ORIGINAL ARTICLE

# Blood serum changes in patients with pain during bone fractures and acute pancreatitis

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**Abstract**

**OBJECTIVES:** Our aim was to evaluate whether some biochemical parameters in the blood serum can establish and discriminate pain intensity of different etiology. **METHODS:** Three groups of patients hospitalised at the Department of Surgery have been investigated: 1) the patients without pain but with the indicated surgical treatment, 2) the patients with acute pancreatitis, which represents severe pain of the visceral type, and 3) the patients with fractures of upper or lower extremities, which represented acute somatic pain. Whole serum proteins, albumin, C-reactive protein, glucose, total cholesterol, LDL-cholesterol, HDL-cholesterol, triacylglyceroles (triglycerides), apolipoprotein-B and electrophoretic levels of α-lipoprotein, β-lipoprotein and pre-β-lipoprotein were analysed immediately after the first clinical inspection in the hospital and then 30 days after treatment. **RESULTS:** The intensity of pain estimated by the visual analogue scale (VAS) was higher in patients with acute pancreatitis than in patients with fractures. In both diagnoses during persisting pain, the products of lipid metabolism such as triacylglyceroles and HDL-cholesterol were enhanced together with glucose levels. Electrophoretic measurements, revealed higher levels of β-lipoproteins in fractures, and increased values of pre-β-lipoproteins and α-lipoproteins in both groups of patients suffering from pain. After 30 days of treatment some indicators decreased, but when compared with normal values, they were still higher, especially in patients with pancreatitis (HDL-cholesterol, triacylglyceroles, pre-β-lipoprotein). In control patients without pain symptoms, an increase of LDL cholesterol, triacylglyceroles and β-lipoprotein were observed during their stay in hospital, which may be considered to be due to hospitalisation stress per se. Acute stress generally influences glucose levels so that their increase cannot be considered as a specific marker of pain intensity. **CONCLUSIONS**: It may be concluded from our investigation, that the biochemical composition of the blood serum is changing during painful states, although the question still remains open to what extent these changes reflect the pain intensity and to what extent they may modulate the perception of pain.

## Introduction

There are no rapid and objective methods of assessing pain intensity, however, several attempts have been proposed including electrophysiological [1] and imaging methods [2] or pupillometry [3]. The routinely used methods are based on psychological questionnaires [4] and on the measurements of subjective feeling of pain represented by the visual analogue scale (VAS) [5].

It was shown in our previous animal experiments, that the biochemical spectrum of blood serum is changing during nociceptive stimulation [6, 7, 8]. In our model of neuropathic pain, the rats were stimulated on five consecutive days by clamping of their hind limbs. Analyses of the blood showed that after such a single acute stimulation, the HDL-cholesterol, triacylglycerols, glucose and free fatty acids were increased. The level of total cholesterol was not changed, however, the LDL-cholesterol level increased during repeated long-lasting stimulation, while the level of HDL-cholesterol decreased [8]. We hypothesized on the basis of these results, that the observed increase of blood serum components might be the result of a non-specific stress effect of acute pain perception.

Pain is the leading symptom in chronic [9] as well as in acute pancreatitis and the pathophysiological mechanisms involved have been recently studied. The pain in chronic pancreatitis is related to NK-1 receptors and to other known neurotransmitters such as substance P and the calcitonine gene-related peptide [10]. The nociceptive signals from pancreas are also transmitted through the dorsal column pathway, but this is not the only route for the nociceptive information from the pancreas [11].

Fractures could be considered as a possible model of acute and postoperative pain and stress [12] because of the similarity in neuroendocrine as well as metabolic responses to surgery, e.g. hyperglycemia, increased synthesis of acute-phase proteins and increased lipolysis and oxidation of fats.

In this paper we discuss some changes of different biochemical parameters of blood serum constituents obtained in animal experiments and compared them with the results obtained in man during different painful states.

## Materials and Methods

The examinations were performed in three groups of patients:

1) The first group represented 10 patients from the Department of Surgery without declared painful symptoms. Their diagnoses concerned a large scale of ordinary symptoms and diseases (thromboses, hernias, varices etc.). The average age was 50.2 ± 7 years, the height 178 ± 8.2 cm, and the weight 84.1 ± 9.6 kg (means ± SD).

2) The second group consisted of 10 patients with a first attack of acute pancreatitis. The average age was 45.5 ± 4.3 years, the height 178.3 ± 5.5 cm, and the weight 93.3 ± 11.8 kg.

3) The third group comprised 10 patients with fractures of lower or upper extremities with an average age of 45.7 ± 7.5 years. Their height and weight were not measured because of the character of their affliction.

Patients without pain were not treated by any analgesics; they only used the drugs for their primary diseases. The patients with acute pancreatitis received 500 ml 0.9% NaCl with Dolsin in doses of 50–100 mg. If necessary, they received either Dolsin or NSAID analgesics only. The patients with fractures immediately received 100 mg of Dolsin *i.m.* on admission and after transfer to the patient`s ward further 100 mg of tramadol were injected *i.m.*. Before the operation, Dolsin was administered as premedication in the dose of 1 mg/kg and the second day after the operation they received doses of 50 mg *i.m.* every 6 hours. Subsequently the patients received only tramadol until their subjective pain symptoms disappeared. The blood samples were withdrawn from all patients immediately on admission and before the administration of any drugs.

