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# Effects of noopept on cognitive functions and pubertal process in rats with diabetes

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ARTICLE INFO

*Chemical compounds studied in this article:*
Insulin: Insulin detemir (Levemir Flexpen-Novo Nordisc- Denmark)
Noopept: GVS-111, *N*-phenylacetyl-L-prolyl glycine ethyl ester (Powder City, USA)
Kiss1 antibody: Biorbyt, UK
GnRH antibody: Santa Cruz- USA
Insulin ELISA rat kits: Elabscience (USA)
FSH ELISA rat kits: Elabscience (USA)
LH ELISA rat kits: Elabscience (USA)
BDNF ELISA rat kits: Elabscience (USA)
NGF ELISA rat kits: Elabscience (USA)

*Keywords:*
Noopept
Puberty
Pubertal timing
Type 1 diabetes

ABSTRACT

*Aim:* Type 1 diabetes (T1DM) is a common chronic disease in childhood. Increasing insulin resistance in puberty gives rise to higher doses of insulin usage in treatment. Of this reason new approaches in treatment are needed. Noopept researches suggest it to have anti-diabetic properties. We tried to determine the effects of noopept on pubertal diabetes.

*Main method:* The research was made with 60 prepubertal, 28 day-old, male, *Sprague Dawley* rats. The rats were divided into randomised 6 groups ($n = 10$/group). i) Control, ii) Diabetes Control, iii) Noopept Control, iv) Diabetes + Noopept, v) Diabetes + Insulin, vi) Diabetes + Insulin + Noopept. T1DM model was induced by streptozotocin on postnatal 28th day. 0.5 mg/kg noopept and 1 IU insulin were administered intraperitoneally for 14 days. Blood glucose and body weight measurements, puberty follow-up and MWM tests were performed. Hippocampus, hypothalamus and testis were evaluated histologically. Hypothalamic GnRH and kisspeptin were studied immunohistochemically. Serum LH, FSH and insulin, hippocampal homogenate NGF and BDNF levels were determined by ELISA.

*Key findings:* Delayed puberty was normalized by noopept ($p < 0.05$). Blood glucose levels were lower in noopept-administered diabetic groups ($p < 0.05$). Noopept decreased HOMA-IR in insulin administered diabetic group ($p < 0.05$). Number of degenerated cells in hippocampus and testis were higher in diabetes control group when compared with other groups ($p < 0.05$). GnRH immunoreactivity in Diabetes + Noopept group was increased when compared to insulin + noopept group ($p = 0.018$). There was no difference in kisspeptin, serum LH, FSH, hippocampal NGF-BDNF levels and spatial learning assessment among groups ($p > 0.05$).

*Significance:* Noopept may have positive effect in treatment of pubertal diabetes.

## 1. Introduction

Puberty is a transitional period between childness and adulthood [1]. Pubertal process, effected from many factors as chronic diseases and drugs, begins with hypothalamo-pituitary-gonadal (HPG) axis activation [1,2]. The primary regulatory hormone of HPG axis is gonadothropin releasing hormone (GnRH). Kisspeptin (Kp) is an important factor in GnRH secretion and puberty [3,4]. Also, Kp levels interact with glucose-dependent insulin secretion and metabolic modulators [5].

Type 1 diabetes (T1DM) is a common childhood chronic disease causing numerous complications [6,7]. A frequently observed complication of T1DM in childhood is cognitive weakness [9]. Widely expressed peptides, neuronal growth factor (NGF) and brain-derived neurotrophic factor (BDNF) have important roles both in cognitive disorders and diabetes [10].

Blood glucose level regulation is the main treatment goal in reducing complications [6]. Insulin, as the main regulatory hormone of blood glucose level regulation, is the primary treatment modality in T1DM. Besides, insulin has important roles in growth, GnRH neuronal function and pubertal processes [8]. Normally, insulin resistance increases in pubertal process. This situation gives rise to; increased GH levels and decreased IGF-1, GnRH and gonadotropic hormone levels [11]. Also, in pubertal period higher doses of insulin usage is required

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in T1DM treatment because of the insulin resistance [11]. Despite the treatment protocols, mainly because of the interaction of insulin with growth-related hormones, growth and pubertal process problems are still observed in T1DM children [12,13]. Hyperinsulinemia, by causing; weight gain, increased hypoglycemia attack frequency, over-stimulation of insulin/IGF-1 receptors in the gonads and increased steroid secretion [14–16] gives rise to long-term complications [16,17]. Due to the undesirable effects of high-dose insulin usage, different/ alternative treatment protocols to normalize insulin sensitivity and insulin resistance in pubertal T1DM are in search [6,17].

Nootropics are widely used agents in cognitive disorders [18]. Noopept is a nootropic dipeptide produced from nonpeptide prototype of vasopressin and piracetam [19,20]. Dipeptides have high biological stability and there exists specific transport systems for dipeptides in intestine (PEPT1) and blood-brain barrier (PEPT2) [19,21,22]. Noopept shows its effect by blocking voltage-dependent calcium and calcium-dependent potassium channels [22]. It has broad neuroprotective activities and cognitive regulatory effects [21]. Noopept administration increases NGF and BDNF levels in long-term [23]. As Noopept has been shown to have anti-oxidant enzyme activity and lipid peroxidase activity, its probable effects on DM has been researched [24]. In adult rats with streptozotocin (STZ)-induced diabetes, noopept has been shown to have positive effect on blood glucose regulation and body weight changes [24]. In two trials, antidiabetic activity of Noopept was found to be similar with DPP-4 inhibitors (commonly used anti-diabetic), [20,25].

However, efficacy of noopept in puberty has not been searched. If similar effects with the previous researches are determined, such an agent may be a probable approach in pubertal process T1DM treatment. In this respect we aimed to determinate the effects of noopept usage on blood glucose, body weight, insulin resistance, HPG axis, pubertal timing, cognitive functions, NGF and BDNF levels in prepubertal type 1 diabetes model.

## 2. Materials and method

### 2.1. Animals

This study was carried out; with approval of Inonu University Animal Ethics Committee (2015/ A- 62) and in accordance with the Declaration of Helsinki. The cages (5 rats in each) were set at 20–22 °C and 12 h light/ dark period. Animals were fed ad libitum with tap water and standard rat diet. All animals received humane care.

The research was made with 60 prepubertal, 28 day-old, male, Sprague Dawley rats. The number of animals in groups was determined according to the Power analysis ($n = 10$). Rats were randomly divided into 6 groups. Groups were: Control (C), Diabetes Control (DC), Noopept Control (N), Diabetes + Noopept (D + N), Diabetes + Insulin (D + I), Diabetes + Noopept + Insulin (D + N + I).

