The relationship of body condition and chewing time with body weight, the level of plasma cocaine and amphetamine regulated transcript, leptin and energy metabolites in cows until reaching the lactation peak

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Abstract

This study examined the relationship of body condition and chewing time (CT) with body weight (BW), the level of cocaine-and amphetamine-regulated transcript (CART), leptin and energy metabolites in cows until reaching the lactation peak. The results showed the greatest loss of BW between days 11 and 45 of lactation and a decrease in the body condition score (BCS) until day 75. Chewing time showed an increase from day 45 with the highest values after lactation peak. The CART and leptin concentrations demonstrate a reduction of both indices ($P < 0.05$) between days 11 and 75. The function of leptin as a factor positively correlating with BW and BCS was found. In the heaviest individuals and those with higher BCS, the blood leptin levels were higher. The results of beta-hydroxybutyrate (BHBA) indicate that cows with the lowest BCS had the most intensive energy transformation. This led to a higher BHBA concentration compared to the cows with high BCS ($P < 0.05$). The obtained results show that CART was most strongly negatively associated with CT ($P < 0.05$). For leptin, the tendency was the opposite and the correlation with CT was not significant. The results suggest that CT may be stronger regulated by CART, which has anorectic properties, than by appetite inhibiting leptin. Significant decrease of body condition during lactation is a great problem for dairy farmers. The explanation of the issue of the participation of CART and leptin in the regulation of body's energy homeostasis may therefore be of importance for milk production.

Blood indicators, energy balance, milk production, neuropeptide

Metabolic profile indicators are of great importance for early identification of energy metabolism disturbances in cows. Knowledge of the processes regulating the secretion of milk and physiological factors determining fat tissue accumulation is particularly important in high-yielding cows. In practice, despite the use of high-energy diets, a strongly negative energy balance is observed in these animals until lactation peak is reached. In addition, it is accompanied by reduced dry matter (DM) intake which increases the energy deficit even more, and insufficient coverage of the organism's nutrient needs (Ellis et al. 2006; Wathes et al. 2007; Leblanc 2010). In response to negative DM intake, a strong loss of the body condition score (BCS) is commonly observed (Patton et al. 2007). This situation results in a decrease in the level of leptin in the blood and the activation of energy reserves accumulated in fat (Vargová et al. 2015). It can also negatively affect the functions of many other organs (Roche et al. 2007). Leptin is the main regulator of feed intake, an inhibitor of lipogenesis and the main stimulator of lipolysis (Ahima and Flier 2000; Ingvartsen and Boisclair 2001; Chilliard et al. 2005). However, leptin functions may be somewhat attenuated by anorectic agents, including the cocaine- and amphetamine-regulated transcript (CART). It is considered as factor affecting the suppression of appetite (Brady et al. 1990; Hwang and Guntz 1997). By regulating appetite, CART has an effect on feed intake and chewing time (CT) which determine the rumen metabolism and

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the energy balance in cattle (Hunter et al. 2004; Nikkhan 2011). One of the sources of CART are neurosecretory cells (DNES) in the hypothalamus and other tissues (e.g. gastrointestinal tract) that release CART into the blood (K a s a c k a and P i o t r o w s k a 2012; Janiuk et al. 2013). In the hypothalamus the presence of CART has been observed in nuclei responsible for nutrition or motor activity and also in the regions of the central nervous system considered to be the most important in the regulation of the cardiovascular system (K o y l u et al. 1998; K a s a c k a and P i o t r o w s k a 2012). Peptide has also been localized in the peripheral nervous system (Wierup et al. 2007), among others locations in the nerve ganglia of the pyloric part of the abomasum in cattle. This presence has been confirmed by the studies conducted by Młynek et al. (2015). According to E l i a s et al. (2001), the location of CART-containing structures which are in the vicinity of leptin secreting receptors, may inhibit the transduction of the leptin signal, therefore, the excessive mobilization of fat reserves in cows during energy deficit periods may also increase the activity of the areas releasing CART (K r i s t e n s e n et al. 1998; Wang et al. 1999).

Most reliable indicators of the cow's energy status are beta-hydroxybutyrate (BHBA) and glucose concentrations. The body fat mobilization in high-yielding cows is associated with non-esterified fatty acids production, which are esterified into triacylglycerols and BHBA (S u m n e r and M c N a m a r a 2007). According to S t e n g ä r d e et al. (2008), the concentration of these parameters is in high correlation with the amount of deposited fat (BCS) during the peripartal period.

