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EFFECTS OF β-ENDORPHIN ON PLASMA GLUCOSE LEVELS

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Abstract

Healthy, and insulin-deficient (streptozotocin-induced diabetic, STZ) Sprague-Dawley rats were used to investigate the effects of different doses of β-endorphin (25 and 50 μg/kg) on plasma β-endorphin, insulin, glucagon, and glucose levels at 15- and 30-min time points. In experimental groups, plasma β-endorphin levels were higher at the 15-min than at the 30-min time point in healthy rats; however, in STZ-diabetic rats, β-endorphin levels were lower at 15 min than at 30 min, indicating that intraperitoneal absorption of β-endorphin differed between healthy and insulin-deficient rats. β-endorphin did not affect plasma glucose, insulin, or glucagon at either dose in the healthy group. In the insulin-deficient rats, β-endorphin at 50 μg/kg reduced plasma glucose levels at the 30-min time point compared to the 25 μg/kg dose, without affecting plasma insulin. Moreover, β-endorphin at 50 μg/kg decreased plasma glucagon levels at the 15-min time point in comparison to the 25 μg/kg dose in insulin-deficient rats. Plasma glucose levels may be reduced in insulin-deficient rats at high β-endorphin levels regardless of insulin status.

Key words: rats, diabetes mellitus, β-endorphin, glucose, insulin, glucagon.

Diabetes mellitus is defined as a metabolic disorder caused by insulin deficiency (Type I) or insulin resistance (Type II). A long duration of hyperglycaemia, one of the most important complications of diabetes, causes micro- and macrovascular degeneration resulting in cardiovascular, nervous, and retinal complications caused by glycosylation of other molecules, like proteins (39). Treatment of diabetes with insulin or oral drugs fails to prevent these complications, thus researchers have sought to investigate components that assist glucose uptake into muscle cells in the absence of insulin.

Insulin secretion is mainly stimulated by glucose and also by other agents like fructose, mannose, ribose, amino acids, fatty acids, and neurotransmitters. Secretion of insulin is pulsative in mammals, and oscillation time in rats is reported to be 13.3 min (9). Half-life of insulin in plasma has been observed to be 9 min in Wistar rats (23). Glucagon, which plasma half-life is reported to be 5–10 min, is mainly stimulated by hypoglycaemia (in humans, glucose levels below 60 mg/kg) (2).

Streptozotocin (STZ) used for the induction of Type I diabetes in a rat model is synthesised by *Streptomyces achromogenes*, with a N-methyl nitrosocarbamyl glucosamine structure (19), and has been shown to increase β-cell polyadenine-diphospho-ribose synthetase activity, causing the destruction of cellular DNA and a decrease in NAD levels in the cell (32, 45). ^{14}C-STZ accumulates abundantly in the liver and kidneys and in smaller amounts in the pancreas, however it binds mostly to pancreatic proteins after intravenous (i.v.) injection (32).

β-endorphin, one of the opioid peptides, is synthesised from preopiomelanocortin in the anterior and intermediate hypophysis (33), hypothalamus (1), other brain regions, gastrointestinal tract, liver, lungs, kidneys (11), and adrenal medulla (21). β-endorphin is a 4,000-Da peptide composed of 31 amino acids in mammals and most other vertebrates (40). In rats, the molecular weight is reported to be 3,500 Da (33, 37). The amino acid sequence may vary among species; the 26 and 27 amino acids at the N-terminal are alanine and tyrosine in humans, whereas in rats they are valine and histidine (3). β-endorphin and enkephalin immunoreactivity levels are, in pmol/g wet tissue, as follows: liver 6.1, hepatocytes 10.1, brain 26.1, pancreas 212, and hyphophysis 59,532 (24). Opioid peptides and their receptors (μ, δ, and κ receptors) were observed to affect pancreatic hormones (24). ß-endorphin has an affinity for μ, δ (27), and σ (44) opioid receptors and is reported to be associated with plasma glucose, insulin, and glucagon levels (41). Researchers have reported a plasma glucose lowering effect of synthetic human β-endorphin (i.v., 12.8 μg/kg) in STZ-diabetic Wistar rats (28). Yu *et al*. (47) reported mediation of β-endorphin by andrographolide, which then resulted in reduced plasma glucose levels in STZ-diabetic rats.