T1DM were induced by a single dose intraperitoneal 50 mg/kg STZ (Sigma Aldrich) -prepared in sodium citrate buffer- administration after 12 h fasting. After three days, fasting blood glucose > 200 mg/dl (Easymax Mini Blood Glucose Meter (EPS Bio Technology Corp.)) was admitted as diabetes. After the identification of diabetic groups, Noopept (Powder City, USA) 0.5 mg/kg in dosage (dissolved in serum physiologic) and/or 1 U long-acting insulin detemir (Levemir Flexpen) were administered intraperitoneally, once daily for 14 days in required groups [24–26]. Insulin detemir (Levemir Flexpen-Novo Nordisc-Denmark) is a novel long acting human insulin analogue which has been shown to be more effective in diabetes treatment among insulin preparates [26]. Intraperitoneal saline was administered in control groups. Morris water maze (MWM) test was performed 30–60 min after the injections.

### 2.2. Experimental process

Blood glucose and body weight measurements were performed on days; 0 (day of STZ injection- postnatal 28th day), 3, 10, 17 of the experiment. To define pubertal process, 'prepuce-glans penis separation' was followed up daily after postnatal 39th day. Insulin resistance was calculated by 'homeostasis model assessment-insulin resistance' (HOMA-IR) = glucose (mg/dL) × insulin (IU/mL) / 405 formula [27].

In 18th (postnatal 46th) day of experiment, animals were sacrificed after 70 mg/kg ketamine and 8 mg/kg xylazine anesthesia, by taking blood from their hearts. The hypothalamus, hippocampus and testis tissues were separated. Left hemisphere hippocampus tissue was kept at −80 °C for biochemical analysis. Hypothalamus, right hemisphere hippocampus and testis tissues were placed in 10% formaldehyde solution for histological examination.

### 2.3. Morris water maze test

The maze is 150 cm in diameter and 60 cm in height. Water was filled to 40 cm, the temperature of the water was adjusted to 24 ± 2 °C. The water was made opaque by nontoxic black dye (Mixol, Germany). The surface area of the maze was divided into 4 equal quadrants. Northwest (NW) quadrant was randomly determined as target quadrant. A 10 cm diametered platform was placed 2 cm below water surface and 30 cm away from the edge of NW quadrant. The platform was covered with a black fibrous cloth in order the rats to feel safe and grip their claws on [28]. The platform was kept stationary for 4-day learning period. Probe-test was performed in the 5th day. During the 4-day learning period, animals were trained to find the hidden platform by 4 trials with 20 min intervals.

In each trial, the rats were placed to the maze from different quadrants and were allowed to swim 90 s. If they did not find the platform in 90 s, they were put on the platform and kept on it for 30 s. During the 4-day learning period, platform finding time was measured in each trial. On the fifth day of experiment (the platform removal day), a single flotation of 90 s for each rat was made after placing the rats into the maze from the opposite point of the previous platform place. Assessment was made by determining the time spent in the platform quadrant and the distance to the former platform place. A computerized video camera system (Ethovision, Noldus) was used to monitor, record and analyze the rats in the pool.

### 2.4. Histological analysis

At the end of the experiment, the right hemisphere hippocampus, hypothalamus and testis tissues were fixed in 10% formalin and were embedded in paraffin. Tissue sections were cut at 4 μm, mounted on slides, stained with hematoxylin-eosin (H-E) for general structure. In the H-E painted hippocampus sections, neurons from 3 randomly selected areas in the pyramidal layer of CA1 region were evaluated and counted. Neurons with round and light nuclei were considered normal, shrunken neurons with picnotic nuclei were considered degenerated [29].

Testicular tissue was considered according to; cell stability, vacuolization/edema in interstitial tissue, spilled cells in tubules, congestion and cell infiltration. Also mean seminiferous tubular diameter (MSTD) and germinal epithelial cell thickness (GECT) were determined in micrometres (μm). In each section, the diameters of 20 separate circular seminiferous tubules were randomly measured using a 20× objective. In each group, 100 tubule diameters were measured.

Histological examinations and evaluations were performed using a Leica DFC-280 research microscope, Leica Q Win Plus and DS-L3 image analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

### 2.5. Immunohistochemical analysis

Four mikrometers of thickness tissue sections were deparaffinized, rehydrated, placed in antigen retrieval solution (citrate buffer, pH 6.0), boiled in a pressure cooker for 20 min and cooled to room temperature for 20 min in order. Then the sections were washed with phosphate-buffered saline (PBS, pH 7.4), afterwards the slides were incubated in 3% hydrogen peroxide solution for 15 min at room temperature for blocking endogenous peroxidase activity and were washed in PBS. After the blockage of non-specific antigen-binding sites with protein block, primary antibodies which do not give cross-reactivity in rats (monoclonal Kiss1 antibody, Biorbyt, UK and polyclonal GnRH antibody, Santa Cruz- USA) were applied for 60 min at room temperature. After being rinsed with PBS, sections were incubated with biotinylated secondary antibody and streptavidin peroxidase for 10 min at room temperature. Samples were visualized with the chromogenic substrates AEC, counterstained with hematoxylin, mounted in glass slide.

Brown staining due to immunoreactivity was observed in the neuron bodies after GnRH and Kp application to hypothalamus sections. Hypothalamic arcuate nucleus was evaluated for Kp. The prevalence (0: %0–25, 1: %26–50, 2: %51–75, 3: %76–100) and severity (0: absent, +1: mild, +2: moderate, +3: severe) of staining immunoreactivity were scored semiquantitatively [30]. Total staining score was calculated by 'prevalence × severity'.

### 2.6. Biochemical analysis

Elabscience (USA) ELISA rat kits were used in measurement of Insulin, FSH, LH, BDNF and NGF levels. No cross-reactivity or interference between rats Insulin, FSH, LH, BDNF, NGF and analogues has been reported to be obtained about the referred kits.

Deep freezed left hemispheric hippocampus was put in glass tubes and 2 ml of 'phosphate buffer' was added. The tissues (approximately 50 mg for each) were homogenized for 3 min at a speed of 16,000 rev/min (IKA, Germany). NGF and BDNF tissue levels were measured by ELISA test from the obtained homogenates.

Insulin, FSH and LH measurements were performed from serum. The results were calculated based on protein levels.

