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Abstract

We report body weights (BW) and blood and serum analyses for 6 fully mature and 8 not-yet-mature captive plains viscachas before and 3, 6 and 9 months after switching from a low-fiber, high-energy diet to a high-fiber, low-energy diet. Initially, body weights, serum glucose, fructosamine and cholesterol levels were above the reference range in the fully mature animals. Furthermore, 4 of these animals had bilateral cataracts. After the diet change, these parameters dropped into the reference range. However, 9 months later, a slightly increased BW became evident again. The findings are consistent with a type II diabetes mellitus and underline the importance of dietary prevention.
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Keywords: hematology, blood chemistry, diabetes, cataract, rodent

Veränderungen nach Futterumstellung in Körpergewicht, Hämatologie und Serologie bei Flachland-Viscachas (*Lagostomus maximus*) in Menschenobhut mit vermutetem Typ II Diabetes


Schlüsselwörter: Hämatologie, Serologie, Diabetes, Katarakt, Nager
INTRODUCTION

The plains viscacha (*Lagostomus maximus*) is a social rodent that inhabits the pampas grasslands of Paraguay, Bolivia, and Argentina. Despite its comparatively large size (for a rodent) and its attractive fur coloration, it is rarely kept in zoological gardens (ISIS, 2001). These animals have been kept and bred successfully at Zurich Zoo since 1964 (Rübel et al., 1989). A comparatively frequent clinical occurrence of bilateral cataracts, glucosuria, and elevations in glucose, cholesterol and fructosamine levels, as well as necropsy findings of fatty livers, led to the suspicion that - like some other rodents (Besselmann and Hatt, 2004), the plains viscacha might be particularly prone to diet-induced diabetes. However, it was only until blood reference values, in particular for fructosamine, for free-ranging viscachas were established (Wenker et al., 2007), that an actual clinical diagnosis based on serum chemistry became possible.

The purpose of this study was to investigate blood analytes in a group of captive plains viscachas with presumptive diabetes type II from the Zurich Zoo in comparison with the data of free-ranging animals, and to observe levels from repeated blood samples after a diet change.

ANIMALS, MATERIAL AND METHODS

Animals and husbandry

Fourteen plains viscachas were available for this study. The animals were classified into mature individuals (> one year of age, exact birth data and age were not available) and not-yet-matured individuals (up to one year of age). The mature viscachas comprised 4 males and 2 females (total 6), the not-yet-matured viscachas comprised 2 animals that had just reached maturity (male and female), 2 subadult females, and 4 juveniles (male and 3 females). They were weighed and bled four times – before the diet change (0), and 3, 6 and 9 months after.

The viscachas were housed indoors at two different locations, with the 6 adults in one and all the other animals in the other group. The animals were kept in indoor enclosures of 23.9 m² and 20.1 m² for the mature and the other animals, respectively. Enclosures had a concrete floor covered with gravel and sand, and were furnished with artificial rocks containing artificial burrows as well as artificial hollow logs, offering the animals a variety of opportunities to hide, climb, and dig.

Feeding

Study animals were fed as a group, not individually. Drinking water was available at all times. The diets used were recorded and analyzed for proximate nutrients (Naumann and Bassler, 1988) in the course of a dissertation project (Besselmann, 2005). The original diet...
consisted of apples, carrots, bread, a commercial mineral/vitamin supplement (Multiforsa M21, Multiforsa AG, 6312 Steinhausen, Switzerland), and rye grass hay and local browse branches ad libitum; however, the apples, carrots and bread alone were provided in amounts theoretically large enough to meet the animas’ estimated energy requirements without any additional hay intake. This ration, the intake of which was not actually determined, contained, calculated without intake of additional hay or branches, in dry matter: 9.0 % crude protein, 5.2 % crude fiber, 1.0 % ether extracts, 3.9 % crude ash and 80.8 % nitrogen-free extracts. After the diet change, the diet consisted of a 1:1 rye grass hay and straw mixture, a vitamin/mineral supplement (Multiforsa M21, Multiforsa AG, 6312 Steinhausen, Switzerland) and a pelleted feed based on alpine meadow hay (PRE ALPIN Lepo, Agrobs GmbH, 82541 Degerndorf, Germany). The actual intake of this diet was measured during three days. It contained, in dry matter (DM): 7.5 % crude protein, 39.2 % crude fiber, 1.7 % ether extracts, 5.6 % crude ash and 46.1 % nitrogen-free extracts. Using data on nutrient content, data on nutrient digestibility measured in viscachas on a mixed diet from Besselmann (2005), and the factorial estimation of digestible energy (DE) for rabbits from Kamphues et al. (2004), DE content of the first ration was estimated at 12.5 MJ/kg DM, and of the second ration at 10.3 MJ/kg DM.