In all three groups of patients, the biochemical data were analyzed twice – immediately after admission and 30 days after the treatment. The assessment of the biochemical spectrum in blood serum included: total cholesterol, HDL and LDL – cholesterol, triacylglycerols (triglycerides), total protein, albumin, glucose, C-reactive protein, apolipoprotein B, α-lipoprotein, β-lipoprotein and pre-β-lipoprotein. The serum albumin levels were determined using bromocresol purple. Total serum proteins were estimated by Roche kits based on the reaction of the biuret reagens with copper ions. Glucose was determined using the enzymes on the principle of the reaction catalysed by hexokinase, and cholesterol was essayed enzymatically using cholesteroloxidase and peroxidase sets (Sentinel). HDL-cholesterol was determined by the direct enzymatic methods using cholesteroloxidase and peroxidase sets (Roche) and LDL-cholesterol and apolipoprotein B were estimated turbidimetrically by the Roche set. CRP was determined using the imunoturbidimetric set (Orion Diagnostic). All biochemical analyses were performed on the analyzer Hitachi 911. The lipoproteins were selected electroforetically on hydrogels (agarose, Sebia) and determined densitometrically using the densitometer Preference (Sebia).

The intensity of pain was measured twice; in the acute phase at the beginning on admission and one week later, using the visual analogue scale (VAS) method with a 100 mm horizontal scale. All patients were discharged from hospital after one month of treatment.

The differences between the groups before and after treatment were tested by the analysis of variance (ANOVA) with repeated measures. Three diagnostic groups represented the between subject variables (factor group), the first and the second blood examination, with and without pain, represented repeated measures

**Table 1.** Means ± S.D. of plasma levels of biochemical parameters in control group, patients with fractures and patients with acute pancreatitis after admission (I) and 30 days after the treatment (II). Results of ANOVA, F statistics and probability, are shown for factors group, treatment and group x treatment interaction. The group comparisons are shown for interactions of factors group x treatment only,  P: pancreatitis; C: controls, F: fractures.

| | Reference Range | Control group | | Fractures | | Pancreatitis | | Group F(2,27) P | Treatment F(1,27) P | G x T F(2,27) P | Group x treatment interaction group comparisons df(1,27) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *I* | *II* | *I* | *II* | *I* | *II* | | | | | |
| *Albumin* | 35 – 50 g/L | 33.3 ± 2.0 | 34.3 ± 4.8 | 49.0 ± 1.6 | 48.2 ± 4.1 | 46.9 ± 11.8 | 44.7 ± 8.2 | 20.38 <0.0001 | 0.27 0.6046 | 0.54 0.5889 | P vs. C / P vs. F / F vs. C | ns / ns / ns |
| *Total protein* | 65 – 85 g/L | 62.9 ± 13.4 | 64.4 ± 10.5 | 79.1 ± 2.0 | 77.7 ± 3.6 | 72.1 ± 7.1 | 71.1 ± 7.7 | 9.04 0.0010 | 0.07 0.7876 | 0.68 0.5165 | P vs. C / P vs. F / F vs. C | ns / ns / ns |
| *LDL cholesterol* | 0.0 - 4.0 mmol/L | 1.5 ± 1.73 | 2.48 ± 1.76 | 5.46 ± 1.43 | 2.54 ± 0.97 | 3.84 ± 0.66 | 2.58 ± 1.72 | 7.31 0.0029 | 12.44 0.0015 | 14.04 0.0001 | P vs. C / P vs. F / F vs. C | F=9.21, p=0.0053 / F=5.04, p=0.0332 / F=27.88, p=0.0001 |
| *HDL cholesterol* | 0.9 – 1.6 mmol/L | 1.87 ± 1.31 | 1.82 ± 1.01 | 3.23 ± 1.57 | 1.2 ± 0.5 | 4.62 ± 2.31 | 3.25 ± 2.51 | 5.07 0.0135 | 24.84 <0.0001 | 6.37 0.0054 | P vs. C / P vs. F / F vs. C | F=5.5, p=0.0266 / ns / F=12.26, p=0.0016 |
| *Total cholesterol* | 3.8 –5.9 mmol/L | 6.2 ± 1.26 | 5.95 ± 0.94 | 7.61 ± 0.72 | 5.75 ± 0.89 | 5.84 ± 1.38 | 5.96 ± 1.27 | 1.6 0.2200 | 18.85 0.0002 | 15.83 <0.0001 | P vs. C / P vs. F / F vs. C | ns / F=28.00, p<0.0001 / F=18.51, p=0.0002 |
| *Triglycerides* | 0.64 – 1.5 mmol/L | 1.33 ± 0.92 | 2.6 ± 1.18 | 3.81 ± 1.86 | 1.92 ± 0.1 | 5.12 ± 2.23 | 3.71 ± 2.01 | 9.44 0.0008 | 4.17 0.0511 | 8.76 0.0012 | P vs. C / P vs. F / F vs. C | F=10.82, p=0.0028 / ns / F=15.1, p=0.0006 |
| *Glucose* | 3.3 – 7.0 mmo/L | 5.53 ± 0.68 | 5.8 ± 0.6 | 7.67 ± 0.89 | 5.52 ± 0.63 | 7.96 ± 3.14 | 8.21 ± 3.74 | 3.63 0.0401 | 9.87 0.0041 | 21.75 <0.0001 | P vs. C / P vs. F / F vs. C | ns / F=32.42, p<0.0001 / F=32.82, p<0.0001 |
| *Apolipoprotein B* | 0.5 – 1.2 g/L | 2.09 ± 0.66 | 2.08 ± 0.83 | 1.01 ± 0.39 | 0.93 ± 0.42 | 1.43 ± 0.22 | 1.25 ± 0.34 | 14.82 <0.0001 | 1.12 0.3003 | 0.34 0.7157 | P vs. C / P vs. F / F vs. C | ns / ns / ns |
| *Alpha-lipoprotein* | 0.23-0.46 U | 0.29 ± 0.07 | 0.33 ± 0.09 | 0.56 ± 0.15 | 0.36 ± 0.07 | 0.67 ± 0.14 | 0.48 ± 0.13 | 27.61 <0.0001 | 16.14 0.0004 | 7.5 0.0026 | P vs. C / P vs. F / F vs. C | F=10.81, p=0.0028 / ns / ns |
| *Beta-lipoprotein* | 0.42-0.63 U | 0.46 ± 0.11 | 0.51 ± 0.12 | 0.69 ± 0.11 | 0.85 ± 1.12 | 0.58 ± 0.19 | 0.44 ± 0.17 | 2.01 0.1532 | 0.05 0.8277 | 0.56 0.5776 | P vs. C / P vs. F / F vs. C | ns / ns / ns |
| *Pre-beta lipoprotein* | 0.03-0.18 U | 0.058 ± 0.029 | 0.099 ± 0.074 | 0.251 ± 0.178 | 0.112 ± 0.067 | 0.407 ± 0.275 | 0.339 ± 0.336 | 11.05 0.0003 | 1.26 0.2723 | 1.12 0.3406 | P vs. C / P vs. F / F vs. C | ns / ns / ns |
| *C-reactive protein* | 0 - 5 mg/L | 14.4 ± 22.0 | 16.2 ± 17.6 | 38.5 ± 44.6 | 17.6 ± 11.0 | 324.2 ± 252.1 | 146.3 ± 101.3 | 15.66 <0.0001 | 10.61 0.0030 | 7.85 0.0021 | P vs. C / P vs. F / F vs. C | F=13.24, p=0.0011 / F=10.11, p=0.0037 / ns |