### 2.7. Statistical analysis

IBM SPSS Windows 22 version was used for statistical evaluation of the research data. Normal distribution of quantitative variables was assessed by Shapiro-Wilk test. Normal distributions were defined by mean ± standard deviation (mean ± Sd), non-normal distributions were defined by median, minimum and maximum values. In comparisons, one way variance analyses followed by Tukey binary comparison method were used when necessary. When the variances were not homogeneous, Welch test and Games-Howel binary comparison methods were used. Changes in time were tested by repeated measures of variance analysis and binary comparisons Bonferroni method when necessary. Wilcoxon, Mann Whitney U, Kruskal Wallis tests were used when parametric test assumptions were not provided. Conover method was used in binary comparisons after Kruskal Wallis. $p < 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Body weight change

Body weights of all groups were measured on experimental days; 0, 3, 10, 17. Weight gain of the Control group was significantly higher than Diabetes Control ($p = 0.025$), Noopept Control ($p = 0.002$), Diabetes + Noopept ($p = 0.043$) and Diabetes + Noopept + Insulin ($p = 0.035$) groups at the end of the experiment. No statistical difference was obtained when the weight gain of the Control group was compared with Diabetes + Insulin ($p = 0.111$) group ($p > 0.05$) (Table 1).

**Table 1**
Body mass change (gr) (Mean ± Sd).

| Group/weight(gr) | 1. day Mean ± Sd | 17. day Mean ± Sd |
|---|---|---|
| Control | 55.20 ± 12.73 | 110.90 ± 14.69[a] |
| Diabetes control | 58.80 ± 12.56 | 101.40 ± 17.08[b] |
| Noopept control | 57.90 ± 9.99 | 107.00 ± 10.08[a] |
| Diabetes + noopept | 64.00 ± 9.04 | 102.50 ± 21.30[a] |
| Diabetes + insulin | 56.10 ± 12.01 | 98.60 ± 24.61 |
| Diabetes + noopept + insulin | 61.50 ± 11.25 | 107.70 ± 18.57[a] |

(a, b different from each other, [a,b] $p \leq 0.05$.)

### 3.2. Change of blood glucose levels

Blood glucose levels in 3rd day measurements increased in all diabetic groups ($p \leq 0.05$). Diabetes Control and Diabetes + Insulin groups did not show statistically significant difference in blood glucose levels in day 3, 10, 17 day measurements. Blood glucose levels of Diabetes + Noopept ($p = 0.07$) and Diabetes + Noopept + Insulin ($p = 0.00$) groups were found to be lower ($p \leq 0.05$) on day 17 when compared with day 3 (Table 2).

### 3.3. Evaluation of insulin level and insulin resistance

Insulin levels of Diabetes Control group was significantly higher than Diabetes + Noopept + Insulin group ($p = 0.05$) at the end of the experiment. Insulin resistance was higher in Diabetes Control group when compared with other groups. Diabetes + Noopept + Insulin group had lower insulin resistance when compared with Diabetes + Insulin group (Table 3).

### 3.4. Immunohistochemical findings – hypothalamus

No change was observed in H-E stained hypothalamus sections (Fig. 1). When diabetic groups were compared, GnRH immunoreactivity of Diabetes + Noopept group was significantly higher than Diabetes + Noopept + Insulin group and it was close to Control group (Tables 4, 5, Fig. 2) ($p = 0.018$). When Kp immunoreactivity in hypothalamic arcuate nucleus was evaluated no statistically significant difference was determined (Tables 4, 5, Figs. 3, 4).

### 3.5. Assessment of FSH and LH Values

No statistically significant difference was determined when FSH ($p = 0.764$) and LH ($p = 0.200$) levels were evaluated among groups (Table 6).

### 3.6. Histological findings of the testis

Testicular tissue of healthy control groups were in normal appearance. In the Diabetes Control group testicular tissue; cell stability in mitotic phase, vacuolization/ edema in interstitial tissue, spilled cells in tubules, congestion and cell infiltration were determined. Being more in the noopept and insulin co-administered group, testicular changes in diabetic groups were reduced in all treatment groups (Fig. 5).

### 3.7. Measurement of seminiferous tubule diameter and germinal epithelial cell thickness

MSTD and GECT of each testis were determined in μm. Both MSTD and GECT values of treatment groups were different from diabetes control group. While values of monotherapy groups were similar among

**Table 2**
Blood glucose (mg/dl) values according to days (Mean ± Sd).

| Group/glucose (mg/dl) | 1. day<br>Mean ± Sd | 3. day<br>Mean ± Sd | 17. day<br>Mean ± Sd |
|---|---|---|---|
| Control | 85.60 ± 9.93 | 108.60 ± 23.82[a] | 83.40 ± 7.08[a] |
| Diabetes control | 101.60 ± 21.16 | 291.30 ± 101.29[b] | 244.60 ± 74.04[b] |
| Noopept control | 91.30 ± 11.66 | 101.20 ± 18.37[a] | 88.60 ± 10.34[a] |
| Diabetes + noopept | 83.40 ± 16.34 | 286.50 ± 138.46[b,x] | 188.30 ± 107.15[b,y] |
| Diabetes + insulin | 91.30 ± 14.08 | 265.20 ± 89.61[b] | 227.60 ± 122.68[b] |
| Diabetes + noopept + insulin | 92.60 ± 25.91 | 335.10 ± 138.54[b,x] | 205.50 ± 90.76[b] |

(a, b different from each other, [a,b] $p \leq 0.05$), (x,y different from each other, [x,y] $p \leq 0.05$).

themselves, insulin and noopept co-administered group had statistically significant difference with control group (Table 7).

### 3.8. Assessment of puberty

On the experiment termination day, 7 of the rats in Diabetes Control group and 2 of the rats in Diabetes + Insulin group had not entered puberty (Table 8). The rate of entering puberty in Diabetes Control group was statistically different from other groups ($p < 0.01$).

### 3.9. Assessment of NGF and BDNF among groups

There was no statistically significant difference among groups when NGF ($p = 0.170$) and BDNF ($p = 0.786$) levels were evaluated (Table 9).

### 3.10. Histological findings of the hippocampus

Neuronal degenerative changes and decrease in neuronal density were observed in the Diabetes Control group. Degenerated neurons were distinguished by contracted/enhanced acidophilic cytoplasms and dark picnotic nuclei. The number of degenerated neurons in Diabetes Control group was found to be higher when compared to Control group ($p = 0.003$). Among the diabetic groups, number of degenerated neurons was decreased in Diabetes + Noopept, Diabetes + Insulin and Diabetes + Noopept + Insulin groups. This decrease was statistically significant only in the Diabetes + Noopept group when compared with Diabetes Control group ($p = 0.015$) (Fig. 6) (Tables 10–11).

### 3.11. Evaluation of Morris water maze tests

There was no statistically significant difference when the groups' platform finding time was evaluated. Although there was no difference, the treatment administered diabetic groups had lower values than Diabetes Control group (Table 12).