The aim of this study was to examine the relationship of body condition and CT with body weight (BW), the level of CART, leptin and energy metabolites in high-yielding dairy cows until reaching the lactation peak. The experimental hypothesis was that the concentrations of CART, leptin, glucose and BHBA are correlated with cow's fat deposition and chewing activity, and are dependent on the lactation phase.

Materials and Methods

Animals, housing, and feeding

The study was conducted under a research protocol approved by the Third Local Ethics Committee in Warsaw, No. 70/2013 of 19 December 2013.

The animals were obtained from a private farm located in eastern Mazovia. The experiment was carried out in 69 Holstein-Friesian (HF) cows whose mean production age was 2.5 lactations ($\pm$ 0.9). The animals did not show any disease symptoms (ketosis, acidosis, perinatal diseases). In the analysed period of lactation, 5 *post partum* phases were distinguished: the first week (1 to 7 days), the second week (8 to 14 days), the second month (31 to 60 days), the third month (61 to 90 days) and the fourth month (91 to 120 days). The material for evaluation of the indices in the individual phases was acquired on days: 4 ± 1.2, 11 ± 2.1, 45 ± 3.5, 75 ± 4.4 and 105 ± 5.3 on average. About 24 h after calving, the cows were transferred to the same production group. The animals were kept in a free-stall barn with separate lying areas where they had free access to water. The cows were milked by an A3 milking robot (Lely, Maassluis, the Netherlands) and fed in concentrated feeding stations. A Juno automatic feed push-up robot (Lely) was used at the feed bunk. The average number of milkings was 2.5 ± 1.2 per day. The roughage was prepared in a feed wagon and given *ad libitum* (5:30 h and 17:30 h). The cows were fed using the total mixed ration (TMR) system in order to ensure the proper amount of all nutrients (Table 1). The nutritional value of the feed was determined each time after the silos with silage were opened. The diet was formulated to meet or exceed the nutrient requirements for a lactating HF breed weighing 550 kg, according to the INRA feeding standards with the use of INRAtion Software for Ruminant Diet Calculation, version 2.03 (DJ Group, Krakow, Poland). The diet in each period of the experiment was determined based on the production of milk standardized for 4.0% fat and 3.5% protein. The average dry matter intake was determined for the group in each period of the experiment. The dry matter intake balance was calculated on the basis of the difference between the weight of given feed and the leavings (kg). Checks were carried out every few days during the experiment. The particle size in the feed was defined according to Heinrichs' procedure (2013). Particle size distribution was as follows: 19 mm: 21.5 to 23.7% $\pm$ 3.35; 8 mm: 27.8 to 28.2% $\pm$ 2.16; 4 mm: 8.7 to 9.05% $\pm$ 1.59; Pan: 39.5 to 41.8% $\pm$ 1.54. The data are mean values for all lactation periods.

Production and body condition monitoring

Transponders were used for identification of the cows in the T4C 3.7 (Lely) management software. The chewing time was measured using noseband-sensor halters (QWES-HR system; Lely). The time of feed intake (head down) was not included in the CT. The body weight was determined using a weighing platform mounted on

Table 1. Components of diets (kg/head/day).

Component	Mean time of *post partum* (day)				
	4	11	45	75	105
Corn silage	30	30	30	30	30
Grass silage	15	15	15	15	15
Wheat meal	3.0	3.0	2.5	2.0	1.5
Full-fat soybean meal	1.0	1.0	1.0	1.0	1.0
Full-fat rapeseed meal	1.0	1.0	1.0	1.0	1.0
Dried molasses beet pulp	4.0	4.0	3.5	2.0	1.0
Post-extraction soybean cake	2.5	2.5	2.0	-	-
Post-extraction rapeseed cake	2.0	2.0	1.5	-	-
DM (%)	45.4	45.4	44.9	43.5	40.6
CP (% of DM)	14.35	14.42	14.5	12.6	12.05
NEL (MJ/kg)	4.54	4.56	4.98	6.32	6.07
ADF (% of DM)	22	21	20	21	22
NDF (% of DM)	29	28	29	29	30
DMI (kg/d)	17.5	18.1	20.89	21.5	22.4
Energy requirement (MJ NEL)	124.0	132.4	142.2	151.1	138.7
Energy input (MJ NEL)	95.2	108.6	124.5	141.0	137.9
Energy balance (MJ NEL)	-28.2	-23.8	-17.7	-3.34	-0.8