of the within subject variables (factor treatment). The changes of biochemical parameters at the beginning of hospitalization and 30 days after treatment were compared by paired two-tailed Student's t-test. All data are presented as mean ± SD.

## Results

**Albumin:** No difference was found between the groups with pain (pancreatitis vs. fractures $F_{(1,27)}=1.30$, p=0.2642). The serum albumin of both groups with pain was significantly higher then in control group but it was still within the physiological range. In neither group was the amount of albumin changed during the followed period ($F_{(1,27)}=0.27$, p=0.6046).

**Total protein:** The statistically significant difference between the groups ($F_{(2,27)}=9.04$, p=0.001) is due to the lower amount of total protein in the control group. The higher level in patients with fractures compared with pancreatitis is marginally significant ($F_{(1,27)}=3.83$, p=0.0606). There was no difference found between the first and second determination after one month ($F_{(1,27)}=0.07$, p=0.7876).

**Total cholesterol:** There was no difference between the groups ($F_{(2,27)}=1.60$, p=0.22), however, both factor treatment ($F_{(1,27)}=18.85$, p=0.0002) and the interaction group x treatment ($F_{(2,27)}=15.83$, p<0.0001) are significant. Total cholesterol is increased only in fractures immediately after injury and these become normalized after 30 days (from 7.61 to 5.75 mmol/l, p<0.0001). However, it is not increased at the beginning of pancreatitis (Fig. 1A).

