SERUM LEPTIN AND GHRELIN LEVELS IN PATIENTS IN THE EARLY STAGES OF ACUTE BILIARY PANCREATITIS AND DIFFERENT DEGREES OF SEVERITY

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Acute pancreatitis (AP) is a potentially fatal disease. In animal experiments leptin and ghrelin were shown to modulate the course of AP.

The aim of the study was to estimate the relationship between the severity of acute biliary pancreatitis (ABP) and serum levels of leptin and ghrelin in non-obese patients in the first seven days of the hospitalization.

Material and methods. The study included nine patients with mild ABP (MABP), eleven patients with severe ABP (SABP) and twenty healthy controls, appropriately matched age, sex and weight. Serum concentrations of leptin and ghrelin were measured in patients on the first, third, fifth, and seventh days of hospitalization using leptin and ghrelin RadioImmunoAssay (RIA) kits.

Results. At admission and throughout the study the mean serum leptin concentration in SABP patients was higher than in the controls but without statistical significance. Serum ghrelin concentrations on admission were significantly lower in patients with ABP than in the controls. We observed steadily increasing serum ghrelin levels in both groups of the patients during the course of ABP.

Conclusions. The results of our study do not support the role of leptin as a marker of the severity of ABP. On the other hand, rising serum ghrelin levels during the course of ABP may be a marker of recovery and an indicator of the healing process.

Key words: acute pancreatitis, leptin, ghrelin
which function as the main storage for fat in the form of triglycerides (10). Obesity is an important risk factor in the severe form of AP. An increase in the volume of intrapancreatic adipose tissue in obese humans with AP was associated with more pronounced pancreatic necrosis and multisystem organ failure (11). In turn, hydrolysis of triglycerides generates free fatty acids (FFA’s). In upper-body obesity subcutaneous fat is the main contributor to circulating FFA’s, and the source of their excessive release (12). Increased levels of mainly unsaturated FFA’s were found to aggravate acute pancreatic (13).

Serum leptin concentrations are high in obese persons (14). Early study showed that AP in rats and humans is also associated with significant increase in plasma leptin level (6). There was a suggestion that serum leptin and ghrelin level could be used as an inflammatory marker of the severity of the disease (15).

Ghrelin is a 28-aminoacid hormone originally isolated from the rat stomach playing major role in the regulation of lipid metabolism and regulation of growth hormone (16, 17). Numerous studies showed that ghrelin has a spectrum of peripheral activities which engage endocrine, cardiovascular and bone systems (18, 19). Role of ghrelin in the course of AP was the subject of several studies (3, 4).

The aim of our investigation was to find out if there is a correlation between the severity of biliary AP and serum leptin and ghrelin level.

**MATERIAL METHODS**

The study included nine patients with the mild form of acute biliary pancreatitis (MABP), 11 patients with the severe form (SABP) (treated at 2nd Department of General Surgery UJCM in 2007-2009 y) and twenty healthy controls matched for age, sex and body mass index (BMI) (tab. 1). The diagnosis of acute biliary pancreatitis (ABP) was based on clinical symptoms, elevated serum amylase activity (more than 3 times above the reference limit), abdominal ultrasonography (USG) and abdominal computed tomography (CT). The severity of AP was assessed according to the Atlanta criteria (20), presence of three or more Ranson’s criteria (21) and eight or more Acute Physiology and Chronic Health Evaluation II (APACHE II) score (22). Abdominal USG was performed on each patient and the progression of morphological changes within the pancreas was evaluated with the Becker scale (23). A CT scan of the upper abdomen was performed on all the patients with SAP. The Balthazar scale (CT grade) was used for evaluation of pancreatic CT findings (24). The occurrence of organ dysfunction (failure) was determined by the MODS score (25).

Venous blood samples were taken from the patients after 12 hrs fasting on the first, third, fifth and seventh day of hospitalization. We missed to obtain the blood samples at the seventh day in one patient with MABP and two patients with SABP. In the control group we collected the blood only once – at the beginning of the study.

Leptin and ghrelin concentrations were measured by using radioimmunoassay (RIA) kit (LINCO Research, Inc. USA). Leptin concentration is expressed as nanograms per milliliter (ng/mL) and ghrelin as picograms per milliliter (pg/mL).