Blood sampling

At each sampling interval, the animals were anaesthetized by mask induction and maintenance with isoflurane in oxygen. They were weighed, and a complete physical and ophthalmologic examination was performed. Blood samples taken from the medial branch of the saphenous vein were subject to complete hematology counts and serum chemistry profiles following the standard procedures outlined in Wenker et al. (2007) for the samples analyzed at the Clinical Laboratory of the Vetsuisse Faculty of the University of Zurich in Switzerland.

Statistical analysis

For each group, a repeated measurements-ANOVA, with subsequent Tukey-Kramer post hoc tests, was used to compare the time points. Furthermore, for each time point, a t-test was used to compare the two groups. Statistical analyses were carried out using Instat 3.0 (GraphPad Software Inc.) and SPSS 12.0 (SPSS Inc., Chicago, IL), respectively. The significance level was set to 0.05.

RESULTS

Matured adults were significantly heavier than growing and recently-matured animals, and before the diet change had almost consistently higher body weights than the reference range for
free-ranging animals of the same sex (Tab 1). Whereas the body weights of the two female
animals dropped and remained within the reference range after the diet change, the drop in
body weight after the diet change was only temporary in the males; the latter all gained weight
again between months 6 and 9, again mostly exceeding the reference range for free-ranging
animals. By contrast, juveniles, subadults and recently-matured adults gained weight more-or-
less consistently throughout the experiment, and were always within the body weight reference
range. Opthalmologic examination revealed that 4 of the 6 adults had bilateral cataracts.

Whereas the blood cell count for the group of younger animals was within the reference
ranges, the fully matured group was characterized by high white blood cell, high monocyte,
and particularly high lymphocyte counts (Tab 2). Although there was substantial variation
between individuals in this group, most individuals had values well above the reference ranges
for these parameters.

Whereas the younger group had serum glucose and fructosamine levels consistently within
the reference range, the fully matured group had levels above the reference range before the
diet change (above reference range for fructosamine for all animals, and for glucose for all but
one individual; Tab 3). These individuals were therefore considered to fall within the diabetic
condition range. In this group, glucose values fell within the reference range directly after the
diet change, as did the average fructosamine value. For four animals of this group, however,
the fructosamine value, though decreasing already at 3 months, dropped into the reference
range only at 9 months after the diet change. For the younger group, cholesterol levels were
always within the reference range, but values for fully matured animals exceeded the reference
range before the diet change. In the latter case, cholesterol only fell within the reference range
6 months after the diet change.