DM - dry matter; CP - crude protein; NEL - netto energy for lactation; ADF - acid detergent fiber; NDF - neutral detergent fibre; DMI - dry matter intake

the floor of the milking stall (A3 sensor system; Lely). Calibration of the devices was performed at the beginning of the experiment. In each period of the experiment, the mean values of the analysed factors (BW, CT and milk yield) were determined based on the data collected individually from each cow. The cows were evaluated for the amount of deposited fat using BCS (Edmonson et al. 1989; Metzner et al. 1993) in the range of 1 to 5 points (1 point = emaciated; 5 point = obese). The BCS was the mean of the ratings performed by two independent persons, carried out at the beginning of each period. For the purposes of the experiment, the division of animals into classes I, II and III in relation to the BCS and CT was made by analysing the normal distribution of a given factor and using general discriminant analysis (GDA) taking into account the analysed lactation phases ($P < 0.05$).

Blood collection and storage

The blood samples were acquired in the morning, on days 4, 11, 45, 75 and 105 *post partum*, about 1 h before feeding and about 2 h before milking. Blood was collected from the abdominal vein using a 10-ml vacuum tube with sodium heparin, and a 1.6 × 3.8-mm needle (Medlab-Products Ltd., Raszyn, Poland). After collection, all blood samples were transported to the laboratory on ice (the time did not exceed 3 h). Plasma was harvested after centrifugation at 2,000 × g for 15 min at 4 °C and frozen at -75 °C. The plasma samples were analysed for the levels of CART and the leptin hormone using the bovine specific ELISA kit (EIAab, Wuhan, China). In the ELISA, fragment 55–102 of CART was recognized. Blood for the determination of glucose was collected into tubes with sodium fluoride and sodium heparin (Medlab-Products Ltd.). The glucose content in the blood was measured using original Randox kits (Randox Laboratories Ltd, Crumlin, UK) and a UV-Vis spectrophotometer (Varian Inc., Palo Alto, CA, USA). Samples to determine BHBA were centrifuged at 1,500 × g at 4 °C for 20 min. The supernatant was collected and stored at -75 °C until it was analysed for BHBA using original Randox kits (Randox Laboratories Ltd.) and a UV-Vis spectrophotometer (Varian Inc.). All samples were measured in triplicate.

Statistical analysis

The data were subjected to statistical analysis using the Statistica 13.1 program (TIBCO Software Inc., USA). The ANOVA model with repeated measurements was applied, taking into account one of the studied factors: *post partum* time, BCS, or CT. A division into study groups within the factors was made using a multidimensional discrimination model. The analysis was based on the mixed linear model taking into account *post partum* time as a permanent effect (covariance type AR) and an animal as a random effect. The tables present the weighted mean values of the analysed indicators, standard error of the mean (SEM), and the *P* value. Differences between means were tested by Tukey's method (the level of significance set at 5%). Pearson's correlation coefficients were calculated for BCS and CT, including BW and blood parameters ($P < 0.05$).

Results

The analysis of indicators characterizing the body condition shows that up to day 45 of lactation, there was a gradual decrease in the BW (Table 2). However, the greatest loss was observed between days 11 and 45 *post partum*. On the other hand, BCS decreased until day 75 of lactation. At the same time, in the period from 4 to 75 days there was an increase in the daily milk production by an average of 11.7 kg. During this period, the cows most often reached the peak milk yield. After this period, the animal's condition began to improve, as evidenced by the increasing values of BW and BCS. The CT data obtained in the analysed *post partum* phases (Table 2) showed a gradual increase of this index from day 45 of lactation with the greatest values usually achieved after the lactation peak (day 105). The CART and leptin concentrations presented in Table 2 demonstrate a significant reduction of both indices between days 11 and 75 *post partum* ($P < 0.05$). During this period, a systematic increase in daily milk production was noted. There was no significant effect of the analysed lactation phases on *post partum* glucose values ($P = 0.146$). As for BHBA, it was shown that its concentration in the cows' blood was relatively higher in the period preceding the lactation peak (day 75 *post partum*). After this time, the value of this parameter clearly decreased by 0.228 mmol·l^{-1} on average ($P < 0.05$).