Additionally, enhanced β-endorphin levels after isofeluric acid administration into STZ-diabetic rats may increase plasma β-endorphin and stimulate the μ-opoid receptors to increase glucose utilisation or/and reduce hepatic gluconeogenesis (30). β-endorphin has been suggested to stimulate glucose uptake into muscle cells (11, 29). Liu and Cheng (31) considered that β-endorphin may have a glucose-lowering effect in rats, mediated by peripheral μ-opioid receptors, however, the

effects of β-endorphin on plasma glucose, glucagon, and insulin levels in rats have not been reported. In addition, the relationship of circulating β-endorphin levels and plasma glucose levels is unknown. In this study, we investigated the effects of rat β-endorphin on plasma glucose, insulin, and glucagon levels, and the plasma glucose lowering effect of the endorphin regardless of insulin condition. Our aim was also to explore the relationship of circulating β-endorphin levels with the parameters in healthy and insulin-deficient rats.

Material and Methods

Male Sprague-Dawley (10–12 weeks old, n=117) rats were used. They were maintained in a temperature-controlled room (21°C ±2) and kept on a 14:10 h light–dark cycle. Food (Purina rat chow) and water were available *ad libitum*. The study protocol was approved by the Ethics Committee of the Veterinary Faculty of the University of Selcuk.

Seventy-five rats were injected intraperitoneally (i.p.) with STZ at a dose of 60 mg/kg (freshly dissolved in 0.1 M citrate buffer, pH 4.5,) to induce insulin deficiency. Blood taken from the tail vein of the rats after 2 weeks was used to determine glucose levels with a glucose analyser *via* glucose-oxidase method. Forty-two rats, with plasma glucose levels of 250 mg/dl or greater, and with signs of polyuria, were considered as diabetic (9). Blood taken from the tail veins of healthy and insulin-deficient rats before the experiment was used to measure plasma glucose as a basis for dividing the rats into groups with equivalent average plasma glucose levels.

Rats were divided into healthy and insulin-deficient groups. Both groups were then divided into control (n=6, each), experimental 1 (n=6, each), and experimental 2 (n=6, each). Healthy and insulin-deficient control groups were administered 0.9% NaCl solution (i.p. 0.2 ml/200 g) and intracardiac (i.c.) blood was taken at 0-, 15-, and 30-min time points. Healthy and insulin-deficient experimental 1 and 2 groups were administered intraperitoneally 25 and 50 μg/kg of β-endorphin (freshly dissolved in 0.9% NaCl), respectively, and blood was taken after 15 and 30 min. Different animal groups were used for different time points.

Animals were anesthetised by thiopental (40 mg/kg), which, as has been reported, does not affect plasma glucose, insulin (6), or β-endorphin (17, 43) levels. Blood samples (1.5 ml), collected by i.c. puncture using a chilled syringe, were transferred into polyethylene test tubes containing EDTA (1–2 mg/mL of blood), aprotinin (500 kIU/mL of blood), and NaF (1 mg/mL of blood), and centrifuged at 1,600×g for 15 min. Except for plasma glucose, which was determined immediately, plasma samples were stored at -80°C for hormone analysis. Radioimmunoassays of β-endorphin (Phoenix Pharmaceuticals, Inc., USA) and insulin (Linco Research, USA) were performed using commercial kits with polyclonal antibodies raised in rabbits with rat β-endorphin and rat insulin. Plasma glucagon (Linco Research, USA) was determined using a commercial kit specific for pancreatic glucagon. Plasma glucose levels were determined according to glucose-oxidase method by spectrophotometric assay (Spinreact SA, Glucose LQ, Spain).

Drugs. STZ, aprotinin, and EDTA were purchased from Sigma-Aldrich Chemical Co. **(USA)**, rat β-endorphin from Bachem AG (Switzerland), thiopental from I.E. Ulagay Medical (Istanbul, Turkey), and NaF from Merck **(USA)**.

Statistics. Statistical analysis of differences among treatments was done using ANOVA followed by the Duncan test with SPSS Programme. For differences between healthy and STZ-diabetic rats, independent t tests were used. β-endorphin values were log10 transformed. All data were presented as means ±SE, and a P value of 0.05 or less was considered to be significant.

Results

Increased ($P<0.001$) plasma glucose and glucagon levels with decreased ($P<0.001$) insulin levels were determined in the insulin-deficient control groups in comparison to the healthy control groups, confirming the Type I diabetes model. Additionally, plasma β-endorphin levels were also lower in STZ-diabetics (Table 1).