No statistical difference was determined when; area swam until the platform was found, distance to the platform at the end of swimming, area swam in the platform quadrant (NW) and the distance to the platform were evaluated on the last day (Table 13) of MWM experiment.

## 4. Discussion

The primary treatment modality for blood glucose level regulation in T1DM is insulin replacement [31]. As a common childhood chronic disease, T1DM causes significant complications as neuropathy, nephropathy and retinopathy even in short term [6]. When compared with pre and postpubertal periods insulin resistance normally increases in puberty. Due to the increased insulin resistance, higher doses of insulin usage are required in T1DM replacement therapy in pubertal period [11]. This situation is one of the main reasons of unexpected weight gain in diabetes [37]. Also, over-stimulation of insulin and IGF-1 receptors in gonads causes increased steroid secretion [16] and this gives rise to complications as hyperandrogenism and policystic ovary syndrome (PCOS) [16,17]. Therefore, alternative protocols in T1DM treatment are in search [32,33].

In the research when blood glucose levels were evaluated, although there was no difference in Diabetes + Insulin group, blood glucose levels were lower in noopept treated diabetic groups. Not having a significant decrease in blood glucose levels in Diabetes + Insulin group of the research may be due to increasing insulin requirements in puberty [31]. The decrease in blood glucose levels of noopept administered diabetic groups is compatible with Ostrovskaya et al.'s study [24]. Also our result indicates noopept and insulin co-administration to be more effective than insulin mono-therapy in blood glucose regulation. This finding makes noopept a new candidate to be used with insulin replacement therapy which can decline daily insulin usage.

Being significantly higher in puberty, insulin resistance is observed averagely in 20% of T1DM patients [34–36]. Pubertal stage diabetics have been shown to be more insulin resistant in every stages of puberty and show pre-diabetic characteristics because of the insulin resistance [11]. Higher insulin resistance in Diabetes Control group of the study shows similarities with previous findings [34]. In the experiment, it has been determined by histological examination that STZ administration did not disturb all beta cells and there existed basal insulin production in diabetic groups (unpublished data). We think that; high insulin levels in diabetes control group are because of the increased insulin resistance and pre-diabetic characteristics of the pubertal stage. We determined that serum insulin levels; did not show difference among Diabetes Control and Diabetes + Insulin group but were lower in the Noopept + Insulin co-administered group when compared with Diabetes

**Table 3**
Evaluation of insulin level and insulin resistance (HOMA-IR) (Mean ± Sd/median (min-max)).

| Group/insulin | Insulin (IU/mL)<br>Mean ± Sd | Group/HOMA-IR | HOMA-IR | |
|---|---|---|---|---|
| | | Median | Minimum | Maximum |
| Control | 2.10 ± 0.53 | 0.42 | 0.01 | 1.13 |
| Diabetes control | 3.94 ± 0.75[a] | 2.23[c] | 0.11 | 4.71 |
| Noopept control | 2.61 ± 0.64 | 0.43 | 0.17 | 1.52 |
| Diabetes + noopept | 1.48 ± 0.40 | 0.36 | 0.02 | 1.45 |
| Diabetes + insulin | 3.40 ± 0.72 | 1.17[x] | 0.40 | 4.29 |
| Diabetes + noopept + insulin | 1.44 ± 0.38[b] | 0.49[y] | 0.10 | 2.22 |

(a, b different from each other [a,b] $p \leq 0.05$, c different from all others [c] $p \leq 0.05$, x,y different from each other [x,y] $p \leq 0.05$.)

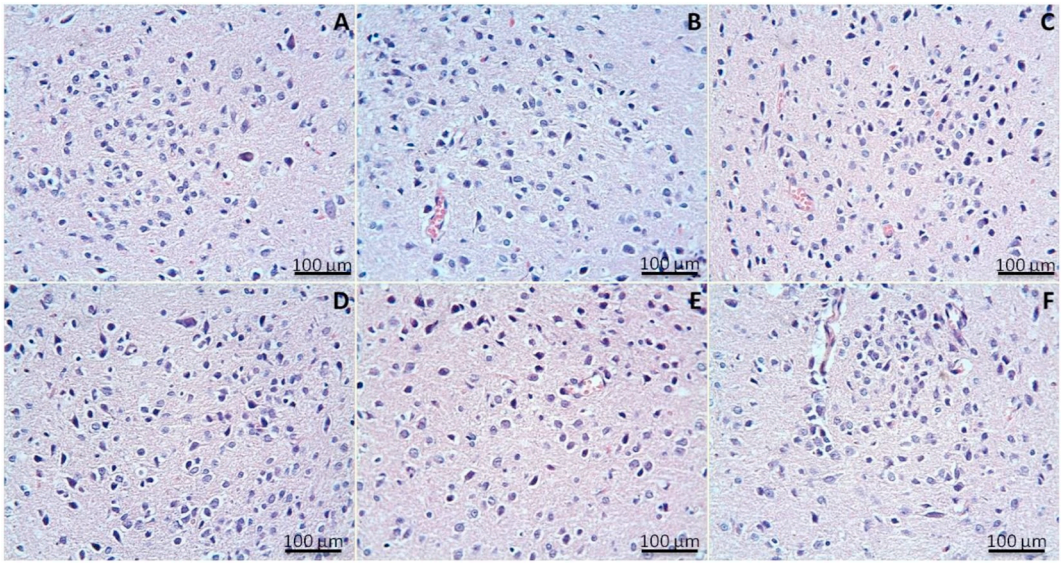


**Fig. 1.** Hypothalamus general appearance. A; Control, B; Diabetes Control, C; Noopept Control, D; Diabetes + Noopept, E; Diabetes + Insulin, F; Diabetes + Noopept + Insulin. (H-Ex40).