**LDL cholesterol:** A significant difference was observed between the different groups of patients ($F_{(2,27)}=7.31$, p=0.0029); both in pancreatitis (3.21 ± 1.42 mmol/l; $F_{(1,27)}=5.33$, p=0.0288) and in fractures (4.0 ± 1.91 mmol/l; $F_{(1,27)}=14.4$, p=0.0008) the levels were significantly higher than in controls (1.99 ± 1.77 mmol/l, Fig.1B). After treatment, both in the group
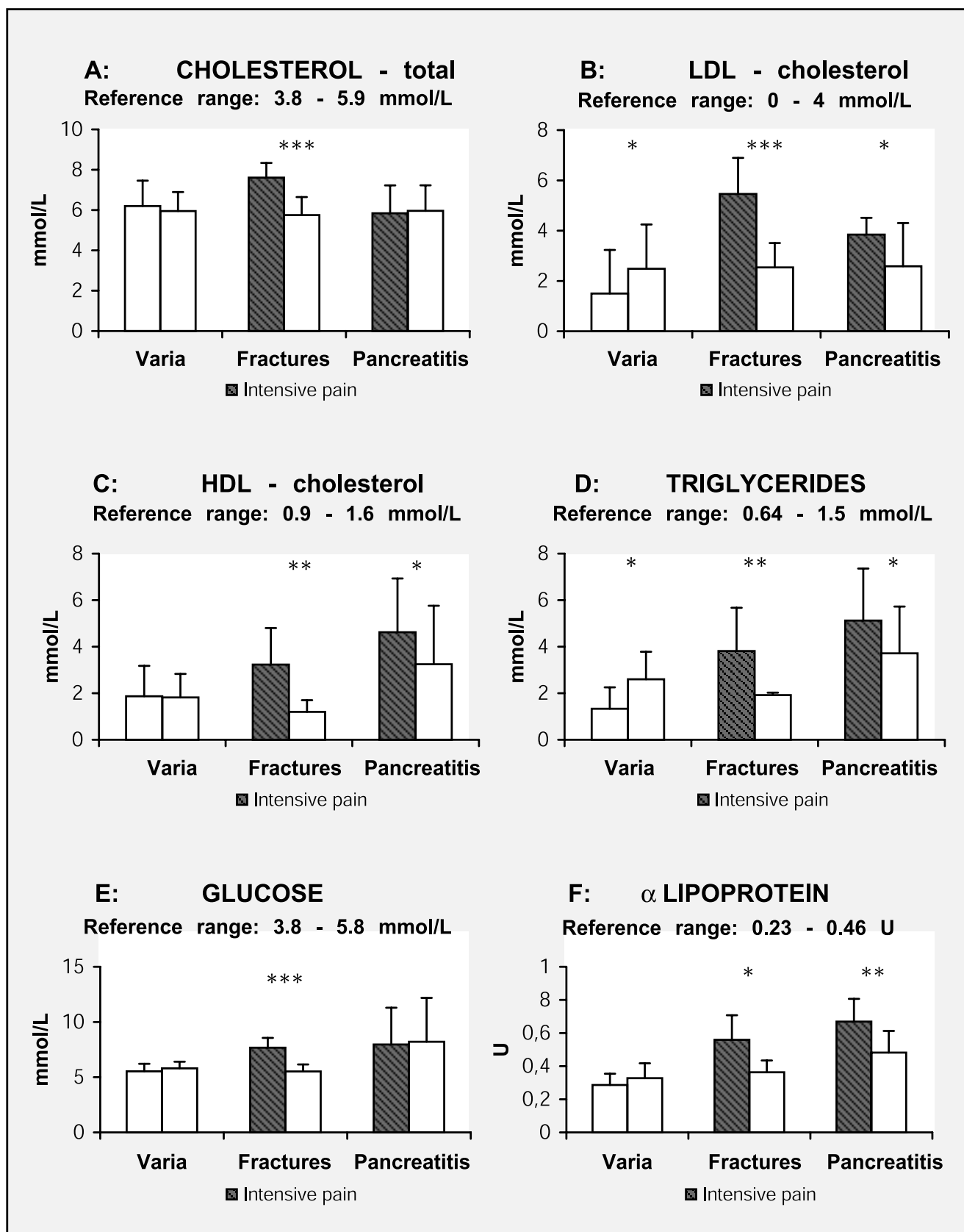
**Figure 1.** Differences in serum levels of total cholesterol (A), LDL-cholesterol (B), HDL-cholesterol (C), triglycerides (D), glucose (E), and alpha-lipoprotein (F) in three groups of patients: varia – patients without declared painful symptoms; patients with fractures of lower or upper extremities and patients with the first attack of acute pancreatitis. Left columns in all three groups represent the levels immediately after admission, right columns 30 days after the treatment. Dashed columns correspond to the period with more intensive pain. Statistically significant differences of paired Student's T-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

with pancreatitis (from 3.84 to 2.58 mmol/l, p= 0.05) and with fractures (from 5.46 to 2.54 mmol/l, p<0.001) serum LDL cholesterol decreased. In the control group of patients, LDL cholesterol kept increasing during their stay in hospital from the starting level of 1.5 to 2.48 mmol/l (p=0.032).

**HDL cholesterol:** This exhibited a similar picture as LDL-cholesterol, with a higher level in pancreatitis cases compared to the controls (3.93 ± 2.45 vs. 1.84 ± 1.14 mmol/l, respectively, $F_{(1,27)}=8.91$, p=0.006) or compared to patients with fractures (2.22 ± 1.54 mmol/l, $F_{(1,27)}=6.03$, p=0.0208).

After 30 days during the period of decreasing intractable pain, the level of HDL in both painful diseases is diminishing (Fig.1C). In the group with fractures, HDL cholesterol decreases to the physiological reference level (from 3.23 to 1.2 mmol/l, p=0.002), while in pancreatitis the observed decrease is still higher than the physiological values (from 4.62 to 3.24 mmol/l, p=0.02). In the control group, the level of HDL-cholesterol was not changed and it was in the normal range. This means that the pancreatitis still represents very strong acute pain, which is long-lasting in character, whereas the very sharp acute pain in fractures continuously decreases.