The effects of β-endorphin on plasma β-endorphin levels. An increase ($P<0.05$, Tables 2–3) in plasma β-endorphin was associated with β-endorphin (25 μg/kg – 50 μg/kg) administration at both time intervals in a dose-dependent manner in healthy and diabetic rats. The 15-min levels were higher ($P<0.05$) than the 30-min levels in healthy rats at both β-endorphin doses, whereas it was lower at 15-min than 30 min levels in STZ-diabetics, an outcome that differed from the results in the healthy animals (Tables 2 and 3).

The effects of β-endorphin on plasma glucagon levels. β-endorphin did not affect plasma glucagon levels in healthy rats but resulted in decreased plasma glucagon in STZ-diabetic rats at 15 min at the 50 μg/kg dose compared to the 25 μg/kg ($P<0.05$, Table 3).

The effects of β-endorphin on plasma insulin levels. Plasma insulin was not affected by β-endorphin in either healthy or STZ-diabetic rats (Tables 2 and 3).

The effects of ß-endorphin on plasma glucose levels. The change in plasma glucose levels as a result of β-endorphin administration (25 μg/kg – 50 μg/kg) was not statistically significant; however, a dose-dependent, slight decrease was found in healthy rats (Table 2). In STZ-diabetic rats, plasma glucose levels were decreased ($P<0.05$) at the 50 μg/kg of β-endorphin dose in comparison to the 25 μg/kg dose at the 30-min time point (Table 3).

Table 1
Baseline (0 min) plasma parameters in healthy (n=6) and insulin-deficient (n=6) control groups

Parameter	Healthy	Insulin-deficient	P
β-endorphin (pg/mL)	48.17 ± 6.06	28.83 ± 3.27	*
Glucose (mg/dl)	108.83 ± 3.79	354.0 ± 23.85	**
Insulin (ng/mL)	0.726 ± 0.021	0.237 ± 0.044	**
Glucagon (pg/mL)	27.67 ± 5.23	83.83 ± 5.75	**

Data are presented as means ± SE; * P< 0.05; ** P< 0.001 vs. healthy rats.

Table 2
Effects of different doses of β-endorphin (β-end) on plasma parameters in healthy rats

Healthy	ß-end pg/mL	Glucose mg/dl	Insulin ng/mL	Glucagon pg/mL
Control 15 min	10.3 ± 1.38 e	104.33 ± 4.55 ab	0.595 ± 0.07	30.67 ± 1.8 ab
Control 30 min	16.5 ± 4.15 e	97.3 ± 5.43 ab	0.572 ± 0.06	33.5 ± 3.21 ab
β-end (25 µg/kg) 15 min	696.67 ± 119.68 b	106.83 ± 4.90 a	0.703 ± 0.05	28.0 ± 4.08 ab
β-end (25 µg/kg) 30 min	357.5 ± 9.66 c	95.67 ± 4.12 ab	0.712 ± 0.04	39.17 ± 6.59a
β-end (50 µg/kg) 15 min	2000.0 ± 77.46 a	102.5 ± 3.78 ab	0.76 ± 0.01	25.83 ± 3.43b
β-end (50 µg/kg) 30 min	1750.00 ±157.06 a	89.67 ± 1.98 b	0.640 ± 0.03	26.17 ± 0.87 ab

Data are presented as means ±SE from each group of 6 animals; a, b, c, d, e - different letters in the same column (between time points) are statistically different (P<0.05).

Table 3
Effects of different doses of β-endorphin (β-end) on plasma parameters in insulin-deficient (STZ-diabetic) rats

Insulin-deficient	ß-end pg/mL	Glucose mg/dl	Insulin ng/mL	Glucagon pg/mL
Control 15 min	37.33 ± 9.27 cd	395.83 ± 9.5 b	0.295 ± 0.05	76.0 ± 4.48 abc
Control 30 min	16.17 ± 2.33d	427.83 ± 9.18 ab	0.295 ± 0.04	64.0 ± 4.09 bc
β-end (25 µg/kg) 15 min	280.0 ± 124.0abc	416.6 ± 9.93 ab	0.238 ± 0.04	78.33 ± 4.54 ab
β-end (25 µg/kg) 30 min	503.33 ± 201.19ab	452.33 ± 13.28 a	0.205 ± 0.02	75.12 ± 6.4 abc
β-end (50 µg/kg) 15 min	207.5 ± 126.72bcd	422.67 ± 8.83 ab	0.346 ± 0.05	60.0 ± 6.2 c
β-end (50 µg/kg) 30 min	758.5 ± 246.27a	399.83 ± 8.65 b	0.285 ± 0.03	58.33 ± 7.82 c

Data are presented as means ±SE from each group of 6 animals; a, b, c, d - different letters in the same column (between time points) are statistically different (P<0.05).

