the various fractions were related to the total protein concentration. Mean serum values measured by the Technicon AXON System and electrophoresis were 76 and 72 g/l for total protein and 46 and 42 g/l for albumin, respectively. The estimation of agreement between the two methods demonstrated a mean difference for total protein of -4 g/1 (SD=3.3) and for albumin of -4.5 g/l (SD=1.7). As the differences approximate the normal distribution, the limits of agreement between the two methods may be given by  $\pm 1.96$  SD from the mean. By using this 95% interval, the electrophoresis method for total protein is expected to give a result from 10 g/l below to 3 g/l above the respective analysis with the Technicon AXON System and from 1 to 8 g/l below for albumin. A paired t-test showed the differences to be significantly different from zero for both total protein and albumin (P=0.006 and P=0.00003, respectively) giving evidence for a true difference between the two methods of measurements.

#### DISCUSSION

Reference values for 28 different plasma biochemical parameters are presented. For some individuals and for some parameters, suspected aberrant values were found, i.e., higher or lower than mean ±3 SD (Lee et al., 1977). Instead of using this as a criterion for excluding aberrant values from the expected normal range, we have chosen to present the data as 90% central range (Table 2). The samples in this study were obtained and treated to avoid high temperatures, light, and hemolysis. They were, however, stored for 5 mo from sampling until analysis took place. The effect on biochemistry parameters of such storage has not been reported for polar bears, but it may be reasonable to assume that some parameters, especially enzymes, may decrease during long term storage, as observed for sera from dogs, northern fur seals (Callorhinus ursinus), and minke (Balaenoptera acutorostrata) (Hunter and Madin, 1978; Thoresen et al., 1995; Tryland and Brun, 2001). The levels of the enzymes aspartate aminotransferase

(AST), alanine aminotransferase (ALT), and LDH in this study were somewhat higher than previously reported (Lee et al., 1977; Kuntze, 1995). This may indicate that storage of samples for 5 mo before analysis did not influence the enzyme levels to a great extent, or that the actual enzyme levels at the time of sampling were higher than measured 5 mo later, and that the enzyme levels presented in Table 2 should be regarded as minimum figures.

The statistical analyses were sensitive to the small and unequal number of animals in the different groups. Larger groups would have increased the power in the study, especially for the condition groups, which was illustrated by the large SD for some variables. Age is often found as a strong confounder in statistical analyses. Because of the small and unbalanced condition-groups, age may therefore have concealed or weakened real differences due to condition.

The higher levels of LDH we found compared to polar bears from Canada (Lee et al., 1977) may be influenced by different trapping and immobilization methods. High levels of LDH in blood are usually associated with general cell damage or necrosis, and this enzyme is present in liver, kidney, pancreas, intestine, cardiac and skeletal muscle, and brain (Bossart et al., 2001). In Canadian polar bears, levels of LDH were significantly higher in bears trapped with foot snares (128–406 U/l) compared to bears caught in culvert traps (37–216 U/l), probably due to muscle trauma caused by the snare (Lee et al., 1977). The bears in this study were chemically immobilized from helicopter, which should minimize stress before immobilization and not cause muscle damage or necrosis. However, the adult polar bears in this study were chased for up to 10 min by helicopter before immobilization and sampling. This induced strenuous exercise, albeit a short period of time may have influenced blood LDH levels for some individuals. The higher levels of LDH found in young individuals may be influenced by the fact that cubs were immobilized subsequent to their mothers, and thus experiencing a longer period of stress and chasing before they were immobilized. Another factor that could have influenced blood biochemical parameters was the drug used for immobilization. In the Canadian samples, phencyclidine was used (Lee et al., 1977), whereas we used tiletamine-zolazepam. Potential influence of these drugs on the blood levels of LDH, as well as on other parameters, is unknown to the authors.