### Table 1. Characteristics of the patients

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>MABP</th>
<th>SABP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>20</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Age mean (range) (years)</td>
<td>52 (25–71)</td>
<td>53 (24–71)</td>
<td>55 (37–74)</td>
</tr>
<tr>
<td>Sex Male / Female (number)</td>
<td>11/9</td>
<td>6/3</td>
<td>5/6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 ±3.2</td>
<td>28.1 ±3.9</td>
<td>26.9 ±1.8</td>
</tr>
<tr>
<td>APACHE II mean</td>
<td>1.37</td>
<td>13.43</td>
<td></td>
</tr>
<tr>
<td>Ranson mean</td>
<td>1.72</td>
<td>4.25</td>
<td></td>
</tr>
<tr>
<td>CT grade (Balthazar’s score: A-E)</td>
<td>C — 2</td>
<td>D — 7</td>
<td>E — 2</td>
</tr>
</tbody>
</table>

MABP – mild acute biliary pancreatitis; SABP – severe acute biliary pancreatitis
The study was approved by Ethics Committee of Jagiellonian University Medical College.

Statistics

As the concentration distributions of both hormones are highly skewed (tab. 2 and fig. 1) prior to the analysis we transformed our data and used natural logarithms rather than the original values. This transformation did not normalize the distribution in case of leptin but was effective in the case of ghrelin. After several trials of fitting different models we assumed the gamma distribution for leptin and the normal distribution for ghrelin (both for the transformed data). In the exploratory part of the analysis we recognized that the concentrations in subsequent days of observation are closely correlated to each patient. Subsequently, we assumed that patient-induced variability might be satisfactorily approximated with the normal distribution (in the case of leptin concentrations variances were assessed separately for the severe and mild group of patients). In our analysis we treated time as a continuous variable. Finally, in statistical modeling we tested three effects: time (days since admission), group membership (mild vs. severe) and interaction. In the gamma distribution we used logarithmic

Table 2. Descriptive statistics of the hormone concentrations. Units of ghrelin – pg/mL, units of leptin – ng/mL

<table>
<thead>
<tr>
<th>Group</th>
<th>Hormone/day</th>
<th>Average</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>Lower quartile</th>
<th>Upper quartile</th>
<th>SD</th>
</tr>
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<tr>
<td></td>
<td>Leptin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP</td>
<td>1</td>
<td>6,8</td>
<td>5,3</td>
<td>1,2</td>
<td>21,7</td>
<td>2,6</td>
<td>5,5</td>
<td>6,7</td>
</tr>
<tr>
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<td>1</td>
<td>24,7</td>
<td>19,7</td>
<td>2,0</td>
<td>65,8</td>
<td>11,7</td>
<td>45,6</td>
<td>20,0</td>
</tr>
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<td>3</td>
<td>5,7</td>
<td>2,2</td>
<td>1,2</td>
<td>16,0</td>
<td>1,6</td>
<td>8,4</td>
<td>5,6</td>
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<tr>
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<td>3</td>
<td>23,5</td>
<td>9,0</td>
<td>1,3</td>
<td>93,2</td>
<td>3,1</td>
<td>29,8</td>
<td>31,7</td>
</tr>
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<td>5</td>
<td>7,6</td>
<td>3,0</td>
<td>1,2</td>
<td>23,8</td>
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<td>15,7</td>
<td>8,5</td>
</tr>
<tr>
<td>SABP</td>
<td>5</td>
<td>23,9</td>
<td>7,5</td>
<td>2,1</td>
<td>93,8</td>
<td>4,6</td>
<td>54,4</td>
<td>31,2</td>
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<tr>
<td>MABP</td>
<td>7</td>
<td>16,7</td>
<td>3,0</td>
<td>1,3</td>
<td>112,9</td>
<td>1,8</td>
<td>5,0</td>
<td>38,9</td>
</tr>
<tr>
<td>SABP</td>
<td>7</td>
<td>18,6</td>
<td>14,5</td>
<td>3,6</td>
<td>49,8</td>
<td>4,5</td>
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<td>16,9</td>
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<tr>
<td></td>
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<td>17,6</td>
<td>13,9</td>
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<td>11,4</td>
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<tr>
<td>Ghrelin</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP</td>
<td>1</td>
<td>269,9</td>
<td>202,5</td>
<td>126,7</td>
<td>616,6</td>
<td>139,2</td>
<td>335,7</td>
<td>165,7</td>
</tr>
<tr>
<td>SABP</td>
<td>1</td>
<td>281,3</td>
<td>290,3</td>
<td>112,0</td>
<td>674,7</td>
<td>137,8</td>
<td>321,3</td>
<td>160,3</td>
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<td>3</td>
<td>318,1</td>
<td>292,3</td>
<td>108,7</td>
<td>941,3</td>
<td>147,7</td>
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<td>251,8</td>
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<td>378,6</td>
<td>272,8</td>
<td>124,3</td>
<td>752,9</td>
<td>182,3</td>
<td>572,0</td>
<td>223,1</td>
</tr>
<tr>
<td>MABP</td>
<td>5</td>
<td>382,9</td>
<td>289,1</td>
<td>88,4</td>
<td>1134,7</td>
<td>219,7</td>
<td>361,0</td>
<td>324,6</td>
</tr>
<tr>
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<td>5</td>
<td>353,6</td>
<td>267,1</td>
<td>102,3</td>
<td>1194,6</td>
<td>171,9</td>
<td>498,0</td>
<td>308,2</td>
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<tr>
<td>MABP</td>
<td>7</td>
<td>390,7</td>
<td>415,6</td>
<td>100,5</td>
<td>811,1</td>
<td>243,1</td>
<td>475,2</td>
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<tr>
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<td>265,8</td>
<td>103,1</td>
<td>826,6</td>
<td>234,7</td>
<td>617,9</td>
<td>236,7</td>
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<tr>
<td></td>
<td></td>
<td>540,9</td>
<td>561,6</td>
<td>289,1</td>
<td>805,6</td>
<td>417,0</td>
<td>645,2</td>
<td>177,1</td>
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</tbody>
</table>