**Table 4**
GnRH and Kisspeptin immunoreactivity score results in hypothalamic neurons (Mean ± Sd/median (minimum-maximum)).

| Group | GnRH immunreactivity | | Kp immunreactivity | |
|---|---|---|---|---|
| | Mean ± Sd | Med (min-max) | Mean ± Sd | Med (min-max) |
| Control | 6.92 ± 3.17 | 6.0 (2.0–12.0) | 6.00 ± 3.29 | 6.0 (1.0–12.0) |
| Diabetes control | 5.8 ± 3.39 | 6.0 (1.0–12.0) | 5.00 ± 3.31 | 4.0 (1.0–12.0) |
| Noopept control | 6.92 ± 3.70 | 8.0 (1.0–12.0) | 5.40 ± 3.37 | 4.0 (1.0–12.0) |
| Diabetes + noopept | 6.52 ± 3.39 | 8.0 (1.0–12.0) | 5.43 ± 3.88 | 4.0 (0.0–12.0) |
| Diabetes + insulin | 5.93 ± 3.13 | 6.0 (1.0–12.0) | 5.40 ± 4.21 | 6.0 (0.0–12.0) |
| Diabetes + noopept + insulin | 4.90 ± 2.84 | 4.0 (1.0–12.0) | 5.00 ± 3.39 | 4.0 (0.0–12.0) |

**Table 5**
GnRH and Kp immunoreactivity comparement among groups (p values).

| Group | GnRH immunreactivity p | Kp immunreactivity p |
|---|---|---|
| Control & noopept control | 0.97 | 0.33 |
| Diabetes control & noopept control | 0.11 | 0.11 |
| Diabetes control & diabetes + noopept | 0.34 | 0.63 |
| Diabetes control & diabetes + insulin | 0.81 | 0.91 |
| Diabetes control & Diabetes + noopept + insulin | 0.19 | 0.95 |
| Diabetes + noopept & diabetes + insulin | 0.38 | 0.80 |
| Diabetes + noopept & Diabetes + noopept + insulin | 0.01* | 0.60 |
| Diabetes + insulin & diabetes + noopept + insulin | 0.08 | 0.86 |

* $p \leq 0.05$.

Control group. The insulin treatment regiment in the experiment was determined by a previous study [26], and it was constant during the whole experiment in order to determine noopept's effect in diabetic groups. We could not find any research about noopept's effect on these topics, but our findings suggest that noopept usage with insulin may be effective in regulating serum insulin levels and insulin resistance. Also, the probable regulating effect of noopept in blood glucose levels may be the reason of lower insulin levels and insulin resistance in noopept treated diabetic groups.

T1DM is normally characterized by weight loss. However, T1DM patients have increasing body mass index (BMI) tendencies especially after 'Diabetes Control and Complications Trial' in 1990's. Because of the vicious cycle between increased insulin resistance and high dose insulin usage in treatment, weight gain has been determined to be three times more with high-dose insulin usage in young people [37]. Also, increase in BMI itself without T1DM has been shown to cause diabetic symptoms in puberty [38]. Preventing weight gain in pubertal T1DM has become an important target according to these findings. Our results indicating lower weight gain in the prepubertal noopept treated diabetic groups, when compared to only insulin treated group, suggests noopept usage to be effective in weight regulation of T1DM.

Drugs as incretin based DPP-4 inhibitors and GLP- 1 analogues which are used in type 2 diabetes treatment are actively investigated about their effects on T1DM [14]. DPP-4 inhibitors and GLP- 1 analogues have been reported to have favorable effects on; blood glucose levels, insulin levels, insulin resistance, weight loss and/or weight gain prevention [14,39]. In two separate studies noopept has been shown to normalize incretin system parameters in diabetes model [20,25]. The obtained results suggest that -incretin effect shown- noopept can be a therapeutic that can be used orally with insulin. However, more

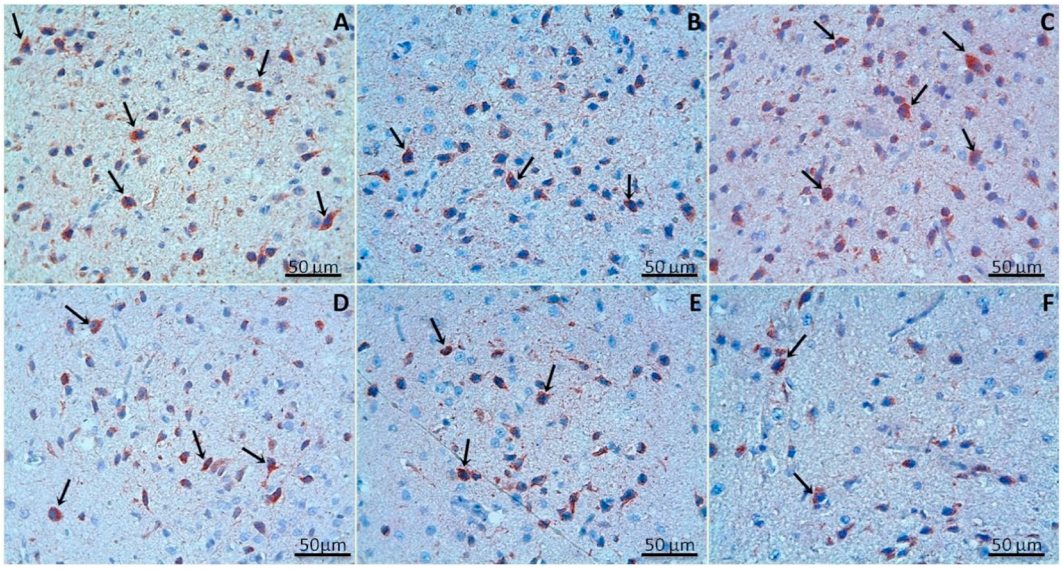


**Fig. 2.** Hypothalamic neurons showing GnRH immunoreactivity (arrows). A; Control, B; Diabetes Control, C; Noopept Control, D; Diabetes + Noopept, E; Diabetes + Insulin, F; Diabetes + Noopept + Insulin. (H-Ex40).

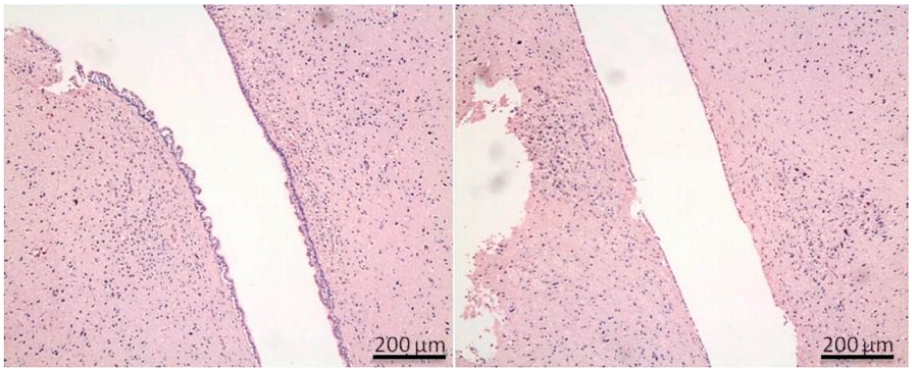


**Fig. 3.** Kisspeptin immunoreactivity in hypothalamic general appearance with 3rd ventricle (kisspeptin neurons stained dark in colour) (H-EX10).