Discussion

In the present study, plasma ß-endorphin levels in healthy rats were similar (48.17 pg/mL) to those identified in other studies (28). Plasma β-endorphin levels after administration of 25 or 50 μg/kg of β-endorphin increased considerably, suggesting that absorption of β-endorphin in healthy rats begins within 15 min after i.p. injection. The decline at 30 min, observed in this study, may be a result of plasma degradation and/or transcapillary diffusion. Transcapillary diffusion of β-endorphin through intracellular fluid (38) and plasma degradation by proteases has been reported (20, 46).

In the current study, plasma β-endorphin levels were lower at 15 min than at 30 min in the STZ-diabetic rats, ($P>0.05$ at 25 μg/kg, $P<0.05$ at 50 μg/kg dose) and the increase in plasma β-endorphin was not as dramatic as that seen in the healthy group. Healthy rats' plasma β-endorphin levels were also higher than the levels observed in the STZ diabetic animals (Tables 2-3). Intermediate hypophysis, hypothalamus, and plasma β-endorphin levels have been reported previously to decrease in STZ-diabetic rats (13, 14). To our knowledge, a change in intraperitoneal absorption of β-endorphin in STZ-diabetic rats has not been previously reported, but the absorption of vitamin D_3 and calcium from the intestines and placental Ca^{++} transport from the mother to the foetus was abolished (34) in diabetics. Diabetes-caused metabolic complications may underline these effects.

In this study the effects of β-endorphin on plasma glucose, insulin, and glucagon levels were not significant in healthy rats, which is consistent with results reported by other researchers (25, 29). In humans, bolus of β-endorphin (i.v.) was reported to raise plasma glucose levels (15), but infusion (i.v., 1 h) did not change (16). These results suggest that the administration route of β-endorphin may influence plasma glucose levels and that different effects may occur in rats and humans.

β-endorphin was previously reported to reduce plasma glucose levels in STZ-diabetic rats (28, 8). In this study, β-endorphin at 50 μg/kg decreased plasma glucose levels at the 30-min time point in STZ-diabetic rats in comparison to the 25 μg/kg dose. This finding is similar to that of Liu *et al.* (28), who reported a glucose-lowering effect of intravenous (i.v.) human β-endorphin in STZ-diabetic rats. They reported the plasma glucose lowering effect of β-endorphin at 15- and 20-min time points in comparison to the 0-min levels without a sham group. In the current study, a plasma glucose lowering effect was seen at 50 μg/kg dose in comparison to 25 μg/kg, at 30 min in STZ group; thus, the absorption after i.p. administration might have taken more time than the i.v. route. Previous work (28) did not report a determination of plasma β-endorphin concentrations necessary for decreasing plasma glucose levels. In this study, 25 μg/kg i.p. of β-endorphin increased plasma glucose slightly, whereas the 50 μg/kg dose decreased ($P>0.05$) it. The insignificant ($P>0.05$) decrease in plasma glucose levels at the 50-μg/kg dose in comparison to the control group can be attributed to the plasma levels of β-endorphin in STZ diabetic rats. Intraperitoneal absorption of β-endorphin is delayed in STZ diabetic animals (lower at 15 min than at 30 min; Table 3) in comparison to healthy ones (higher at 15 min than at 30 min; Table 2). The β-endorphin levels necessary to reduce plasma glucose levels in STZ diabetic animals may not be sufficient at these time points.

β-endorphin has been reported to increase glucose uptake of mouse and rat muscle cells i*n vitro* (11, 29). The endorphin increased 2-[^{3}H]-glucose uptake in contracted and resting phrenic muscle cells, and the contracted muscle cells were more sensitive than resting cells (11). In STZ-diabetic rats, the μ agonist loperamid increased glucose uptake into soleus muscle cells (29). Glucose is transported into the cells *via* GLUT proteins. So far twelve GLUT proteins have been identified (35). GLUT4 (26, 22) and recently GLUT8 (5), and GLUT12 (36) are reported to be insulin sensitive. The K_m of GLUT4 for glucose is 5 mM (26, 22). Metformin, the effects of which are reversed by the μ antagonist naloxonazine, increased plasma β-endorphin and GLUT4 mRNA levels in rat soleus muscle cells (8). Stimulation of GLUT4 protein~~s~~ in the muscle cells and increased glucose uptake can be considered as possible mechanisms underlying β-endorphin's effects. In exercise involving muscle contraction, β-endorphin (4-bouix) and epinephrine (42-watt) levels are also increased. Epinephrine increases plasma glucose levels to supply the energy needs of muscle cells during exercise, and the rise in β-endorphin may relate to increased glucose uptake into muscle cells in addition to insulin function.