Fig. 1. Subject specific profiles of leptin concentrations. The solid black line indicates mean in the control group (17.6 ng/mL). The dashed blue line denotes the arithmetic mean calculated for each day independently.
and in the normal distribution we used identity link functions. To find out whether the concentrations of leptin and ghrelin are correlated we used canonical correlation analysis. In the statistical analysis we used the GLIMMIX and CANCORR procedures from the SAS package.

RESULTS

In general, the leptin and ghrelin concentration distributions are right-tailed which is evident when comparing averages and medians (averages are more on the right) – exceptions are noticeable on the first and the last day of observation in the severe group in the case of ghrelin (tab. 1). In the studied groups, high average concentration values of both hormones are associated with high values of SD. The hormone concentrations range from 88.4 to 1194 pg/mL (for ghrelin) and 1.2 and 112.9 ng/mL (for leptin).

Leptin

Our analysis revealed that the form of the disease had a borderline-significant influence (F_{1,18}=5.44; p=0.0315). Patients with the severe form have a lower concentration of leptin compared to those patients with the mild form (fig. 1). Additionally, the group of patients with MABP is more diversified compared to those patients with SABP (mild: variance=0.90, SEE=0.47; severe: variance=0.15, SEE=0.08). We failed to find any influence of time since admission on the concentration of leptin (F_{1,55}=0.16, p=0.6909) nor interaction between time and group membership (F_{1,55}=0.09, p=0.7705).

Ghrelin

We lack evidence that the form of the disease influences the level of ghrelin in the average patient (F_{1,58}=0.21, p=0.6462). Our estimation of variance between patients is 0.31 with SEE=0.11. Analysis confirmed that as the process of healing progresses, patients have higher and higher concentrations of the hormone (F_{1,58}=6.52; p=0.0133) (fig. 2). We calculated that the natural logarithm of ghrelin concentration increases by 0.04 (SEE=0.16) units per day. Our analysis does not confirm that the rate of change differs across groups of patients (F_{1,56}=0.88, p=0.3532).

Correlation of leptin and ghrelin

We found the canonical correlation coefficient between concentrations to be 0.80. The linear combination of ghrelin explains 16.1% of the linear combination of leptin (and 26.6% vice versa). One-dimensional correlations between ghrelin and leptin for four days are generally negative and range from −0.36 (when referring to measurements taken on the day of admission) and +0.13 (when correlating second measurements of ghrelin and fourth of leptin). The data do not support a hypothesis about any form of correlation between both studied hormones – the test of significance of canonical (multidimensional) correlation is $\chi^2=17.9$ df=16 p=0.3303 and all one-dimensional coefficients are non-significant (the smallest p values referring to the most negative correlation is 0.1564).

At admission and throughout the study the mean serum leptin concentration in SABP patients was higher than in the controls but
Without statistical significance. The mean serum leptin level was significantly lower in the mild ABP patients compared to the controls on the first and third days of the study. Also show that the greater variability (SD) was in SABP (tab. 3).

The serum ghrelin concentrations were lower in both patient groups with ABP than in the controls. There was a significant difference in the SABP group on analyzed each day, while the mild ABP patients showed a difference on the first and third days. The mean serum ghrelin concentrations were rising in both patient groups on the consecutive days of the study (tab. 4).

No statistically significant difference is found in the concentration of leptin and ghrelin between the groups of patients with severe and mild forms of pancreatitis.