**Triacylglycerols (triglycerides):** The levels of TAG changed in different groups differentially (group x treatment interaction, $F_{(2,27)}=8.76$, p=0.0012). In pancreatitis and in fractures, the values significantly decreased (from 5.12 to 3.72 mmol/l, p=0.0567, from 3.81 to 1.92 mmol/l, p=0.0097, respectively), while in controls, the level of TAG after 30 days increasead (from 1.33 to 2.6 mmol/l, p=0.0295) above the upper level of normal reference value. In patients with fractures after one month of the treatment, the levels of triglycerides are completely normalized, while in pancreatitis thy still remind increased (Fig.1D).

**Glucose:** Higher levels were found in pancreatitis, which significantly differed from the control group (8.08 ± 3.37 vs. 5.66 ± 0.64 mmol/l, $F_{(1,27)}=7.14$, p=0.0126), however, there were no changes after 30 days (from 7.96 to 8.21 mmol/l, p=0.6122). The higher levels in fractures decreased 30 days after the treatment (from 7.67 to 5.52 mmol/l, p<0.0001), so that values returned to the normal range (Fig.1E).

**Apolipoprotein B:** The levels in the control group (2.08 ± 0.73 g/l) were higher if compared with patients with pancreatitis (1.34 ± 0.29 g/l, $F_{(1,27)}=12.86$, p=0.0013) and with fractures (0.97 ± 0.39 g/l, $F_{(1,27)}=28.52$, p<0.0001). No changes were observed during the treatment period ($F_{(1,27)}=1.12$, p=0.3003).

**Alpha-lipoproteins:** Higher values were increased in the pancreatitis group (0.57 ± 0.16 U, $F_{(1,27)}=54.83$, p<0.0001) and patients with fractures (0.46 ± 0.15 U, $F_{(1,27)}=18.05$, p=0.0002) when compared with the control group (0.31 ± 0.08 U). In both groups, the levels decreased after one month, in fractures the levels became completely normalized (from 0.56 to 0.36 U, p=0.0128), but in pancreatitis were still in the upper range of normality (from 0.67 to 0.48 U, p=0.0037) (Fig.1F).
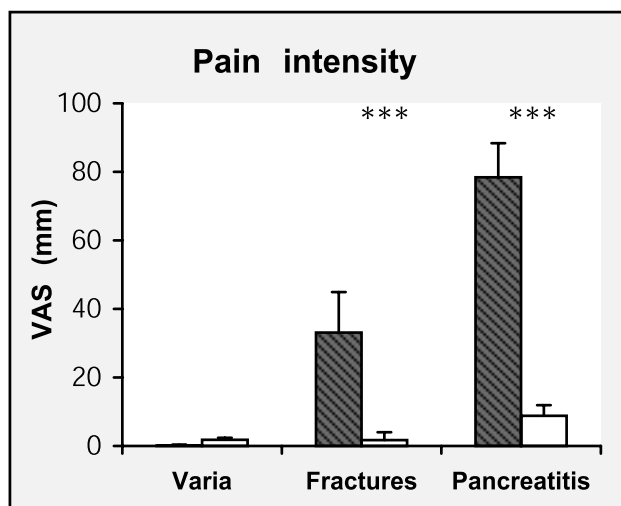


**Figure 2.** Differences in pain intensity in three groups of patients measured by visual analogue scale (VAS) in the acute phase on admission (left columns) and one week later (right columns).

**Beta-lipoproteins:** According to ANOVA, neither the factor group ($F_{(2,27)}=2.01$, p=0.1532) nor treatment ($F_{(1,27)}=0.05$, p=0.8277) were significant. If compared with the physiological values, beta-lipoproteins were increased only in the group with fractures and they did not change during the treatment. The initial and final levels were within the physiological limits in both the control group and the group with pancreatitis. During treatment, the levels of beta-lipoproteins decreased in pancreatitis (from 0.58 to 0.44 U, p=0.0399), while they were slightly increased in the controls (from 0.46 to 0.51 U, p=0.0278).

**Pre-betalipoproteins:** we found significant differences in factor group only ($F_{(2,27)}=11.05$, p=0.0003) not in factor treatment ($F_{(1,27)}=1.26$, p=0.2723). The normal levels of pre-betalipoproteins were found only in the control groups. Both diagnostic groups with pain had higher starting levels and they became normalized only in fractures (from 0.251 to 0.112 U, p=0.0356). In patients with pancreatitis if compared with controls, almost two-fold levels were found and they still persisted after 30 days (from 0.407 to 0.339 U, p=0.6249).

**C-reactive protein:** All factors of ANOVA were significant, group ($F_{(2,27)}=15.66$, p<0.0001), treatment ($F_{(1,27)}=10.11$, p=0.0037) and group x treatment interaction ($F_{(2,27)}=7.85$, p=0.0021). In all groups, the level of the C-reactive protein was higher than normal. After the treatment, it decreased only in the group with pancreatitis (from 324.2 to 146.3 mg/l, p=0.0148), but it was still higher if compared with the control group (from 14.4 to 16.2 mg/l). In patients with fractures, the decrease was not significant (from 38.5 to 17.6 mg/l, p=0.1186).

**VAS:** The intensity of pain assessed on a visual analogue scale was significantly higher in pancreatitis than in fractures (72.9 ± 12.9 vs. 59.2 ± 13.2, respectively, t=2.35, p=0.0304 – non-pair Student's t-test) and than in the controls, which had practically zero pain score (Fig. 2).