Our findings of significantly higher plasma glucagon levels in diabetic animals in comparison to healthy rats are similar to those of other researchers (18). In mice, 5 d administration of STZ (40 mg/kg) affected the morphology of α-cells. After 28 d, nearly all of the β-cells were destroyed, and the α-cells had expanded by 2–3 times (8) in the "islands. The increase in glucagon level in STZ-diabetic animals may relate to the expansion of α-cells into the Langerhans islands, resulting in more glucagon production. β-endorphin did not affect plasma glucagon significantly in healthy rats in the current study, which is consistent with the results of other researchers (12). In STZ-diabetic rats, β-endorphin at 50 μg/kg significantly reduced plasma glucagon levels at the 15-min time point in comparison to the 25 μg/kg dose, which has not been previously reported. However, β-endorphin has been suggested to decrease the gluconeogenetic key enzyme PEPCK mRNA levels in the liver, causing reduced glucagon secretion (7).

In conclusion, the intraperitoneal administration of β-endorphin increased plasma β-endorphin levels dramatically in healthy rats in comparison to insulin-deficient (STZ-diabetic) rats. The degradation time of β-endorphin was increased at high plasma β-endorphin levels in both healthy and insulin-deficient rats, the rise of the endorphin after administration required more time to peak. β-endorphin did not affect plasma glucose, insulin, or glucagon levels in healthy rats at normal glucose levels; however, it had a slight glucose-

lowering effect without affecting plasma insulin in insulin-deficient rats at high plasma glucose levels. The mechanism of action of β-endorphin on plasma glucose and the involvement of GLUT protein levels require further investigation.