**DISCUSSION**

It has long been known that serum leptin levels are correlated with body fat mass, and that obese subjects have increased serum leptin concentrations (15). It is to be noted that both groups of our patients as well as the control group included subjects with BMI levels below 30, therefore excluding obesity as a confounding factor in the determination of serum leptin levels. The examined group of patients with AP was also etiologically homogeneous, including patients with biliary AP.

At admission the mean serum leptin concentration in severe ABP patients was higher than in the controls but without statistical significance. The level of serum leptin did not significantly change over the study period, and there was no correlation between the serum leptin and ghrelin and the severity of the disease.

From the outset, several, sometimes conflicting, animal and human studies were concerned with the role of leptin and ghrelin in AP.

In 2002 Konturek et al. assessed plasma leptin levels in rats with cerulein-induced pancreatitis (CIP), healthy subjects, and patients with AP (6). Their study showed that AP in rats and humans was associated with a marked increase in leptin plasma levels.

Subsequent animal studies showed that the exogenous leptin protected the pancreas and lungs from damage induced by CIP, modulating cytokine production, attenuating inflammatory changes and decreasing nitric oxide (NO) levels (6, 7). Kerem et al. in a study on Wistar rats with induced AP concluded that increased serum leptin and ghrelin levels could be used as a marker to show the severity of pancreatitis (15).

### Table 3. The concentration of serum leptin (ng/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>17.6±9.71</td>
<td>17.6±9.71</td>
<td>17.6±9.71</td>
<td>17.6±9.71</td>
</tr>
<tr>
<td>SABP</td>
<td>24.7±19.99</td>
<td>23.5±31.73</td>
<td>23.9±31.19</td>
<td>18.6±16.88</td>
</tr>
<tr>
<td>MABP</td>
<td>6.8±6.69</td>
<td>5.7±5.59</td>
<td>7.6±8.47</td>
<td>16.7±38.90</td>
</tr>
</tbody>
</table>

p-values (statistically significant in bold)

Control vs. SABP 0.9561 0.5597 0.6176 0.6343
Control vs. MABP 0.0343 0.0208 0.0524 0.1043

Means ± SD for each group calculated across groups independently. P-values are based on two-sided Student t tests calculated for log-transformed data.

### Table 4. The concentration of serum ghrelin (pg/mL)

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>540.9±177.09</td>
<td>540.9±177.09</td>
<td>540.9±177.09</td>
<td>540.9±177.09</td>
</tr>
<tr>
<td>SABP</td>
<td>281.3±160.31</td>
<td>378.6±223.11</td>
<td>353.6±308.22</td>
<td>366.5±236.74</td>
</tr>
<tr>
<td>MABP</td>
<td>269.9±165.72</td>
<td>318.1±251.77</td>
<td>382.9±324.63</td>
<td>390.7±231.67</td>
</tr>
</tbody>
</table>

p-values (statistically significant in bold)

Control vs. SABP 0.0026 0.0549 0.0270 0.0399
Control vs. MABP 0.0023 0.0140 0.0612 0.0918

Means ±SD for each group calculated across groups independently. P-values are based on two-sided Student t tests calculated for log-transformed data.
Ghrelin may act indirectly by its well-known action as a potent stimulator of growth hormone (GH) release and insulin-like growth factor-1 (IGF-1). A hypophysectomy in rats delayed the healing of the pancreas and abolished the therapeutic effect of ghrelin (4).

Lee et al. investigated the correlation between the severity of AP and serum ghrelin levels in a group of 53 patients (30). The patients were divided into non risk and risk groups based on known risk factors for severe acute pancreatitis (namely, Ranson’s score, the APACHE II score, and the CT severity index). At admission, serum ghrelin levels were significantly higher in the risk group but after 48 hours, and at discharge, there was no difference in serum ghrelin levels between the two groups. The overall serum ghrelin levels were significantly lower at admission than at discharge.

Liu et al. observed that when the patients with acute pancreatitis recovered, the plasma ghrelin concentration increased significantly (31).

Daniel et al. evaluated the serum ghrelin levels in 32 patients with alcoholic AP and in 30 matched controls (32). The serum ghrelin concentrations on the first, third and fifth days of the study were comparable and significantly higher than in the controls.

In our study the serum ghrelin concentrations on admission were lower in both groups of the patients with ABP than in the controls. We observed steadily increasing serum ghrelin levels during the course of ABP. A significant difference (p=0.0133) in the concentration of ghrelin on admission and on the seventh day was found in both groups of the patients. Similar findings were reported in other studies (30, 31).

The results of our study do not support the role of leptin as a marker of the severity of ABP. On the other hand, rising serum ghrelin levels during the course of ABP may be a marker of recovery and an indicator of the healing process.

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