## Discussion

It can be concluded from these results, that the most important changes in pain-related syndromes exhibit changes in the serum lipid spectrum. In cases, when lipids participate in processes involving cell membranes characteristics, they involve both triacylglycerols and HDL- and LDL-cholesterol, but total cholesterol is less affected. These changes become normalized during the treatment, but they do not necessarily attain physiological values. Proteins are influenced to a lesser extent, but if they are related to lipids the effect of nociceptive stimulation is substantially higher, concerning especially alpha-lipoproteins and apolipoprotein. Glucose metabolism is affected especially in fractures.

The association between pain and altered glucose metabolism is frequently discussed but the actual physiological mechanism is still open. Almost all patients with impaired glucose tolerance report neuropathic pain. Singleton et al. [13] proposed an alternative hypothesis that chronic pain, either through cortisol or other mechanisms, causes glucose dysregulation. Increased glycaemia after painful trauma results in impaired metabolism with changed insulin sensitivity, which is related to the magnitude of traumatic stress. It has been reported that a systemic glucose injection enhances morphine-induced antinociceptive effects in rats [14] but on the other hand intravenous injection of tramadol in fasting streptozocine-induced diabetic rats decreased glucose level. If the opioid receptors were blocked by the pretreatment with naloxon this effect disappeared [15].

Greisen et al. [16] have shown that acute painful electrical stimulation in humans increases serum cortisol, epinephrine and free fatty acids, and decreases insulin sensitivity by affecting nonoxidative glucose metabolism. Lankisch et al. [17] demonstrated that pancreatic necrosis is correlated with high blood glucose. Patients with normal blood glucose on admission to the hospital are unlikely to have pancreatic necrosis. Brisinda et al. [18] used multiparametric criteria in acute pancreatitis for its prognosis. Out of those investigated, only blood glucose and serum albumin were also increased as in our study. Pezzilli at al. [19] provided simultaneous serum assays of lipase and interleukin-6 ad they concluded that both of them are very important for early diagnoses and could be also used for the prognoses of acute pancreatitis.

It therefore seems, that the analyzed parameters could play an important diagnostic role in different types of pain and their treatment. It has been proved that several biochemical characteristics of pain are compensated or normalized after the treatment both in pancreatitis and in fractures, but it should be stressed that the normalization is not complete. Pancreatitis is one of the most intractable types of pain known and this disease also has very important metabolic consequences. But the analyzed serum parameters seem to be rather pain- specific than diagnose-specific. In pancreatitis, enzymatic changes and preferentially immediate changes of alpha-amylase and phosphorylase are especially affected, oxidative metabolism being substantially influenced. Painful stimulation also evokes changes associated with stress, which include the production of acetyl-coenzyme A and cholesterol. All other changes are probably caused by alterations of cell oxidative metabolism. It is possible that the changes, which have been reported here, were mediated by this mechanism.

The period when the patients declared diminishing of pain correlated with the biochemical normalization, but this did not concern all markers. Evident were the increasing values of LDL-cholesterol in control patients. This was probably due to changes in the living style during the hospitalization stress. It is known from experiments on animals that transport stress increases the levels of HDL- and LDL-cholesterol and catecholamines in their blood. The increased levels are still present three weeks after the transportation stress [20].

In rats, the levels of serum lipoproteins, triacylglycerols, total cholesterol, VLDL, HDL and LDL cholesterol are not only changed after painful stimulation [8, 6], but also after the immobilization stress. A detailed comparison of the results obtained in animals and man have shown that there is no absolute analogy. Ruiz de Gordoa et al. [21] did not describe any increase of total cholesterol in female rats, but only a mild and significant increase of VLDL and LDL-cholesterol and triacylglycerols. Increasing levels of HDL cholesterol and acute phase reaction following acute pancreatitis and myocardial infarction have similar characteristics [22]. In man, it has been shown that the psychological stress, similar to the infusion of adrenaline, increases serum lipids and stimulates lipolysis, which increases the levels of free-fatty acids, VLDL-, LDL-, HDL-cholesterol and apoprotein B [23]. Psychological stress together with the increasing level of adrenaline correlates with the rising level of free-fatty acids and triacylglycerols. As far as our patients are concerned, we can speculate about the additive effect of pain and stress. The controls, which are represented by the group of patients without any pain symptoms, were exposed only to the psychological hospitalization stress, while the other two groups also felt very strong pain.

Balon [24] and Vevera et al. [25] have demonstrated that there exists a relationship between the level of cholesterol and behavior. In patients with depressions, with a suicidal tendency and with violent behavior, decreased level of cholesterol has been shown [26, 27, 28, 29, 30, 31, 32, 33, 34] and higher pain threshold [35]. This is in good agreement with the present study, because the level of cholesterol in our patients with intensive pain was on contrary increased. We can speculate about the more general relationship between the aggressive behavior (oriented in or out of the person), the perception of pain and the level of cholesterol. Van de Vijver et al. [36] studied the seasonal variation in LDL oxidation and antioxidant status in men. He concluded that LDL oxidation parameters did not change over the year.