Table 2. Production and body condition indicators, chewing time and blood parameters in cows during lactation.

Indicator	Mean time of *post partum* (day)					SEM	*P* <
	4 (n = 69)	11 (n = 69)	45 (n = 69)	75 (n = 69)	105 (n = 69)		
BW (kg)	544[a]	539[bc]	526[b]	533[b]	541[c]	2.6	0.001
Milk production (l/d)	25.4[a]	31.2[b]	34.3[c]	37.1[d]	33.2[cb]	2.1	0.004
BCS (point)	2.5[a]	2.1[b]	2.0[b]	1.7[b]	2.3[c]	0.6	0.012
CT (min/d)	759[a]	752[a]	769[b]	779[c]	788[d]	3.9	0.008
Blood indicator:							
CART (ng·ml^{-1})	1.296[a]	1.240[a]	0.961[b]	0.711[c]	0.712[c]	0.021	0.021
Leptin (ng·ml^{-1})	3.392[a]	3.414[a]	3.301[b]	3.226[c]	3.204[c]	0.047	0.016
Glucose (mmol·l^{-1})	3.443[a]	2.441[b]	2.719[b]	2.939[b]	3.059[c]	0.035	0.146
BHBA (mmol·l^{-1})	0.884[a]	0.977[a]	0.846[b]	0.807[b]	0.579[c]	0.029	0.002

SEM - standard error of the mean; BW - body weight; BCS - body condition score; CT- chewing time; CART - cocaine- and amphetamine-regulated transcript; BHBA - beta-hydroxybutyrate; [a,b,c,d]- means in the row with different superscripts differ significantly ($P < 0.05$)

Table 3 shows the summary of the BW results in the tested cows, the level of peptides involved in energy metabolism, the concentration of glucose and BHBA depending on the BCS. In the BCS classes (I to III), the mean values ranged from 1.89 to 3.57 points. These data indicate that in the heaviest individuals and those with higher BCS, the blood CART concentrations were lower and leptin levels were higher. The mean CART concentration in the blood plasma of cows ranked in the first BCS class (< 2.4) was 1.148 ng·ml^{-1}. In comparison, the blood of individuals with a BCS higher than 2.4 points contained by 0.453 ng·ml^{-1} lower mean CART concentration ($P < 0.05$). The blood of individuals ranking in the class > 2.4 points (II and III) was characterized by a higher content of leptin ($P < 0.05$). The glucose values differed significantly in BCS classes II and III in comparison with class I. These tendencies of changes of the analysed indicators depending on the BCS (Table 3) were confirmed by the values of the correlation coefficient presented in Table 4. The results of the BHBA concentration in the blood plasma shown in Table 3

Table 3. Pearson's correlation coefficients for BCS with BW and blood indicators.

| Indicator | BCS (point) | | | SEM | $P <$ | Correlation coefficient |
	I < 2.4 (n = 19)	II 2.4 to 3.4 (n = 38)	III > 3.4 (n = 12)			BCS ×
Mean day of lactation	38	78	91	4.97	0.021	0.318*
BCS (point)	1.89[a]	2.83[b]	3.57[c]	0.03	0.000	-
BW (kg)	531[a]	537[b]	542[b]	4.60	0.024	0.376*
Blood indicator:						
CART (ng·ml^{-1})	1.148[a]	0.712[b]	0.679[b]	0.021	0.008	-0.520*
Leptin (ng·ml^{-1})	3.254[a]	3.335[b]	3.376[c]	0.033	0.002	0.359*
Glucose (mmol·l^{-1})	2.541[a]	3.024[b]	3.204[b]	0.035	0.015	0.485*
BHBA (mmol·l^{-1})	0.873[a]	0.736[b]	0.619[c]	0.013	0.000	-0.357*

BCS - body condition score; SEM - standard error of the mean; BW - body weight; CART - cocaine- and amphetamine-regulated transcript; BHBA - beta-hydroxybutyrate; [a,b,c] - means in the row with different superscripts differ significantly ($P < 0.05$); * $P < 0.05$

Table 4. Pearson's correlation coefficients for CT with BW and blood indicators.

