CIRCULATING APELIN LEVELS AND METABOLIC PARAMETERS IN RESPONSE TO EXERCISE IN NORMAL AND DIABETIC RATS

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ABSTRACT

Background: Apelin is a peptide hormone recently identified as an endogenous ligand for the G_i protein-coupled, receptor named APJ. Apelin and APJ have a widespread distribution in the body; apelin is considered a beneficial adipokine related to glucose and lipid metabolism. Exercise was proved to increase apelin expression in cardiac myocytes, but its effect on circulating apelin levels is not known up to the date.

Aim: This work was done to explore the effect of continuous and intermittent exercise on circulating apelin levels and some metabolic parameters in normal and Streptozotocine induced diabetic rats. **Design**: A total number 60 adult, healthy, male albino rats. The rats were divided into 2 main groups **Group I lean group:**(n= 24) it was further subdivided into 3equal subgroups (n= 8): **Group I A:** lean control group. **Group I B:** Rats were exposed to continuous exercise**. Group I C:** Rats were exposed to intermittent exercise. **Group II Streptozotocine- induced (STZ) diabetic group:** 36 rats were used. After induction of diabetes, it was further subdivided into 3equal subgroups (n= 12): **Group III A**: control STZ diabetic group. **Group III B:** Rats were exposed to continuous exercise **Group III C:** Rats were exposed to intermittent exercise. The animals were maintained for 4 weeks and then blood samples were collected for measurement of: Serum apelin, insulin, glucose, total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), high density lipoproteins (HDL); body mass index (BMI) and HOMA-IR were calculated. **Results:** STZ induced diabetic control group showed significantly lower serum apelin ($p < 0.001$) serum insulin ($p < 0.001$), calculated HOMA-IR ($p < 0.01$) and serum HDL ($p < 0.001$) with serum glucose, total cholesterol, triglycerides and LDL levels significantly higher($p < 0.001$) than control lean group. In lean groups exercise caused significant increase in serum apelin and HDL levels, with significant decrease ($P < 0.05$) in serum LDL, calculated HOMA-IR (intermittent) and BMI (intermittent). In STZ induced diabetic groups exercise caused significant ($P< 0.05$) elevation of serum apelin, insulin, HOMA-IR and HDL with significant decrease ($P< 0.05$) in total cholesterol, triglycerides and LDL. **Conclusion:** STZ induced diabetes results in decrease serum apelin levels and disturbed glucolipid metabolism, but exercise was able to elevate serum apelin levels nearly toward normal values, also it was able to correct partially the disturbed glucolipid metabolism and the effect of intermittent pattern was more profound than that of continuous pattern. Further studies in both animals and humans are needed to explore the related mechanisms.

Keywords: Apelin; exercise; STZ diabetes; adipokines.

INTRODUCTION

pelin is a novel bioactive peptide originally Asecreted from white adipose tissues and many other tissues as stomach **(1).** It is isolated as the endogenous ligand of the orphan G protein- coupled receptor (APJ) **(2)**. Apelin is a multifunction neuropeptide that regulates body fluid homeostasis, food intake, cardiovascular system, respiratory and biological rhythm **(3).** The association between apelin levels and glucose concentrations and insulin sensitivity provides evidence that apelin may play a role in the pathogenesis of diabetes **(4).** Also, acute injection of apelin has a powerful glucose-lowering effect **(5)**.

It is documented that physical exercise promotes beneficial effects in lipid profile **(6).** Reports on the effect of exercise on the glucose-related metabolic parameters in type 1 diabetes remain controversial, especially in animal models of diabetes **(7, 8).** Moreover, the effect of exercise on increasing the apelin/APJ system in cardiovascular tissue

was proved by **Zhang et al. (9).** However, the effect of exercise on the circulating apelin levels not studied yet. So, this study was designed to explore the effect of exercise training on circulating apelin levels and some metabolic parameters in normal and STZ- induced diabetic rats.