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References

1. Angelogianni P., Li H.L., Gianoulakis C.: Ontogenesis of proopiomelanocortin and its processing to β-endorphin by the fetal and neonatal rat brain. Neuroendocrinology 2000, **72**, 231-241.
2. Anonymous: www.ctf.istanbul.edu.tr/farma/pankreas.pdf
3. Anonymous: www.bachem.com/bachem/bachem/joust/index.cfm?idland=216.
4. Bouix O., Lenoir A.N., Kerdelhue B., Orsetti A.: Endogenous opioid peptides stimulate post-exercise insulin response to glucose in rats. Int J Sports Med 1996, **17**, 80-84.
5. Carayannopoulos M.O., Chi M.M.Y., Cui Y., Pingsterhaus J.M., McKnigth R.A., Mueckler M., Devaskar S.U., Moley K.H.: GLUT 8 is a glucose transporter responsible for insulin stimulated glucose uptake in the blastocytes. Proc Natl Acad Sci USA 2000, **97**, 7313-7318.
6. Cardoso A., Carvalho C.R.O., Velloso L.A., Brenelli S.L., Saad M.J.A., Carvalheira J.B.C.: Effect of thiopental and dietyl ether on early steps of insulin action in liver and muscle of the intact rat. Life Sci 2005, **76**, 2287-2297.
7. Cheng J.T., Haung C.C., Liu I.M., Tzeng T.F., Chang C.J.: Novel mechanism for plasma glucose-lowering action of metformin in streptozotocin–induced diabetic rats. Diabetes 2006, **55**, 819-825.
8. Cheng J.T., Liu I.M., Tzeng T.F., Chen W.C., Hayakawa S., Yamamoto T.: Release of β-endorphin by caffeic acid to lower plasma glucose in streptozotocin-induced diabetic rats. Horm Metab Res 2003, **35**, 251-258.
9. Chou H.F., McGivern R., Berman N., Ipp E.: Oscillations of circulating plasma insulin concentrations in the rat. Life Sci 1991, **48**, 1463-1469.
10. Debold C.R., Nickolson W.E., Orth D.N.: Immunoreactive POMC peptides and POMC-like messenger ribonucleic acid are present in many rat non-pituitary tissues. Endocrinology 1988, **122**, 2648-2657.
11. Evans A.A.L., Khan S., Smith M.E.: Evidence for s hormonal action of β-endorphin to increase glucose uptake in resting and contracting skeletal muscle, J Endocrinol 1997, **155**, 387-392.
12. Fatouros I., Goldfarb A.H., Jamurtas A.Z., Gao J.: β-endorphin infusion alters pancreatic hormone and glucose levels during exercise in rats. Eur J Physiol 1997, **76**, 203-208.
13. Forman L.J., Estlilow S., Lewis M., Vasilenko P.: Streptozocin diabetes alters immunoreactive β-endorphin levels and pain perception after 8 week in female rats. Diabetes 1986, **35**, 1309-1313.
14. Forman L.J., Marquis D.E., Stevens R., Adler R., Vasilenko P.: Diabetes induced by streptozotocin results in a decrease in immunoreactive β-endorphin levels in the pituitary and hypothalamus of female rats. Diabetes 1985, **34**, 1104-1107.
15. Guigliano D., Cozzolino D., Salvatore T., Ceriello A., Torella R.: Dual effects of β-endorphin on insulin secretion in man. Horm Metab Res 1987, **19**, 502-503.
16. Guigliano D., Cozzolino D., Salvatore T., Ceriello A., Torella R., Franchimont P., Lefebvre P., Donofrio F.: Physical elevations of plasma β-endorphin alter glucose metabolism in obese, but not normal-weight subjects. Metabolism 1992, **41**, 184-190.
17. Helman A., Giraud P., Nicolaidis S., Oliver C., Assan R.: Glucagon release after stimulation of the lateral hypothalamic area in rats: predominant β-adrenergic transmission and involvement endorphin pathways. Endocrinology 1983, **113**, 1-5.
18. Hemmings S.J., Spafford D.: Neonatal STZ model of type II diabetes mellitus in the Fischer 344 rats: characteristics and assessment of the status of the hepatic adrenergic receptors. Int J Biochem Cell Biol 2000, **32,** 905-919.
19. Hinz M., Katsilambros N., Maier V., Schatz H., Pfeiffer E.F.: Significance of streptozotocin induced nicotinamide-adenine dinucleotide (NAD) degradation in mouse pancreatic islands. FEBS Letters 1973, **30**, 225-228.
20. Houghten R.A., Swann R.W., Li C.H.: β-endorphin: stability, clearence behavior and entry into the central nervous system after intravenous injection of the tritiated peptide in rats and rabbits. Proc Natl Acad Sci USA 1980, **77**, 4588-4591.
21. Hsu C.T., Liu I.M., Cheng J.T.: Increase of β-endorphin biosynthesis in the adrenal gland of streptozotocin-induced diabetic rats. Neurosci Letters 2002, **318**, 57-60.
22. James D.E., Brown R., Navarro J., Pilch P.F.: Insulin regulatable tissues express a unique insulin-sensitive glucose transport protein. Nature 1988, **333**, 183-185.
23. Jones R.G., Ilic V., Williamson D.H.: Physiological significance of altered insulin metabolism in the conscious rat during lactation. Biochemic J 1984, **220**, 455-460.
24. Khawaja X., Green I.C., Thorpe J.R., Titheradge M.A.: The occurrence and receptor specifity of endogenous opioid peptides within pancreas and liver of the rat. Biochem J 1990, **267**, 233-240.
25. Khawaja X., Gren I.: Dual action of β-endorphin on insulin release in genetically obese and lean mice. Peptides 1991, **12**, 227-233.
26. Klip A., Walker D., Ransome K.J., Schroer D.W., Leinhard G.E.: Identification of the glucose transporter in rat skeletal muscle. Arch Biochem Biophys 1983, **226**, 198-205.
27. Kubik R.B., Shippenberg T.S., Herz A.: Involvement of central mu and delta opioid receptors in mediating the reinforcing effects of β-endorphin in the rat. Eur J Pharmacol 1990, **175**, 63-69.
28. Liu I.M., Chi T.C., Chen Y.C., Lu F.H., Cheng J.T.: Activation of opioid μ-receptor to lower plasma glucose in streptozotocin-induced diabetic rats. Neurosci Letters 1999, **265**, 183-186.
29. Liu I.M., Niu C.S., Kuo D.H., Cheng J.T.: Investigations of the mechanism of the reduction of plasma glucose by cold-stress in streptozotocin-induced diabetic rats. Neuroscience 1999, **92**, 1137-1142.
30. Liu M., Chen W.C., Cheng J.T.: Mediation of β-endorphin by isoferulic acid to lower plasma glucose in streptozotocin-induced diabetic rats. J Pharmacol Exp Ther 2003, **307**, 1196-1204.
31. Liu I.M., Cheng J.T.: Mediation of endogenous β-endorphin in the plasma glucose-lowering action of