| Indicator | CT (min/day) | | | SEM | $P <$ | Correlation coefficient |
	I (n = 15)	II (n = 30)	III (n = 24)			CT ×
Mean day of lactation	34	62	87	5.48	0.019	0.267*
CT (min/d)	695[a]	774[b]	844[c]	3.9	0.000	-
BW (kg)	527[a]	534[b]	549[c]	2.6	0.007	0.338*
Blood indicator:						
CART (ng·ml^{-1})	1.352[a]	0.954[b]	0.686[c]	0.021	0.000	-0.667*
Leptin (ng·ml^{-1})	3.127[a]	3.210[a]	3.419[b]	0.047	0.025	0.242
Glucose (mmol·l^{-1})	2.313[a]	2.747[b]	3.114[c]	0.035	0.005	0.482*
BHBA (mmol·l^{-1})	0.904[a]	0.798[b]	0.764[b]	0.052	0.005	-0.201

CT - chewing time; SEM - standard error of the mean; BW - body weight; CART - cocaine- and amphetamine-regulated transcript; BHBA - beta-hydroxybutyrate; [a,b,c] - means in the row with different superscripts differ significantly ($P < 0.05$); * $P < 0.05$

indicate that cows with the lowest BCS (1.89 points) more frequently had the most intensive energy transformation. This led to a higher BHBA concentration compared to cows in good condition and a consequently high BCS ($P < 0.05$). A decreasing tendency of BHBA serum concentration, which was observed in our studies with the simultaneous increase in BCS, was confirmed by the value of the correlation coefficient ($P < 0.05$, Table 3). Table 4 presents the concentrations of these compounds depending on CT. Longer CT ($P < 0.05$) was observed in individuals with higher BW. This was confirmed by the value of correlation coefficient ($P < 0.05$). The data in Table 4 show that CART was most strongly negatively associated with CT ($P < 0.05$). There was a clear tendency for the value of this parameter to decrease with the prolongation of CT ($P < 0.05$). The differences of CART between the CT classes ranged from 0.389 to 0.268 ($P < 0.05$). The level of glucose in individuals with a longer CT was higher, which was confirmed by the differences between the analysed CT classes ($P < 0.05$) and the value of the correlation coefficient ($P < 0.05$). The results of the negative correlation between the concentration of

CART and leptin in the blood are shown in Fig. 1 (Plate IV) ($P < 0.05$). Fig. 2 (Plate IV) and Fig. 3 (Plate V) demonstrating the relationship between the glucose concentration in the blood show a stronger link with CART concentration (Fig. 2) than with glucose and leptin concentrations (Fig. 3).

Discussion

The function of leptin as a factor positively correlating with BW and the amount of fat accumulated in the body, was demonstrated in the studies by Daniel et al. (2002) and Delavaud et al. (2002). A higher blood leptin level in the heaviest individuals with higher BCS in the present experiment also coincides with the results reported by Reist et al. (2003) and Vargová et al. (2015). In contrast, Holtenius et al. (2003) did not find a positive correlation between BCS and blood leptin concentration.

As for BHBA, which was used to describe lipid metabolism in the body, the concentration in the blood plasma indicates that cows with the weaker body condition more frequently had the most intensive energy transformation. This led to the higher BHBA level compared to cows in good condition. A similar relationship was demonstrated in the study by Samanć et al. (2011). The decreasing tendency of BHBA serum concentration with a simultaneous increase in BCS is in line with the results obtained by Kessel et al. (2008) and Vargová et al. (2015).

Given the peculiarity of cattle digestion and the increasing energy deficit during the first weeks of lactation, high yielding cows should devote the most time to chewing food. This stimulates the functions of the digestive tract and increases the use of ingredients from feed. The effect of CT on energy metabolism may be manifested, among others, by changes in the concentration of glucose and BHBA in the blood. The level of these parameters is, however, indirectly dependent on peptides and hormones, such as CART and leptin, which are involved in the regulation of lipid metabolism, and can significantly regulate the feeling of satiety (Ahima and Flier 2000; Ingvartsen and Boisclair 2001; Chelikani et al. 2004). The strongly negatively correlation between CART and CT observed in this study can partially explain the presence of CART in the nerve ganglia of the pyloric part of the abomasum, which was demonstrated in earlier studies (Młynek et al. 2015). The higher level of glucose in cows with a longer chewing time appears to be similar to that observed in earlier studies, which found that the quantity and quality of feed had an impact on the process of chewing and determined the level of glucose in the blood of lactating cows (Frohli and Blum 1988; McGuire et al. 1995; Chelikani et al. 2004). The stability of the glucose level in the body, which is one of the most important energy management substrates, is an indicator of body metabolic homeostasis. In the present experiment, the blood glucose concentration shows a stronger correlation with the CART concentration than with the leptin concentration. Thus, it can be hypothesized that by shortening the duration of the processes related to food intake and CT, CART delays the release of glucose into blood. This results in a lower glucose concentration in the final energy metabolism balance felt as an energy deficit and induction of the mechanisms of lipid transformation in which leptin, released by adipocytes, is involved.