Male polar bears had significantly higher levels of P than females. Although statistically significant, the differences are small compared to the 90% central range for this parameter. Females had about two times higher lipase levels than males. Lipase is produced by the gastric mucosa and the pancreas. Lipase degradation is performed in the kidneys. In dogs, a two to three fold increase in lipase levels is seen in cases of acute pancreatic necrosis or renal failure (Duncan et al., 1994). High levels of lipase may be associated with pancreatic and renal disease in marine mammal species, although available data on lipase levels in other bear species or in other marine mammals are very limited (Bossart et al., 2001). No differences in lipase levels were found between sexes in free-ranging polar bears in Canada (Lee et al., 1977). The same was the situation for minke whales, but in these animals, a mean lipase level of 34 U/l (SD=31) was found in 15 non-lipemic samples (nine females and six males), whereas lower lipase levels were recorded in lipemic samples (Tryland and Brun, 2001). The differences between male and female polar bears cannot be explained by lipemia because none of the samples were classified as lipemic.

Several parameters varied significantly among the three age groups (Table 3). The levels of ALP and Ca decreased with age, being significantly higher in young individuals compared to middle-aged and older bears. Alkaline phosphatase exists in different isoenzymes and is responsible for

the hydrolysis of monophosphate esters in different tissues. The isoenzyme from bone is produced by osteoblasts and blood levels may be three times higher in young and rapidly growing animals compared to adults (Duncan et al., 1994), which corresponds with our findings and those of free-ranging polar bears in Canada (Lee et al., 1977).

We found no significant difference in total protein correlated to age. The globulin levels, however, increased with age, whereas the albumin levels were lower in older bears compared to younger individuals. For Canadian polar bears, age differences for protein were not reported (Lee et al., 1977); but in black bears (U. americanus) from Pennsylvania (USA), globulin levels were significantly higher in adults compared to cubs and yearlings (Storm et al., 1988), which were similar to our data. Storm et al. (1988) also reported lower blood levels of albumin and total protein in cubs compared to yearlings which we did not find for young polar bears compared to middle-aged and older individuals. This difference could be explained by differing age classifications between the studies. Although not significant, we found a tendency of higher CK levels in young individuals (<6 yr) compared to individuals >13 yr. An increase in blood CK levels is often associated with cardiac or skeletal muscle injuries, although a wide range in blood CK have been associated with capture, struggling, and blood sampling of sea otters (Enhydra lutris) (Williams and Pulley, 1983). The higher levels found in young individuals may therefore be a result of the increased stress and chasing of cubs before immobilization compared to adult bears.

There was a linear relationship between age as a continuous variable and different response variables. This suggests that, underlying the differences revealed when grouping the animals in three different age categories, there was a significant linear annual increase or decrease in the parameter. The  $\mathbb{R}^2$  values also showed that age,

as a model variable, explained a considerable proportion of the total variability of the parameter.

Several biochemical parameters varied with condition (Table 5), but only total protein increased significantly from lean individuals compared to obese individuals, which seems to be a result of a similar increase in albumin. The amount of triglycerides was higher in lean polar bears compared to obese individuals. This may be an indication of a fasting hyperlipidemia, which is found in animals that have been fasting for at least 12 hr and which is different from a transient rise following a meal rich in fats (Duncan et al., 1994). The levels of non-esterified fatty acids was lower in lean bears compared to obese bears, which may reflect a lower dietary intake of fats for these bears during a period of fasting.

Like black bears and brown bears (U.arctos), polar bears experience seasonal periods of low food availability. They have evolved physiologic adaptations to starvation and in periods of food abundance they build up adipose depots to provide energy when food is scarce (Derocher et al., 1990). In a fasting state, such as during hibernation and other periods of limited food availability, urea is continuously formed, but urea nitrogen is recycled into plasma proteins, which results in reduced urine formation and a low U/C ratio (Nelson et al., 1983b). In a study of the ability of polar bears to cope with long periods of fasting, samples from 668 polar bears from Canada were analyzed for blood urea and creatinine (Ramsay et al., 1991). The levels of urea (mean 4.7 mmol/l) and creatinine (mean 107 µmol/l) in our study were lower than the levels reported for any season in Canada, while the mean U/C ratio at Svalbard was 10.9, which is in contrast to the mean U/C ratio of 38.9 for Canadian bears sampled on the sea ice with abundant food availability (Ramsay et al., 1991). The mean U/C ratio for the Svalbard polar bears thus indicates a general fasting physiology at the time of sampling. However,

there was no significant correlation between the U/C ratio or plasma urea levels and condition (lean or obese), but we found a tendency of higher level of creatinine in obese individuals compared to individuals in normal condition.