MATERIALS AND METHODS

Animals:

This study was carried out on a total number of 60adult (body weight, 180-200 gm) healthy male albino rats. Animals were kept under hygienic conditions in steel wire cages in the animal house of the faculty of medicine Zagazig University. All rats had free access to water and chow. Rats were kept at comfortable temperature (20 to 24 ºC) and were maintained on a 12 hr light/dark cycle **(10).** The rats were accommodated to laboratory conditions for three weeks before starting the experimental regimen **(11).** The rats were divided into2 main groups:

Group I (lean group) :(n= 24): rats had free access to standard chow. It was further subdivided into 3equal subgroups (n= 8): **Group II A**: control group with sedentary life. **Group II B:** Rats were exposed to continuous exercise**.** Continuous protocol consisted of swimming, at 28–32 Cº, one hour/day, and five days/week for four weeks **(12).** The animals of the continuous group swam for15, 30, and45 min on the first, second, and third days to adapt. The swimming period was then increased to 60 min/day **(13). Group II C:** Rats were exposed to intermittent exercise**.** Intermittent protocol consisted of three sessions for 10, 20, and 30 min on the first, second, and third days to adapt. The swimming period was 60 min/day, divided into three daily sessions (3 × 20 min/ day) with 4 h of controlled intervals between the exercise periods throughout the experimental period. The rats were kept in their home cages all the time between the exercise periods, performed at 7 am, 11:30 am, and 3:30 pm. The exercise protocols were performed for 5 days/week for four weeks (**13)**. **Group II (STZ induced diabetic) group:** 36 rats were used; animals had free access to standard chow. After induction of diabetes by single intra-peritoneal injection of freshly prepared solution of Streptozotocine 65 mg/kg (Sigma Chemical, St. Louis, MO, Sigma-Aldrich, U.S.A.) of body weight dissolved in 0.2 mmol/L sodium citrate (ADWIC Laboratory Chemicals, Egypt.), at PH 4.5 **(14)**. Three days later, diabetes induction was confirmed by measurement of blood glucose level in each animal (from blood sampled from the tail vein) with the One Touch Ultra Glucometer **(15)** and rats with blood glucose levels more than 250 mg/dl were selected for experiments **(8)**, it was then further subdivided into 3equal subgroups (n= 12):**Group II A**: control group with sedentary life, 5 rats died the remaining were 7rats at the end of 4 weeks (death rate was 41.66%).**Group II B:** Rats were exposed to continuous exercise (as in group I B) 3 rats died the remaining were 9 rats at the end of 4 weeks (death rate was 25%)**.Group II C:** rats were exposed to intermittent exercise (as in group I C) 3 rats died the remaining were 9 rats at the end of 4 weeks (death rate was 25%)**.**

Serum analysis:

At the end of the experimental period (at the end of 4th week) after overnight fasting, at 8:00 a.m, blood samples were obtained from sinus orbitus vein of each rat after ether (Merck, Darmstadt, Germany) inhalation **(16).**The blood samples were allowed to clot at room temperature before centrifuging at approximately 3000 rpm for 15 minutes. The serum was stored at -20° C until used for:

1-Estimation of serum apelin-12 levels: According to the methods of **Porstmann & Kiessig, (17)** Apelin 12 Kits, PHOENIX

PHARMACEUTICALS, INC. 330 Beach Rd Burlingame, California USA.

2-Estimation of serum insulin level: According to the methods of **(18)**. KAP1251-INS-EASIA (Rat) (Enzyme Amplified Sensitivity Immunoassay) Kits (BioSource Europe S.A., Belgium).

3-Estimation of fasting serum glucose: According to the methods of (**19)**. Glucose enzymatic **(**GOD-PAP**) -**liquizyme Kits (Rat) (Biotechnology, Egypt).

4-Homeostasis model assessment (HOMA) index: It was calculated to estimate insulin resistance from the equation:

HOMA= fasting serum insulin (μIU/mL) x [fasting serum glucose (mmol/L)/22.5] **(20).**

5- Determination of serum cholesterol levels: According to the methods of (**19)**. Cholesterol RTU 61218 (Rat) kits: (bioMerieux S.A., Lyon, France).

6-Determination of serum Triglycerides: According to the methods of **(21).** Triglycerides ESPAS SL (Rat) kits (Elttech S.A., Sees, France.)

7- Determination of serum) HDL-cholesterol: According to the methods of **(22).** Stanbio HDL-cholesterol procedure No. 0599 (Rat) kits (Stanbio laboratory Inc., San Antonio, Texas, USA)

8- Determination of serum low density lipoprotein cholesterol (LDL): According to **Friedwald et al. (23)**, LDL was calculated as follows: **LDL=TC-HDL-TG\5**.

Statistical analysis:

Data were expressed as mean ± SD, and statistically analyzed by One-way analysis of variance (ANOVA) followed by LSD test using SPSS for widows version 11.5. Differences were considered to be significant at $P < 0.05$.

RESULTS

Figure (1): serum apelin level in all studied groups, apelin was significantly lower ($P < 0.001$) in control STZ- diabetic than lean control. It was significantly increased ($P < 0.001$) in continuous and intermittent exercise in lean and STZ- diabetic compared to their control group, with intermittent lean and STZ- diabetic exercising groups apelin was significantly increased ($P < 0.001$, $P < 0.01$) than continuous lean and STZ diabetic exercising animals respectively.