The negative correlation between the concentration of CART and leptin in the blood obtained in this study suggests that the functions of this anorectic neuropeptide may have an antagonizing effect on leptin signal transduction. This may partly explain the stronger and negative correlation between CART and the BCS and the weaker correlation between leptin and this index as well as the correlation with CT. These results are in line with the outcomes of the study conducted by Elias et al. (2001).

This study confirmed that both CART and leptin levels are strongly but oppositely correlated with a cow's fat deposition and are dependent on the lactation phase. In the case

of CART, the correlation with BCS is strongly negative and the same relationship applies to BHBA. For leptin, the correlation with BCS is positive and the same type of correlation occurs for glucose concentration. The results obtained in the present experiment suggest that chewing may be more strongly regulated by CART which has anorectic properties, than by appetite inhibiting leptin, but to fully confirm this conclusion, it is necessary to conduct further research in this direction. The problem for dairy farmers is the reduced dry matter intake of cows until the peak of lactation is reached. This results in a negative energy balance and excessive activation of fat reserves which causes a significant decrease of body condition. The explanation of the issue of the participation of CART and leptin in the regulation of the body's energy homeostasis may therefore be of great importance for milk production.

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Plate IV
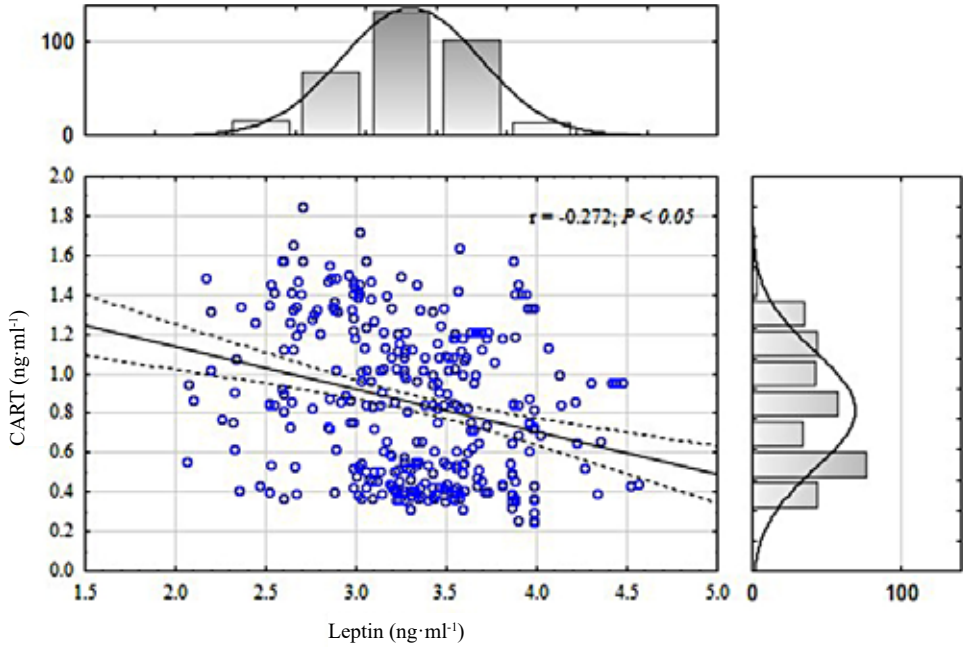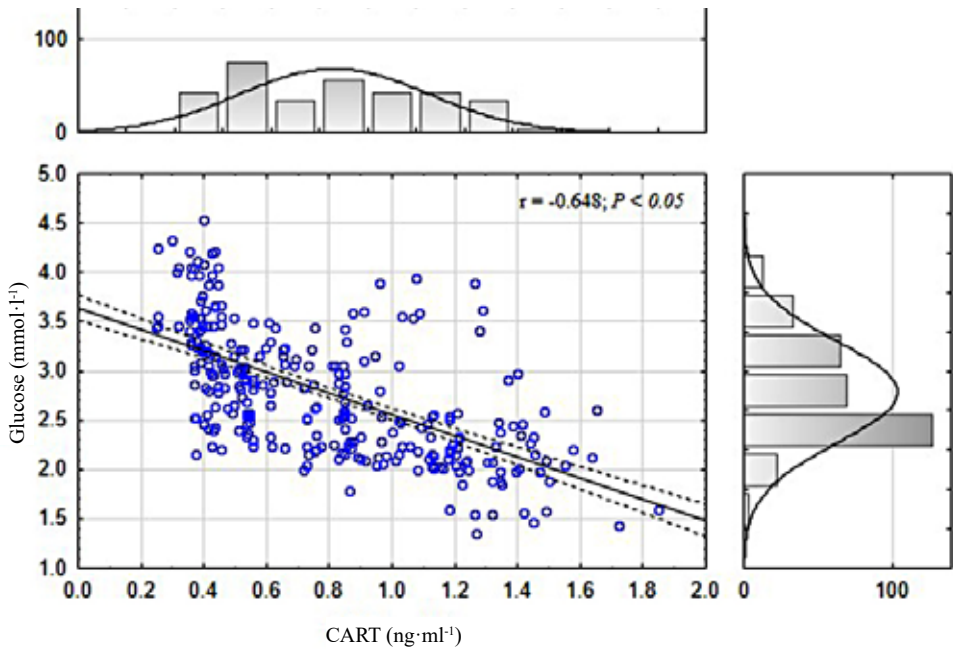Młynek K. et al.: The relationship... pp. 31-38