We found higher levels of AST, ALT, LDH, and CK than reported by Lee et al. (1977). A rise in some or all of these parameters and cortisol has been reported in seals, whales, and other wildlife species under different kinds of stress, such as immobilization and translocation (Geraci and Medway, 1973; St. Aubin et al., 1979; Morton et al., 1995; Bossart et al., 2001). These elevated levels may thus be a result of stress related to immobilization method, although none of the bears in this study, with exception for some of the cubs, were chased extensively before they were darted. In a study on cortisol as a stress indicator in 18 different wildlife species, it was concluded that chemical capture was less stressful than physical restraint (Morton et al., 1995). It thus seems unlikely that the polar bears in this study were more stressed than polar bears caught by footsnares or culvert traps prior to immobilization (Lee et al., 1977). Cortisol levels found for polar bears in this study (90% central range 151-883 nmol/l) were within the range reported for polar bears in Canada as well as from other marine mammals, and a wide variation is generally reported (St Aubin, 2001). None of the polar bears had extremely high levels of cortisol, and the individual variation may have been related to differences in chase time, but unfortunately, individual chase time was not recorded in this study. Cortisol levels were higher in black bears in winter than in summer, and generally higher than found for polar bears (Harlow et al., 1990). It is uncertain whether this difference in cortisol levels is due to less capture stress among polar bears or species variability. No sex or age-related differences in cortisol levels were found.

Plasma total protein values found in this study were slightly higher than previously

reported for polar bears (Lee et al., 1977) and other bear species (Storm et al., 1988; Huber et al., 1997), although even higher levels have been reported for polar bears in captivity (Kuntze and Hunsdorf, 1985). Our higher total protein levels may be related to the presence of fibringen in the plasma samples, which is removed from serum samples analyzed in most studies. Total protein values found in our study showed relatively large variation, from 52 g/l to 82 g/l. Hemolysis, lipemia, and sample dehydration may artificially increase protein levels (Bossart et al., 2001), but none of these factors were pertinent to our study. Lipemia may also interfere with blood analysis, but our polar bears samples were not lipemic. The protein profile was analyzed by two different methods shown to give statistically different results. However, when comparing the results with data obtained from polar bears in Canada (Lee et al., 1977) and from polar bears in captivity (Kuntze, 1995), similar ranges for total protein were found, 59–82 g/l, and 72– 83 g/l, respectively, although slightly higher values and less variation were reported for captive individuals. In our data, we found significantly higher levels of total protein in animals in good condition compared to individuals in poor condition. Although the groups of obese and lean individuals are small, this suggests that the generally higher values found for bears in captivity (Kuntze, 1995) are a result of good quality and stable food availability compared to free-ranging animals.

The difference between the methods (Technicon AXON System and electrophoresis) measuring total protein and albumin was surprising. For these two parameters, we have chosen to present the Technicon AXON measurements (Table 2), since this automated measurement equipment is most common in clinical health assessments. However, the demonstrated differences show that clinical biochemistry data may depend on the measurement methods used and that they should be interpreted with care and merely be used together

with clinical examinations as indications of abnormality, unless huge deviations from the expected ranges are found.

Baseline information on plasma biochemistry parameters for polar bears from Svalbard is presented. Several parameters, such as ALP and Ca, varied with age and were higher in young bears (<6 yr) compared to middle-aged and older bears (>13 yr), whereas an annual decrease in ALP, Ca, and K was demonstrated by linear regression. Total protein levels were higher in obese compared to lean individuals, and were generally lower than reported for polar bears in captivity, probably reflecting different food availability and supporting the U/C ratio findings that these bears generally were in a fasting physiologic state at the time of sampling. This information will be especially valuable for future studies among polar bears at Svalbard as well as other polar bear populations, and should also represent a tool in the health assessments of captive polar bears.

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