Blood urea nitrogen values for most individuals were below the reference range
throughout the study. The same was observed for total protein levels for the younger group. In
both groups, amylase levels, which were actually below the reference range in the younger
group before the diet change, increased after the diet change to a level similar to the mean
measured in free-ranging animals. Glutamate dehydrogenase (GLDH) was within the reference
range for the fully mature group, but below it for the younger group. In both groups, GLDH
levels increased above the reference range after the diet change, only dropping back within the
reference range 9 months after the diet change. In the younger group, aspartate-aminotransferase
(ASAT) and alanine-aminotransfersase (ALAT) decreased over time. Lactate dehydrogenase
(LDH) decreased in both groups over time.
The viscachas of this study were separated into two groups, distinguishing animals that were still growing or had just about completed their growth from animals that had been on the original diet not only during growth but also during their adult life. In contrast to growing animals, fully matured animals had body weights, and serum glucose, fructosamine and cholesterol levels above the reference range, and four out of six had bilateral cataracts. The findings are consistent with type II diabetes mellitus (T2DM). Given reports on diabetes susceptibility in other rodents, including cataract development (Schmidt-Nielsen et al., 1964; Weir, 1974; Shafir and Adler, 1983; Barnett et al., 1994; Kalman et al., 1996; Krugner-Higby et al., 2000) support this hypothesis. As serum glucose values alone are difficult to interpret (possible increase due to the handling stress is likely in wild animals), fructosamine proved to be particularly useful as a reliable long-term indicator for hyperglycemia, reflecting glycemic control over the preceding 2-3 weeks as reported in dogs and cats (Reusch et al., 1993). The fact that parameters associated with T2DM or prolonged hyperglycemia, including body weight, serum glucose, fructosamine, and cholesterol, decreased after the diet change is in accord with similar dietary intervention studies in other rodents (Barnett et al., 1994; Bar-On et al., 1999; Walder et al., 2002).

In the group of growing animals, such elevated parameters were not found. Based on these four parameters alone, one would suggest that the condition only develops if growth reaches completion and surplus energy is directed towards adipose tissue stores only. However, a closer look at other parameters measured in this study, as explained further down below, suggests that an incipient diabetic state did potentially characterize the juvenile animals on the old diet as well.

In human cases with diabetes mellitus linked to pancreatic tissue damage, elevated serum amylase levels have been reported (Abou-Seif and Youssef, 2004). On the other hand, decreased amylase values have been reported in diabetic humans (Foo et al., 1980; Aughsteen et al., 2005), rats (Mori et al., 2003) and guinea pigs (Balk et al., 1975) and in particular, reduced pancreatic amylase secretion was noted in human juvenile-onset diabetes mellitus (Frier et al., 1976). Therefore, the below-reference range serum amylase activities measured also in our juvenile viscachas could be considered an indication that a diabetic condition was already beginning to manifest itself. Activities of the hepatic enzymes AST and ALT in juveniles also decreased after the diet change, a trend that has been reported to occur in insulin-treated experimental diabetic rats (Mori et al., 2003). Additionally, LDH in both juvenile and adult viscachas showed a similar trend (Tab 3) to that usually reported for experimental rats, i.e. to increase in diabetic specimens and decrease with antidiabetic treatment (Stanely et al., 2000;
Narendhirakannan et al., 2006). In sum, it seems likely that even juvenile viscachas kept on the old diet were in the initial metabolic stages of T2DM.

Based on findings of the first clinical examinations and blood samples, the diet change resulted in a body weight decrease in the fully matured group but did not appear to compromise growth in the younger group. Concomitant with the drop in serum parameters already discussed, body weight changes could indicate that such a low-energy diet is adequate even for growth in this species, and presents a possible prophylactic measure in these animals. The fact that body weights of the fully matured animals had increased again 9 months after the diet change might indicate that even such a diet should be provided in more restricted amounts, or that components with a higher digestibility should be reduced even further. We recommend strictly limited diets for successful long-term control of the reported problem in this species.

Blood urea nitrogen values in this study were lower than those measured in free-ranging animals. For free-ranging animals, it was speculated that capture and handling of the animals, including a potential dehydration, might have led to an increase of BUN levels (Wenker et al., 2007), but differences in dietary protein between captive and free-ranging animals, or even renal damage as a consequence of diabetic conditions, could also be implicated. After the diet change, which also represented a decrease in dietary protein concentration, the BUN of both captive viscacha groups dropped temporarily and then increased again. This either reflects a change in the protein content of the roughage used, or it could present an adjustment period during which the animals learned to compensate for lower protein levels by increasing total intake. In other rodents, it has been shown that the gastrointestinal tract does adapt to diets of lower nutritional quality within several months, in particular by increasing in both length and volume (Karasov and McWilliams, 2005). However, there are no reports about the time period that animals, habituated to a diet high in energy, will actually need to adjust to a new diet of lower quality. The development in body weight in the adult animals suggests that such an adaptation might actually occur only much later than would be expected in free-ranging animals that are adapted to seasonal fluctuations in forage quality. For studies on the dietary flexibility of a species, in which captive animals (like animals from zoos) are used, these results indicate that findings based on diet changes need to be evaluated over long time periods.