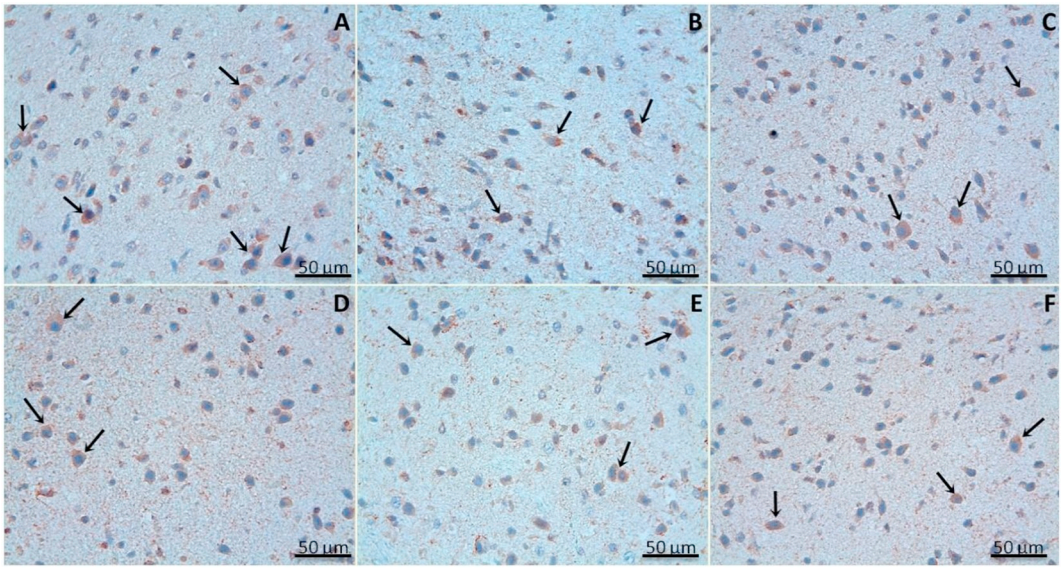


**Fig. 4.** Hypothalamic neurons showing kisspeptin immunoreactivity (arrows). A; Control, B; Diabetes Control, C; Noopept Control, D; Diabetes + Noopept, E; Diabetes + Insulin, F; Diabetes + Noopept + Insulin. (×40).

**Table 6**
Evaluation of FSH and LH values among groups.

| Group | FSH Median(mIU/ml) mean ± Sd | Min- Max | LH Median(mIU/ml) mean ± Sd | Min-Max |
|---|---|---|---|---|
| Control | 319.34 ± 48.56 | 252.99–382.38 | 1.71 ± 1.69 | 0.00–4.03 |
| Diabetes control | 298.60 ± 55.76 | 189.95–355.84 | 1.58 ± 1.47 | 0.00–3.82 |
| Noopept control | 317.02 ± 35.87 | 256.31–355.84 | 1.26 ± 1.24 | 0.00–3.48 |
| Diabetes + noopept | 309.72 ± 54.30 | 229.76–392.34 | 0.64 ± 0.71 | 0.00–1.68 |
| Diabetes + insulin | 286.90 ± 68.99 | 173.36–408.93 | 0.54 ± 0.82 | 0.00–1.89 |
| Diabetes + noopept + insulin | 315.69 ± 52.65 | 243.04–432.15 | 1.93 ± 1.44 | 0.00–3.73 |

$p > 0.05$.

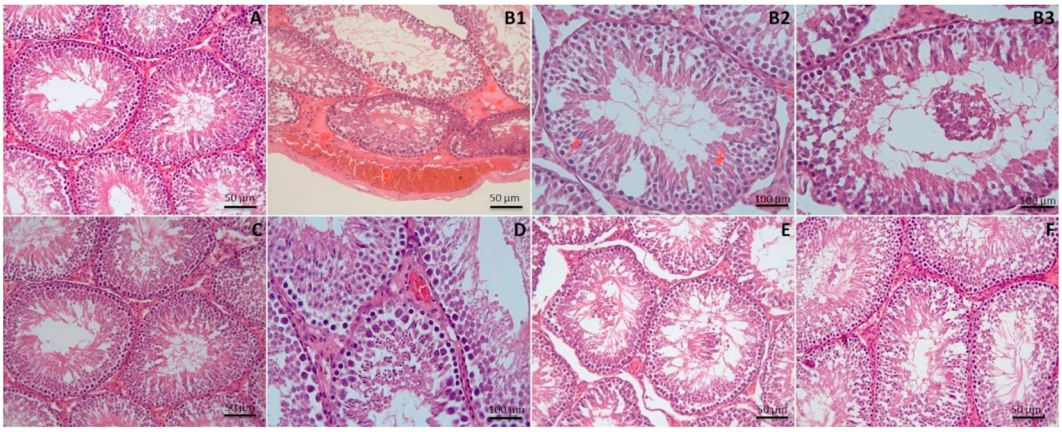


**Fig. 5.** Testis histology (H-E) A; Control (×20), B1; Diabetes Control (×20), B2; Diabetes Control (×40) cells in mitotic phase (arrows), B3; Diabetes Control (×40) spilled cells in tubules, C; Noopept Control (×20), D; Diabetes + Noopept (×20), E; Diabetes + Insulin (×20), F; Diabetes + Noopept + Insulin. (×20).

**Table 7**
The effect of diabetes, insulin and noopept on MSTD and GECT (Mean ± Sd).

| Group | MSTD (μm) | GECT (μm) |
|---|---|---|
| Control | 215.54 ± 1.69[a] | 60.86 ± 1.03[a] |
| Diabetes | 175.75 ± 2.29[b] | 35.80 ± 0.96[b] |
| Noopept | 208.35 ± 1.40[a] | 55.37 ± 1.13[a] |
| Diabetes + insulin | 184.11 ± 2.05[c] | 47.96 ± 0.65[c] |
| Diabetes + noopept | 192.52 ± 1.87[c] | 45.34 ± 0.73[c] |
| Diabetes + insulin + noopept | 204.46 ± 1.49[a] | 51.77 ± 0.82[a] |

The mean differences the values bearing different superscript letters within the same column are statistically significant ($p \leq 0.0001$).

**Table 8**
Evaluation of entrance time to puberty (Mean ± Sd).

| Group | mean ± Sd (day) | Puberty #/group # |
|---|---|---|
| Control | 43.80 ± 1.14 | 10/10 |
| Diabetes control | 44.00 ± 1.00 | 3/10* |
| Noopept control | 43.10 ± 0.88 | 10/10 |
| Diabetes + noopept | 43.30 ± 0.82 | 10/10 |
| Diabetes + insulin | 43.88 ± 0.83 | 8/10 |
| Diabetes + noopept + insulin | 45.50 ± 0.71 | 10/10 |

* $p < 0.01$

extensive work is needed to be done in this regard.