herbal products observed in type 1-like diabetic rats. eCAM 2008, pp. 1-9.
32. Masiello P., Karunanayake E.H., Bergamini E., Hearse D.J., Mellows G.: ^{12}C-streptozotocin: its distribution and interaction with nucleic acids and proteins. Biochem Pharmacol 1981, **30**, 1907-1913.
33. Murray R.K.: Pituiter ve hipotalamik hormonlar, 'Harper'ın Biyokimyası', Ed: Murray R.K., Mayes P., Granner D., Rodwell V., Barış Kitabevi Yayım ve Dağıtım, Cerrahpaşa, İstanbul, 1993, pp. 603-604.
34. Ohara N.: Impaired intestinal active calcium absorption and reduction of serum 1alpha, 25(OH)2D3 in streptozotocin induced diabetic pregnant rats with hypocalcemia in their fetuses. Clin Exp Obstet Gynecol 2000, **27**, 100-102.
35. Pessin J.E., Bell G.: Mammalian facilitative glucose transporter family. Structure and molecular regulation. Ann Rev Physiol 1992, **54**, 911-930.
36. Rogers S., Macheda M., Docherty S.E., Carty M.D., Henderson M.A., Soeller W.C., Gibbs E.M., James D.E., Best J.D.: Identification of a novel glucose transporter-like protein-GLUT12. Am J Physiol Endocrinol Metab 2002, **283**, 733-738.
37. Rubinstein M., Stein S., Udenfriend S.: Isolation and characterisation of opioid peptides from rat pituitary: β-endorphin. Proc Natl Acad Sci USA 1977, **74**, 4969-4972.
38. Sato H., Sugiyama Y., Sawada Y., Iga T., Hanano M.: Physiologically based pharmacokinetics of radioiodinated β-endorphin in rats. Drug Metab Disp 1987, **15**, 540-550.
39. Stanaway S.E.R.S., Gill G.V.: Protein glycosylation in diabetes mellitus: biochemical and clinical considerations. Pract Diab Int 2000, **17**, 21-25.
40. Van Den Burg E.H., Metz J.R., Arends R.J., Devreese B., Vandenberghe I., Van Beeumen J., Bonga S.E.W.: Identification of β-endorphins in the pituitary gland and blood plasma of common carp (*Cyprinus carpio*). J Endocrinol 2001, **169**, 271-280.
41. Watkins W.B., Bruni J.F., Yen S.S.C.: β-endorphin and somatostatin in the pancreatic D-cell colocalization by immunocytochemistry. J Histochem Cytochem 1980, **28**, 1170-1174.
42. Watt M.J., Hargreaves M.: Effect of epinephrine on glucose disposal during exercise in humans: role of muscle glycogen. Am J Physiol Endocrinol Metab 2002, **283**, 578-583.
43. Way W.L., Hosobuchi Y., Johnson B.H., Eger E.I., Bloom F.E.: Anesthesia does not increase opioid peptides in cerebrospinal fluid of humans. Anesthesiology 1984, **60**, 43-45.
44. Wiesner J.B., Robert M.: Behavioral specificity of β-endorphin suppression of sexual behavior: differential receptor antagonism. Pharmacol Biochem Behav 1986, **24**, 1235-1239.
45. Yamamoto H., Uchigata Y., Okamoto H.: Streptozotocin and alloxan induced DNA strand breaks and poly (ADP_RIBOSE) synthetase in pancreatic islets. Nature 1981, **294**, 284-286.
46. Young E., Houghten R.A., Akil H.: Degradation of [^{3}H] β-endorphin in rat plasma is increased with chronic stress, Eur J Pharmacol 1989, **167**, 229-236.
47. Yu B.C., Chang C.K., Su C.F., Cheng J.T.: Mediation of β-endorphin in andrographolide-induced plasma glucose lowering action in type I diabetes-like animals. Naunyn-Schmiedebergs Arch Pharmakol 2008, **377**, 529-540.