Fig. 1. The correlation coefficient of leptin and cocaine- and amphetamine-regulated transcript (CART) contents and their distribution

Fig. 2. The correlation coefficient of glucose and cocaine- and amphetamine-regulated transcript (CART) content and their distribution
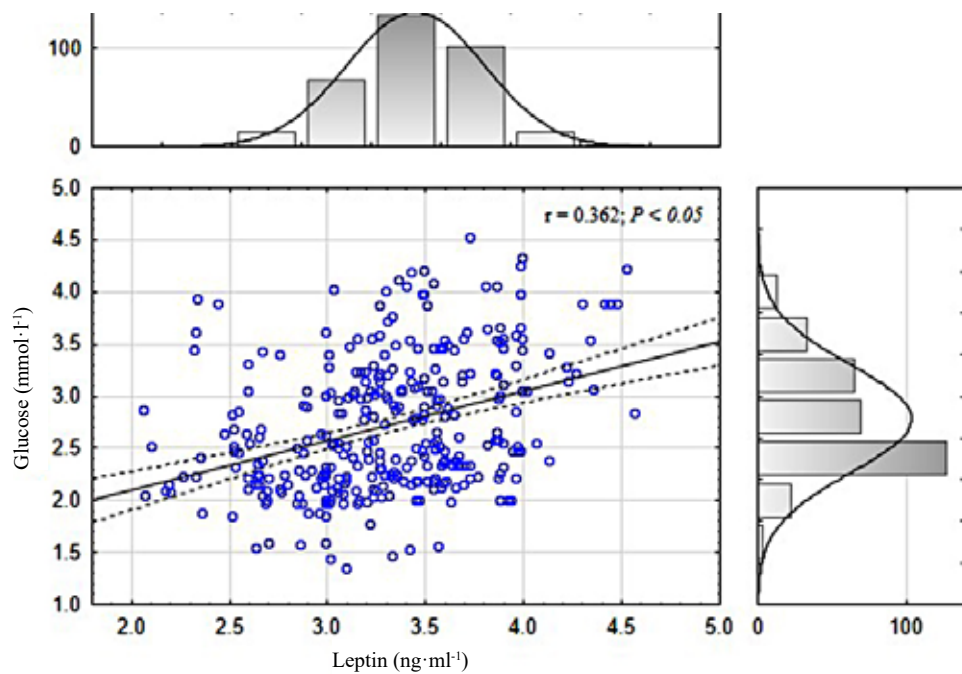
Plate V

Fig. 3. The correlation coefficient of glucose and leptin content and their distribution