We did not find difference in immunohistochemical expression of Kp in hypothalamic arcuate nucleus. GLP-1 has been shown to increase Kp mRNA expression in fetal rat brain [40]. A recent study indicated that while Kp concentrations at nanomolar level suppress glucose-dependent insulin secretion, micromolar concentrations stimulate glucose-dependent insulin secretion [5]. It has been reported that type 2 diabetic patients' liver Kp expressions vary independent of treatment, and these patients have higher Kp production in liver with higher Kp concentrations in circulation [5]. These studies suggest that Kp-Kp receptor activity may have an effect on pubertal insulin resistance by creating incretin resistance in pancreatic beta cells [5]. At this point 'Kp antagonists' usage in diabetes treatment is questioned [8]. The fact that there is no difference in Kp levels in the study may basicly be interpreted as, the pubertal levels of Kp did not change in T1DM. But the defined mechanism causing Kp-insulin-incretin effect resistance may also be effecting Kp expression in T1DM. Increased insulin resistance in pubertal T1DM causes type 2 diabetic features in these patients. In this respect, we think that the diabetes model we used is suitable for pubertal period and Kp-insulin-incretin effect may have occurred in the pubertal period. At this point, while increase of Kp in the control and treatment groups may be due to the pubertal process, Kp elevation related to insulin resistance may be mentioned in the untreated diabetic group. This topic is an important subject to make researches on.

Insulin also plays an important role in GnRH secretion mechanism [41]. There was no statistically significant difference among groups, both in GnRH immunoreactivity and FSH- LH levels in the study. In the evaluation of diabetic groups, GnRH immunoreactivity was lower in insulin and noopept co-administered diabetic group when compared with insulin monotherapy group. Diabetic complications on the pituitary and testicular glands of prepubertal and postpubertal rats have been shown to take place in long time periods as numerous weeks and months [42]. Moreover, in a study about gonadal steroid levels, FSH ratios in pubertal T1DM boys were similar to those of healthy controls, while LH concentrations were found to be similar or low [43]. The lack of difference between FSH and LH levels in our study is similar with these results. Also we think that, low puberty entering ratios in Diabetes Control and Diabetes + Insulin groups in termination day may be an important factor affecting HPG hormone results.

Insulin therapy has been shown to increase gonadotropin and gonadal steroid levels in animal and human studies [44,45]. We determined that GnRH immunoreactivity was higher in

**Table 9**
Evaluation of NGF and BDNF values among groups.

| Group | NGF mean ± Sd (pg/ml) | Min-Max | BDNF mean ± Sd (pg/ml) | Min-Max |
|---|---|---|---|---|
| Control | 0.34 ± 0.22 | −0.01–0.71 | 2.80 ± 1.60 | 0.64–6.41 |
| Diabetes control | 0.34 ± 0.18 | 0.11–0.65 | 3.39 ± 1.89 | 0.77–7.50 |
| Noopept control | 0.25 ± 0.21 | −0.07–0.65 | 1.87 ± 1.23 | 0.12–3.70 |
| Diabetes + noopept | 0.28 ± 0.18 | 0.03–0.59 | 2.21 ± 2.12 | 0.03–6.66 |
| Diabetes + insulin | 0.26 ± 0.22 | 0.00–0.63 | 1.72 ± 1.64 | 0.18–4.63 |
| Diabetes + noopept + insulin | 0.27 ± 0.21 | 0.01–0.71 | 2.07 ± 1.18 | 0.19–3.86 |

$p > 0.05$.

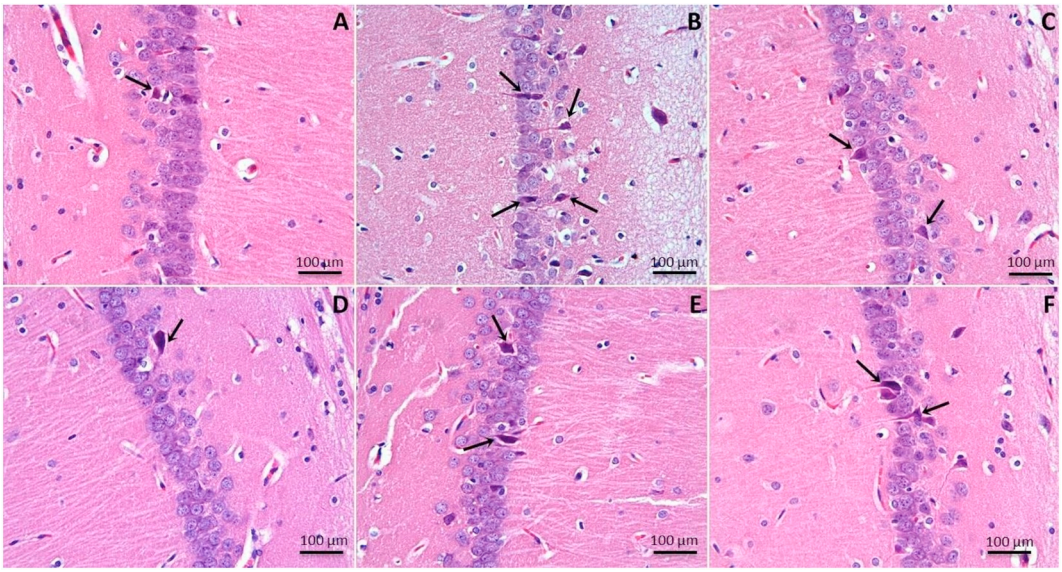


**Fig. 6.** The appearance of the CA1 region of the hippocampus. A; Control, B; Diabetes Control, C; Noopept Control, D; Diabetes + Noopept, E; Diabetes + Insulin ve F; Diabetes + Noopept + Insulin. Neurons in the pyramidal layer generally have normal morphological characteristictic in control group. Degenerated neuron number is increased in pyramidal layer of Diabetes Control group. Number of degenerated neurons in the pyramidal layer of Noopept, Diabetes + Noopept, Diabetes + Insulin and Diabetes + Noopept + Insulin groups are decreased (arrows point to degenerated neurons). H-E ×40.

**Table 10**
Number of degenerated neurons in hippocampus (Mean ± Sd).

| Group | Degenerated cell number Mean ± Sd |
|---|---|
| Control | 4.15 ± 3.46 |
| Diabetes control | 13.94 ± 9.00* |
| Noopept control | 8.93 ± 4.01 |
| Diabetes + noopept | 6.09 ± 2.75 |
| Diabetes + insulin | 9.74 ± 5.81 |
| Diabetes + noopept + insulin | 10.08 ± 7.77 |

* $p = 0.03$.