Wenker et al. (2007) found that the free-ranging viscachas they investigated had a predominantly neutrophilic white blood cell count. This contrasts with the lymphocyte-dominated white blood cell count of chinchilla. These authors speculated that this difference might represent a stress response in the form of neutrophil release during capture and handling. The captive viscachas in this study show similar absolute neutrophil counts as the free-ranging...
animals. Captive animals had distinctively higher total leukocyte, monocyte and lymphocyte counts, a trend especially pronounced in the fully matured animals. While different disease processes not further investigated in this study cannot be ruled out, and might be considered likely due to the high variation in these parameters between individuals, this finding is nevertheless striking. This could indicate that animals with a potentially developing or established T2DM are more susceptible to other diseases in general.

One other factor might play a minor, additional role in the development of potential T2DM in captive plains viscachas – the lack of exercise in comparison to free-ranging animals. In sand rats, it has been demonstrated that physical exercise has a prophylactic effect against T2DM (Heled et al., 2002). While exercise opportunities in the form of running wheels are common practice in the husbandry of small laboratory or pet rodents (Brown and Donnelly, 2004; Gebhardt-Henrich et al., 2005), they are hardly ever used in zoological gardens. This might represent a valuable enrichment strategy, the effect and the acceptance of which – both by the animals and the public – warrants further investigation.

Finally, e.g. in the sand rat, the susceptibility to T2DM has been shown to vary between the genetic lineages within the species (Walder et al., 2000). Although the small sample set of this study does not allow genetic evaluation, hereditary factors might play a role in the proneness to develop T2DM in viscachas as well.

In conclusion, the results of this study suggest that the diet change at Zurich zoo was an important measure to reduce the diabetogenic state in the captive plains viscacha. However, in particular the incipient relapse in body weight in the adult animals raises the question whether this diet change can actually be considered satisfactorily in the long run. This will have to be answered in the future by a comparison of medical records and necropsy reports from before and after the diet change.

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LITERATURE CITED

Balk M. W., Lang C. M., White W. J., Munger B. L.: Exocrine pancreatic dysfunction in

Bar-On H., Den-Sasson R., Ziv E., Arar N., Shafrir E.: Irreversibility of nutritionally induced
NIDDM in Psammomys obesus is related to beta-cell apoptosis. Pancreas 1999, 18:
259-265.

Barnett M., Collier G. R., Zimet P., O’Dea K.: The effect of restricting energy intake on


Besselmann D: Untersuchungen zur Anatomie und Verdauungsphysiologie des Flachland-
Viscacha (Lagostomus maximus). Dissertation, University of Zurich, 2005.


Foo Y., Rosalki S. B., Ramdial L., Mikhailidis D., Dandona P.: Serum isoamylase activities in


Gebhardt-Henrich S. G., Vonlanthen E. M., Steiger A.: How does the running wheel affect the

Heled Y., Shapiro Y., Shani Y., Moran D. S., Lang zam L., Braiman L., Sampson S. S.,
Meyerovitch J.: Physical exercise prevents the development of type 2 diabetes mellitus in

Kalman R., Lazrovici G., Baron H., Ziv E.: The sand rat (Psammomys obesus): morphologic,
physiologic, and biochemical characteristics of a model for type-II diabetes mellitus.

Kamphues J., Coenen M., Kienzle E., Pallauf J., Simon O., Zentek J.: Supplemente zu
Vorlesungen und Übungen in der Tierernährung, 10. Aufl. M. & H. Shaper, Alfeld-

Karasov W. H., McWilliams S. R.: Digestive constraints in mammalian and avian ecology. In:
Physiological and ecological adaptations to feeding in vertebrates. Eds. J. M. Starck

Krugner-Higby L., Shadoan M., Carlson C., Gendron A., Sofia P., Marler C., J W.: Type 2
diabetes mellitus, hyperlipidemia, and extremity lesions in California mice
(Peromyscus californicus) fed commercial mouse diets. Comp. Med. 2000, 50: 412-
418.