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Visceral pain is less tolerable and its affective component is more potent. Patients with acute pancreatitis suffer subjectively more than patients with fractures (see the levels of VAS). From the group of patients with fractures, the maximum perception of pain on the VAS scale was reported by patient with fractures of ribs and injured pleura, which is comparable with visceral pain, as far as intensity is concerned. Patients with bone fractures have increased systemic oxidative stress, which induces the formation of oxidized low-density lipoproteins, and they could alter fracture healing. Reactive oxygen species play an important role in the regulation of bone synthesis and remodeling [37]. Production of free oxygen radicals depends on the pain intensity in rats [38].

A significant difference in the level of CRP was found in our patients with pancreatitis and fractures. Kaw and Singh [39] also described an increase of CRP in acute pancreatitis. Its level depended on the severity of pancreatitis. Lechleitner et al. [40] showed that severe pancreatitis is followed by hypertriglyceridemia. It always has a worse prognosis that the pancreatitis with lower levels of triglycerides. It has been concluded from in vitro experiments that the pancreatic lipase in acute pancreatitis influences the tissue and serum triglycerides and generates non-esterified fatty acids, which could cause necrosis of the pancreas and adipose tissue. Significant hypertriglyceridemia elicited by intravenous injection of human VLDL never deteriorated in the course of cerulein-induced pancreatitis in rats. Non-esterified fatty acids did not differ in the pancreatic tissue from the controls [41]. It is possible to state from our results that a specific relationship exists between the pain provoked by acute pancreatitis (visceral pain) and fractures (somatic pain) as judged by some blood serum markers, which are routinely followed in biochemical laboratories. These changes are very probably specific, because they are not induced by hospitalization stress itself and they are present in such different kinds of pain accompanying, e.g. pancreatitis and fractures.

Pain evokes changes in the blood biochemical spectrum. The question arises as to what extent these changes could reciprocally influence pain perception. Our contemporary observations confirmed our previous findings in animals. Our results also demonstrate that the pain situation of different etiology can provoke similar biochemical changes. The intensity of these changes may reflect the intensity of pain and therefore they could serve as objective parameters for comparing of the pain intensity – algosity – of the different pathological states.

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REFERENCES

1 Handwerker HO, Kobal G. Psychophysiology of experimentally induced pain. Physiol Rev 1993; **73**:639–671.
2 Casey KL, Bushnell MC, editors. Pain Imaging. Progress in pain research and management. Vol. 18. Seattle: IASP Press; 2000.
3 Ellermeier W, Westphal W. Gender differences in pain ratings and pupil reactions to painful pressure stimuli. Pain 1995; **61**: 435–439.
4 Melzack R. The McGill pain questionnaire. In: Melzack R, editor. Pain measurement and assessment. New York: Raven Press; 1983. p. 41–48.
5 Bijur PE, Silver W, Gallagher EJ. Reliability of the visual analog scale for measurement of acute pain. Acad Emerg Med 2001; **8**: 1153–1157.
6 Rokyta R, Kubík V, Steinerová A, Hrabetová S, Hrabe J, Štastná H. Les changements du spectre des lipides après la stimulation nociceptive. Archives de Physiologie, Biochemie et Biophysique 1990; Suppl:A160.
7 Rokyta R, Holecek V, Krejcová I, Kubík V, Štastná H, Steinerová A et al. Biochemical changes in rat model of neuropatic pain. J Physiol 1997; **3**:504P.
8 Rokyta R. Biochemical and electrophysiological changes in animal models of chronic and visceral pain. In: Kleppinger J, editor. Pain clinical aspects and therapeutical issues, Part III. Linz: Selva Verlag; 1998. p. 19–30.
9 Di Sebastiano P, Friess H, Di Mola FF, Innocenti P, Büchler MW. Mechanisms of pain in chronic pancreatitis. Ann Ital Chir 2000; **71**:11–16.
10 Shrikhande SV, Friess H, Di Mola FF, Tempia-Caliera A, Conejo Garcia JR, Zhu Z et al. NK-1 receptor gene expression is related to pain in chronic pancreatitis. Pain 2001; **91**:209–217.
11 Houghton AK, Wang C-C, Westlund KN. Do nociceptive signals from the pancreas travel in the dorsal column? Pain 2001; **89**: 207–220.
12 Cousins M, Power I. Acute and postoperative pain. In: Wall PD, Melzack R, editors. Textbook of pain, 4th edition. Edinburgh: Churchill Livingstone; 1999. p. 447–491.
13 Singleton JR, Smith AG, Bromberg MB. Increased prevalence of impaired glucose tolerance in patients with painful sensory neuropathy. Diabetes Care 2001; **24**:1448–1453.
14 Yamamoto RT, Kanarek RB. Systemic glucose injection enhances morphine-induced antinociception in rats. Soc Neurosci Abstr, Vol. 27, Program No. 614.7, 2001.
15 Cheng J-T, Liu I-M, Chi T-C, Tzeng T-F, Lu F-H, Chang CJ. Plasma glucose-lowering effect of tramadol in streptozocin-induced diabetic rats. Diabetes 2001; **50**:2815–2821.
16 Greisen J, Schmitz O, Jensen TS, Vilstrup H, Grofte T, Juhl CB. Acute pain induces insulin resistance in humans. Anesthesiology 2001; **95**:578–584.
17 Lankisch PG, Blum T, Bruns A, Droge M, Brinkmann G, Struckmann K et al. Has blood glucose level measured on admission to hospital in a patient with acute pancreatitis any prognostic value? Pancreatology 2001; **1**:224–229.
18 Brisinda G, Maria G, Ferrante A, Civello IM. Evaluation of prognostic factors in patients with acute pancreatitis. Hepatogastroenterology 1999; **46**:1990–1997.
19 Pezzilli R, Morselli-Labate AM, Miniero R, Barakat B, Fiocch M, Cappelletti O. Simultaneous serum assays of lipase and interleukin-6 for early diagnosis and prognosis of acute pancreatitis. Clin Chem 1999; **45**:1762–1767.
20 Olubadewo JO, Wingard M, Washington B, Tsai CS, Robinson TJ, Ochillo RF. The effects of stress on lipoproteins and catecholamines in rats. Cell Mol Biol 1994; **40**:1201–1206.
21 Ruiz de Gordoa JC, Santafe J, Segarra Domenech J, Santisteban A. Modification of rat plasma lipoproteins induced by acute immobilization stress. Psychosom Med 1994; **56**:486–492.
22 Fahie-Wilson M, Mills R, Wilson K. HDL cholesterol and the acute phase following myocardial infarction and acute pancreatitis; Clin Chim Acta 1987; **167**:197–209.
23 McCann BS, Magee MS, Broyles FC, Vaughan M, Albers JJ, Knopp RH. Acute psychological stress and epinephrine infusion in normolipidemic and hyperlipidemic men: effects on plasma