**Table 11**
Cross-group comparison of number of degenerated neurons (*p* values).

| Compared groups | P |
|---|---|
| Control & noopept control | 0.126 |
| Diabetes control & control* | 0.003 |
| Diabetes control & diabetes + noopept* | 0.015 |
| Diabetes control & diabetes + insulin | 0.788 |
| Diabetes control & diabetes + noopept + insulin | 0.905 |
| Diabetes + noopept & diabetes + insulin | 0.488 |
| Diabetes + noopept & diabetes + noopept + insulin | 0.477 |
| Diabetes + insulin & diabetes + noopept + insulin | 1.000 |

* $p \leq 0.05$.

**Table 12**
Evaluation of groups' platform finding time (*sec*) (Mean ± Sd).

| Day | 1. day* (13. day of experiment) Mean ± Sd | 4. day* (16. day of experiment) Mean ± Sd |
|---|---|---|
| Control | 65.57 ± 13.63 | 25.99 ± 14.12 |
| Diabetes control | 68.38 ± 19.76 | 34.06 ± 15.03 |
| Noopept control | 73.23 ± 15.87 | 19.57 ± 8.17 |
| Diabetes + noopept | 73.22 ± 19.32 | 21.65 ± 6.16 |
| Diabetes + insulin | 78.57 ± 15.74 | 23.15 ± 15.44 |
| Diabetes + noopept + insulin | 76.64 ± 15.25 | 23.27 ± 7.46 |

* $p \leq 0.05$.

**Table 13**
Area swam in the platform quadrant in the fifth day ($m^2$) (Mean ± Sd).

| Group | Mean ± Sd |
|---|---|
| Control | 17.09 ± 5.30 |
| Diabetes control | 9.63 ± 5.43 |
| Noopept control | 14.91 ± 6.29 |
| Diabetes + noopept | 13.90 ± 6.19 |
| Diabetes + insulin | 12.68 ± 6.78 |
| Diabetes + noopept + insulin | 13.69 ± 5.30 |

$p > 0.05$.

Diabetes + Noopept group when compared to noopept and insulin co-administered diabetic group. This conclusion suggests that noopept may provide positive effects on HPG axis. Our results show similarity with the conclusion that incretin-based substances may be effective in gonadal steroid disorders such as PCOS [46].

Diabetes has negative effects on reproductive system. We determined widespread degeneration in histological examination of testicular tissue of diabetes control group, similar to former researches [45,47]. Besides, while noopept and insulin monotherapy groups' histological testicular tissue evaluation showed statistically significant difference when compared with diabetes control group, noopept and insulin co-administered group was similar with normal control groups. Determining noopept's preventive effect on testicular tissue is a supportive outcome in noopept's usage in T1DM.

Our results are similar with studies in which puberty is delayed despite insulin therapy [36,48]. In the study, all groups had statistically significant difference in pubertal entrance when compared to diabetes control group. Although there was no difference among the groups in Kp- GnRH immunohistochemical studies and in FSH- LH findings, noopept's regulatory effect in the pubertal process may be suggesting that noopept may have different effecting mechanisms on puberty. In this respect, it will be important to investigate and compare the efficacy of noopept with metformin, GLP- 1 agonists and other therapeutic agents researched in T1DM treatment.

In the STZ-induced diabetes model, proapoptotic transcriptional regulators have been shown to be significantly activated within one week and remain elevated to twelve weeks [49]. Our finding of increased number of hippocampal degenerative cells in diabetes is similar with former studies [49,50]. The neuroprotective effects of incretin-based substances suggest that they may be effective in cognitive disorders of T1DM [51]. We determined that while there was no effect of insulin, noopept had positive effects in prevention of neuronal degeneration. This situation highlights noopept in the prophylaxis and treatment of diabetic neuropathy.

While Ostrovskaya et al. determined increases in hippocampal NGF and BDNF levels with 28-day noopept usage after diabetes induction; we did not find difference [23]. Also, there was no statistically significant difference in MWM tests. Studies in diabetic adult rat brain have shown decreases in NGF [52] and BDNF [53] levels due to ischemia and oxidative stress. In Zhao et al.'s study, it was shown that while NGF precursors increased in DM, mature NGF proteins decreased [54]. Although MWM results are inconsistent with Alzheimer research [22], findings suggest that noopept usage may have positive effects on pubertal cognitive functions. Analyzing the former studies, we decided that while the duration of diabetes for two weeks is adequate for the onset of neuronal degeneration, it is inadequate in terms of NGF-BDNF level changes and cognitive impairment process. By this regard more detailed studies shall be necessary in determination of noopept's effect on NGF- BDNF levels and cognitive functions.

## 5. Conclusion

In summary our results indicate that noopept has positive effects in the management of pubertal T1DM. Further studies about noopept may be useful, in modulating treatment of pubertal T1DM and in prevention of chronic complications. It would be valuable to investigate the mechanism of cellular effects of noopept and make comparative studies with other anti-diabetic drugs. Noopept has been shown to have anti-diabetic effects, both in pubertal timing and testicular tissue. Studying the efficacy of the noopept's effects on endocrine and energy mechanism will help to understand its effects on HPG axes. Studies on noopept's incretin activity will provide insight to their effects on glucose metabolism. Researches on NGF and BDNF proteins with improved molecular mechanisms at receptor and mRNA levels will elucidate the efficacy of noopept on neurodegeneration and cognitive functions.

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Perihan Gurbuz, Halil Duzova, Cemile Ceren Gül, Asli Cetin Taslidere. Effects of noopept on glucose, insulin, insulin resistance and pancreas of streptozotocin-induced diabetic prepubertal rats. Europhysiology 2018. The QEII Centre, London, UK. 14-16. 09. 2018.

## Statement of ethics

This study was carried out with approval of Inonu University Animal Ethics Committee (2015/ A- 62). The authors have no ethical conflicts to disclose.

## Declaration of Conflict of Interest

The authors have no conflicts of interest to declare.

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## Author contributions

Dr. Gurbuz reviewed the current literature, did animal experiments, drafted the initial manuscript and approved the final manuscript as submitted. Gul Busra Kaya helped in animal experiments. Azibe Yildiz performed histological and immunohistochemical researches about hypothalamus and hippocampus. Pınar Cakan performed ELISA tests. Merve Durhan helped in Morris test. Cemile Ceren Gul and Aslı Cetin Taslıdere did histological analysis of testis. Aslı Cetin Taslıdere performed statistical analysis of testicular research. Harika Gozde Gozukara Bag performed statistical analysis of all other researches except testis. Dr. Duzova conceptualized this perspective piece, reviewed and revised the manuscript, and approved the final manuscript as submitted.

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