Mori D. M., Baviera A. M., Oliveira Ramalho L. T. d., Vendramini R. C., Brunetti I. L., Pepato

Narendhirakannan R. T., Subramanian S., Kandaswamy M.: Biochemical evaluation of
antidiabetogenic properties of some commonly used Indian plants on streptozotocin-
1157.

Naumann K., Bassler R.: Handbuch der landwirtschaftlichen Versuchs- und
Untersuchungemethodik. III. Die chemische Untersuchung von Futtermitteln.
VDLUFA Verlag, Darmstadt, Germany, 1988.


Rübel A., Hauser B., Ossent P.: Viscachas (Lagostomus maximus), their biology, husbandry,


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Fax: 061 2810005, wenker@zoobasel.ch
Table 1. Body weights of the viscachas (Lagostomus maximus) used in this study before (0) and 3, 6, and 9 months after the diet change.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Cataract?</th>
<th>Stage</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>(sex)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (m)</td>
<td>yes</td>
<td>adult, matured</td>
<td>9050</td>
<td>7600</td>
<td>6400</td>
<td>6650</td>
</tr>
<tr>
<td>2 (m)</td>
<td>yes</td>
<td>adult, matured</td>
<td>8700</td>
<td>7600</td>
<td>6800</td>
<td>7450</td>
</tr>
<tr>
<td>3 (m)</td>
<td>no</td>
<td>adult, matured</td>
<td>7500</td>
<td>6550</td>
<td>6500</td>
<td>7600</td>
</tr>
<tr>
<td>4 (m)</td>
<td>yes</td>
<td>adult, matured</td>
<td>6300</td>
<td>5900</td>
<td>5850</td>
<td>6600</td>
</tr>
<tr>
<td>5 (f)</td>
<td>yes</td>
<td>adult, matured</td>
<td>4900</td>
<td>4100</td>
<td>3950</td>
<td>3800</td>
</tr>
<tr>
<td>6 (f)</td>
<td>no</td>
<td>adult, matured</td>
<td>4200</td>
<td>4200</td>
<td>3600</td>
<td>3950</td>
</tr>
<tr>
<td>7 (m)</td>
<td>no</td>
<td>adult, just-matured</td>
<td>4000</td>
<td>4000</td>
<td>4300</td>
<td>4500</td>
</tr>
<tr>
<td>8 (f)</td>
<td>no</td>
<td>adult, just-matured</td>
<td>3400</td>
<td>3300</td>
<td>3650</td>
<td>3400</td>
</tr>
<tr>
<td>9 (f)</td>
<td>no</td>
<td>subadult</td>
<td>2900</td>
<td>2680</td>
<td>2700</td>
<td>2800</td>
</tr>
<tr>
<td>10 (f)</td>
<td>no</td>
<td>subadult</td>
<td>2500</td>
<td>2650</td>
<td>3100</td>
<td>2850</td>
</tr>
<tr>
<td>11 (m)</td>
<td>no</td>
<td>juvenile</td>
<td>1600</td>
<td>1800</td>
<td>2200</td>
<td>2500</td>
</tr>
<tr>
<td>12 (f)</td>
<td>no</td>
<td>juvenile</td>
<td>1500</td>
<td>1700</td>
<td>2100</td>
<td>2400</td>
</tr>
<tr>
<td>13 (f)</td>
<td>no</td>
<td>juvenile</td>
<td>1400</td>
<td>1500</td>
<td>1700</td>
<td>1900</td>
</tr>
<tr>
<td>14 (f)</td>
<td>no</td>
<td>juvenile</td>
<td>1300</td>
<td>1600</td>
<td>2000</td>
<td>2100</td>
</tr>
</tbody>
</table>

Mean 1-6: 6775\(^a\) ±1989\(^a\) 5992\(^a\) ±1567\(^a\) 5517\(^b\) ±1388\(^b\) 6008\(^ab\) ±1702\(^b\)

SD: ±1031 ±911 ±904 ±828

Mean 7-14: 2325\(^a\) 2404\(^a\) 2719\(^b\) 2806\(^b\)

Different superscripts within a row indicate significant differences between the time periods; for all time periods, the difference between the two groups (animals 1-6 vs. animals 7-14) was significant.