Table (1): The studied parameters in all groups expressed in mean ± SD, in both figure and table:

***Significant versus group I A.**

 # Significant versus subgroup A in the same group.

$ Significant versus subgroup B in the same group.

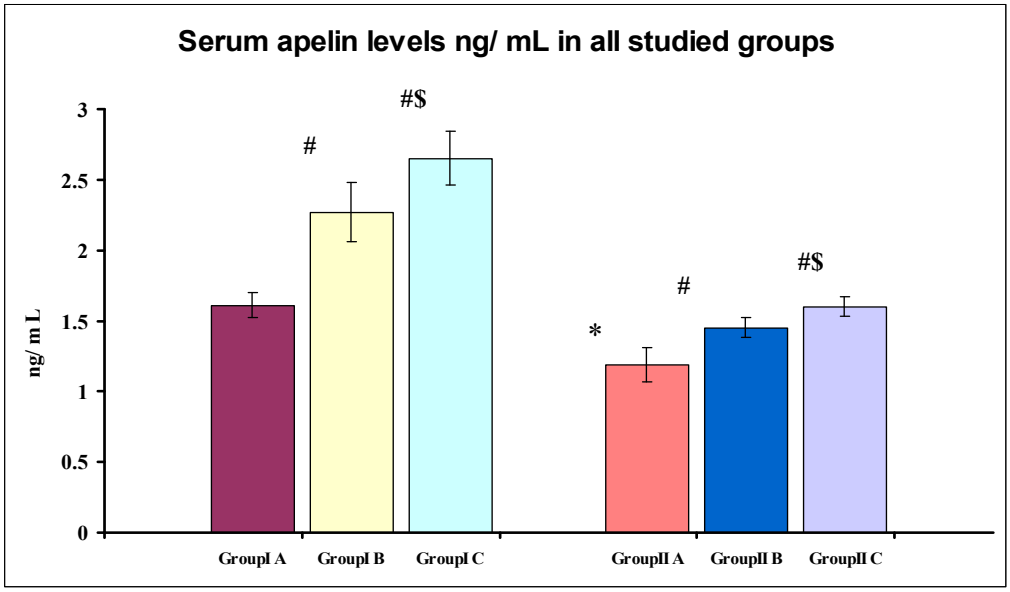

Table (1): The studied parameters in all groups

	Group I (Lean)			Group II (STZ- Induced diabetes)		
	I A	**I B**	**I C**	**II A**	**II B**	**II C**
Insulin (µU/mL)	19.5±3	18.78±1.7	18.4±2.2	1.4±.5*	1.8±.6	2.3±.6#
Glucose (mg/dL)	82.1±17	75.7±10.3	77.3±9.9	439.7±115*	418.8±84	356±75
HOMA-IR	3.9±0.2	3.6±0.17#	3. 5±0.7#	1.4±0.4*	1.8±0.3	1.9±0.3#
CT (mg/dL)	103.5±8	100.4±9	96±6	268.6±27*	213.1±27#	194±11#
TG (mg/dL)	57.1±7	52.8±4	51.5±3	86.1±8*	75.3±6#	62.1±5#$
HDL (mg/dL)	55.1±6	62.8±4#	61.3±6#	41.1±4*	53.3±8#	58.8±5#
LDL (mg/dL)	37.2±12	26.9±6#	24.6±9#	212.6±33*	146.5±34#	123.5±15#
BMI (gm/cm^2)	0.58±0.04	0.56±0.05	0.52±0.04#	0.49±0.04	0.50±0.04	0.53±0.05

DISCUSSION

In the present study, serum apelin levels in STZ induced diabetic control group, were significantly lower than that of lean control group. It was reported by **Zou and Shao (24)** that, insulin-deficient mice (Streptozotocine-treated) had low apelin mRNA levels in adipose tissue and **Castan-laurell et al. (25)** found that adipocytes of insulin-deficient mice (Streptozotocine-treated) had lower apelin mRNA levels than controls and smaller fat cells; they considered fat cell size a parameter affecting apelin mRNA expression in adipocyte. This

hypothesis was strengthened by the finding in obese hyperinsulinemic mice which exhibit hypertrophied adipocytes and increased plasma apelin levels **(26)** and this may explain our finding that, no significant change in calculated BMI in STZ induced diabetic control group in comparison to lean control group.