lipid and apoprotein concentrations. Psychosom Med 1995; **57:** 165–176.

24 Balon R. Cholesterol, mental illness and violence. Psychiatrie 2000; **2:**83–90.

25 Vevera J, Papežová H, Žukov I, Linhartová L. Cholesterol a násilné chování. (in Czech) Ces a slov psychiat 2001; **97:**69–73.

26 Engelberg H. Low serum cholesterol and suicide. Lancet 1992; **339:**727–729.

27 Fowkes FGR, Leng GC, Donnan PT, Deary IJ, Riemersma RA, Housley E. Serum cholesterol, triglycerides, and aggression in the general population. Lancet 1992; **340:**995–998.

28 Hawthon K, Cowen P, Owens P, Bond A, Elliott M. Low serum cholesterol and suicide. Br J Psychiatry 1993; **162:**818–825.

29 Boston PF, Dursum SM, Reveley MA. Cholesterol and mental disorder. Br J Psychiatry 1996; **169:**682–689.

30 Kunugi H, Takei H, Aoki H, Nanko S. Low serum cholesterol in suicide attempters. Biol Psychiatry 1997; **41:**196–200.

31 Mufti RM, Balon R, Arfkem C. Low cholesterol and violence. Psychiatr Serv 1998; **49:**221–224.

32 Alvarez JC, Cremniter D, Lesieur R, Gregoire A, Gilton A, Macquin-Mavier I et al. Low blood cholesterol and low platelet serotonin levels in violent suicide attempters. Biol Psychiatry 1999; **45:**1066–1069.

33 Linhartová L. Hladina cholesterolu a riziko suicidia – klinická a katamnestická studie. (in Czech) Psychiatrie 1999; **3:**150–152.

34 Tanskanen A, Vartiainen E, Tuomilehto J, Viinamaki H, Lehtonen J, Puska P. High serum cholesterol and risk of suicide. Am J Psychiatry 2000; **157:**648–650.

35 Orbach I, Mikulincer M, King R, Cohen D, Stein D. Threshold and tolerance of physical pain in suicidal and nonsuicidal adolescents. J Consult Clin Psychol 1997; **65:**646–652.

36 Van de Vijver LP, Van Duyvenvoorde W, Buytenhek R, Van der Laarse A, Kardinaal AF, Van den Berg H et al. Seasonal variation in low density lipoprotein oxidation and antioxidant status. Free Radic Res 1997; **27:**89–96.

37 Wildburger R, Borovic S, Zarkovic N, Tatzber F. Post-traumatic dynamic changes in the antibody titer against oxidized low density lipoproteins. Wien Klin Wochenschr 2000; **112:**798–803.

38 Rokyta R, Holecek V, Pekárková I, Krejcová I Racek J, Trefil L, et al. Free radicals after painful stimulation are influenced by antioxidants and analgesics. Neuroendocrinol Lett 2003; **24:** 348–353.

39 Kaw M, Singh S. Serum lipase, C-reactive protein, and interleukin-6 levels in ERCP-induced pancreatitis. Gastrointest Endosc 2001; **54:**435–440.

40 Lechleitner M, Ladner E, Seyr M, Hoppichler F, Foger B, Hackel JM. Hypertriglyzeridämie und akute Pankreatitis. Acta Med Austriaca 1994; **21:**125–128.

41 Paye F, Chariot J, Molas G, Benessiano J, Roze C. Release of nonesterified fatty acids during cerulein-induced pancreatitis in rats. Dig Dis Sci 1996; **41:**1959–1965.