* Reference body weights for free-ranging viscachas from Wenker et al. (2007) are, for males 4600 (range 2900-6600) g and for females 3100 (range 1800-4200) g.
Table 2. Red and white blood cell count of captive plains viscachas (Lagostomus maximus) as compared to the reference range established for free-ranging individuals before (0) and 3, 6, and 9 months after the diet change.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference range</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>34-42</td>
<td>34±3</td>
<td>34±3</td>
<td>38±3</td>
<td>33±3</td>
<td>32±2</td>
<td>35±3</td>
<td>35±3</td>
<td>34±3</td>
</tr>
<tr>
<td>RBC (10^6/μl)</td>
<td>4.44-5.64</td>
<td>4.79±0.46</td>
<td>4.34±0.36</td>
<td>4.81±0.40</td>
<td>4.53±0.50</td>
<td>4.28±0.27</td>
<td>4.73±0.32</td>
<td>4.57±0.38</td>
<td></td>
</tr>
<tr>
<td>WBC (10^3/μl)</td>
<td>8.2-17.8</td>
<td>18.0±6.6</td>
<td>25.5±16.0</td>
<td>23.7±20.2</td>
<td>30.5±28.0</td>
<td>11.6±3.4</td>
<td>12.0±2.0</td>
<td>13.1±3.5</td>
<td>13.5±1.9</td>
</tr>
<tr>
<td>Neutrophils (/μl)</td>
<td>6384-13667</td>
<td>8322±2405</td>
<td>8801±2912</td>
<td>8537±3365</td>
<td>9485±2110</td>
<td>6159±2538</td>
<td>6659±1741</td>
<td>6385±1780</td>
<td>7330±1635</td>
</tr>
<tr>
<td>Monocytes (/μl)</td>
<td>196-865</td>
<td>1712±1362</td>
<td>1215±656</td>
<td>1669±1187</td>
<td>959±485</td>
<td>237±180</td>
<td>454±213</td>
<td>302±181</td>
<td>316±159</td>
</tr>
<tr>
<td>Lymphocytes (/μl)</td>
<td>532-4049</td>
<td>7449±4245</td>
<td>14876±15518</td>
<td>13142±16210</td>
<td>19734±26462</td>
<td>4974±2048</td>
<td>4694±1663</td>
<td>6132±3245</td>
<td>5670±1776</td>
</tr>
</tbody>
</table>