Concerning glucose metabolic parameters, in STZ induced diabetic control group serum glucose levels were significantly higher but serum insulin and calculated HOMA-IR levels were significantly lower in comparison to that of lean control group. This is supported by **Valentovic et al. (27)** and **Iizuka et al. (28)**.These changes in glucose, insulin and consequently HOMA-IR are related to the destruction of pancreatic β by STZ.

Our results showed that, serum cholesterol, triglycerides and LDL-cholesterol levels in STZ induced diabetic control groups were significantly higher than that of lean control group with significant decrease in serum HDL-cholesterol levels, in agreement with **Baydas et al. (29)** and **Ahmed et al. (30).** Quantitative lipid abnormalities in STZ diabetic rats are due to insulin deficiency, since insulin plays a central role in the regulation of lipid metabolism **(31).** Also, the dyslipidemic lipid profile particularly high LDL was associated with low serum apelin level in non-obese and healthy patients **(32)**. This may be also another factor involved in low serum apelin in STZ diabetic rats.

Our study revealed that continuous and intermittent exercises significantly increase serum apelin levels in lean and STZ induced diabetic groups and intermittent exercise was significantly more effective than continuous exercise in increasing serum apelin levels. In accordance to **Zhang et al. (9)** who found that exercise training significantly increased the expression of apelin and APJ in the spontaneously hypertensive rats myocardial and vascular tissues and increased the apelin content in plasma also, **David and Lindsey (33)** found that exercise increases apelin expression in white adipose tissue.

Moreover, serum insulin levels were significantly increased in intermittent exercise of STZ induced diabetic group compared to its control group, this in line with the study of **Coskun et al. (8)** who reported that exercise has protective and / or therapeutic effect in diabetes by decreasing the oxidative stress and preserving pancreatic β cells integrity. The increased insulin levels may be effective in increasing apelin levels in these groups, since **Boucher et al. (34)** proved a direct positive action of insulin on adipocyte apelin production both in vivo in mice as well as in vitro in both human and murine adipocytes.

Our study showed that, serum cholesterol, triglycerides and LDL were significantly decreased in continuous and intermittent exercises of STZ induced diabetic groups in comparison to its control group, HDL-cholesterol levels were significantly increased in continuous and intermittent exercises of all groups this is supported by the study of **Russell and Amy, (35)** and **Pels et al. (36)** respectively. The improvement in lipid profile particularly in LDL may be of benefit in elevation of serum apelin in exercise groups as reported by **Tasci et al. (32)**, that LDL lowering result in elevation of serum apelin in healthy people with isolated dyslipidemia.

Collectively, the present study proved that continuous and intermittent exercises resulted in a significant rise in serum apelin levels in both lean and STZ induced diabetic rats. This effect may be attributed to direct effect of exercise on apelin secretion, or indirect through changes in metabolic parameters since, continuous and intermittent exercises resulted in a significant improvement in metabolic

parameters in lean and STZ induced diabetic rats **(37, 38)** and intermittent exercise was more efficient than continuous exercise in reducing the adverse effects of sedentary life. This is in line with **Sene-Fiorese et al. (13)** who stated that intermittent exercise is more efficient in comparison to continuous exercise to have health beneficial effects as improvement in lipid profile (by increase in lipoprotein lipase activity) and reduced food intake, body mass gain, visceral and central adiposity.

In addition, the raised apelin level in exercise groups may help in correction of the disturbed metabolic derangement especially in STZ diabetic rats as apelin exerts direct metabolic effects in human and mouse adipocytes via APJ receptor activation, which appears to reinforce insulin action **(39)**. Also, apelin has a powerful glucose-lowering effect associated with enhanced glucose utilization in skeletal muscle and adipose tissues **(5)**.

Beside the direct and indirect metabolic effects, exercise can regulate circulating apelin levels through other possible mechanisms. Such as increasing PGC-1α (transcription factor), which up regulates the expression and secretion of apelin in human white adipocytes **(40, 41)**.Also, the expression and secretion of IL6 from skeletal muscle has been shown to increase dramatically during exercise **(42).** Furthermore, **Han et al. (43)** found that IL6 increases enteric apelin mRNA levels, which are mediated by the Jak/Stat signaling pathway and exercise training in normal rats increases TNFα in adipose tissue **(44)**, in turn TNFα increases apelin expression in both human and mouse adipocytes and increases apelin plasma levels in mice **(39)**.

It could be concluded that, low serum apelin levels in type I diabetes are related to decreased fat cell mass and disturbed glucolipid metabolism. Exercise particularly intermittent was able to restore apelin levels either directly or through improvement of metabolic parameters, further studies in animals and humans are needed to explore the detailed mechanisms.

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