Different small superscripts (abc) within a row indicate significant differences between the time periods in a group; different capital superscripts (AB) indicate significant differences between the two groups.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reference range</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.7-11.2</td>
<td>14.2±3.7</td>
<td>9.0±0.9</td>
<td>8.2±0.7</td>
<td>9.1±1.5</td>
<td>7.4±1.5</td>
<td>8.5±1.0</td>
<td>7.4±1.3</td>
<td>8.2±0.9</td>
</tr>
<tr>
<td>Fructosamine (μmol/L)</td>
<td>161-297</td>
<td>348±34</td>
<td>281±33</td>
<td>281±39</td>
<td>278±13</td>
<td>276±19</td>
<td>253±26</td>
<td>248±33</td>
<td>255±24</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>10.1-23.7</td>
<td>9.4±2.0</td>
<td>3.8±0.5</td>
<td>4.1±0.9</td>
<td>7.2±2.1</td>
<td>9.4±1.3</td>
<td>8.8±1.7</td>
<td>4.0±0.9</td>
<td>5.7±1.0</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>94-219</td>
<td>139±21</td>
<td>179b±32</td>
<td>171abc±22</td>
<td>154c±25</td>
<td>120±21</td>
<td>136abc±17</td>
<td>144abc±18</td>
<td>140b±19</td>
</tr>
<tr>
<td>Total Protein (g/L)</td>
<td>61-77</td>
<td>69±5</td>
<td>68±7</td>
<td>64±9</td>
<td>69±6</td>
<td>54abc±5</td>
<td>52ab±4</td>
<td>56abd±3</td>
<td>58b±5</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.5-2.1</td>
<td>3.2±1.4</td>
<td>2.3ab±0.9</td>
<td>1.4b±0.6</td>
<td>2.0abc±0.2</td>
<td>1.0b±0.2</td>
<td>1.1b±0.2</td>
<td>1.4±0.4</td>
<td>1.1b±0.2</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.2-2.4</td>
<td>1.2±0.6</td>
<td>1.2±0.3</td>
<td>0.8±0.1</td>
<td>1.2±0.3</td>
<td>0.4ab±0.1</td>
<td>0.3ab±0.1</td>
<td>0.7b±0.3</td>
<td>0.6abd±0.2</td>
</tr>
<tr>
<td>Alkaline Phosphatase (IU/L)</td>
<td>20-316</td>
<td>31±6</td>
<td>70±31</td>
<td>64±57</td>
<td>33±13</td>
<td>41b±8</td>
<td>48±11</td>
<td>45±19</td>
<td>39±9</td>
</tr>
<tr>
<td>Amylase (IU/L)</td>
<td>377-5091</td>
<td>673±142</td>
<td>531±130</td>
<td>1373±224</td>
<td>1345±262</td>
<td>195±123</td>
<td>249ab±62</td>
<td>937abc±184</td>
<td>707ab±204</td>
</tr>
<tr>
<td>Glutamate Dehydrogenase (IU/L)</td>
<td>17.3-39.0</td>
<td>15.4±6.8</td>
<td>57.3±42.0</td>
<td>50.1±40.2</td>
<td>36.7±19.3</td>
<td>45.7b±21.1</td>
<td>45.6±21.8</td>
<td>112.4±112.2</td>
<td>36.0±24.1</td>
</tr>
<tr>
<td>Aspartate aminotransferase (IU/L)</td>
<td>86-215</td>
<td>74±29</td>
<td>92±29</td>
<td>65±15</td>
<td>65±7</td>
<td>129ab±34</td>
<td>103ab±28</td>
<td>78bc±12</td>
<td>64c±12</td>
</tr>
<tr>
<td>Alanine aminotransferase (IU/L)</td>
<td>29±6</td>
<td>42±25</td>
<td>33ab±13</td>
<td>18±5</td>
<td>22ab±7</td>
<td>51±16</td>
<td>39±6</td>
<td>23b±5</td>
<td>22b±6</td>
</tr>
<tr>
<td>Creatine Kinase (IU/L)</td>
<td>4620-48600</td>
<td>4343±4135</td>
<td>2736±1407</td>
<td>2312±1246</td>
<td>2080±1282</td>
<td>2945±2690</td>
<td>2225±884</td>
<td>1694±1446</td>
<td>3256±3803</td>
</tr>
<tr>
<td>γ Glutamyl-transferase (IU/L)</td>
<td>1±3</td>
<td>2±1</td>
<td>2±1</td>
<td>2±1</td>
<td>1±1</td>
<td>1±1</td>
<td>2±1</td>
<td>2±1</td>
<td>1±0</td>
</tr>
<tr>
<td>Lactate Dehydrogenase (IU/L)</td>
<td>801-2530</td>
<td>610±168</td>
<td>621±77</td>
<td>375abc±153</td>
<td>324±117</td>
<td>763±406</td>
<td>618±179</td>
<td>572abc±125</td>
<td>411b±177</td>
</tr>
</tbody>
</table>

Different small superscripts (abc) within a row indicate significant differences between the time periods in a group; different capital superscripts (AB) indicate significant differences between the two groups.