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Relationship of Decreased Circulating Apelin Levels with Growth Hormone, Insulin-like Growth Factor, Carotid Intima-media Thickness, and Epicardial Fat Thickness in Acromegaly

Akromegalide Dolaşımdaki Apelin Düzeylerinin Büyüme Hormonu, İnsülin Benzeri Büyüme Faktörü, Karotis İntima Media Kalınlığı ve Epikardiyal Yağ Kalınlığı ile İlişkisi

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Abstract

Objective: Acromegaly is a rare disorder that results from an overproduction of growth hormone. Patients with acromegaly are highly susceptible to the risk of vascular diseases and metabolic dysfunctions. Apelin, an adipose tissue-derived peptide, is related to insulin resistance, metabolic and cardiovascular disorders. This study aimed to investigate apelin level alterations in subjects with active acromegaly, controlled acromegaly and in subjects without acromegaly, employed as controls, and to identify the presence of any possible relationship between apelin and hormonal or cardio-metabolic parameters.

Material and Methods: A total of 42 active and 32 controlled acromegalic patients, and 40 age- and body mass index-matched controls were recruited in this cross-sectional study. The hormonal and metabolic characteristics of the subjects as well as the carotid intima-media thickness and epicardial fat thickness were thoroughly evaluated. Apelin levels were also measured using ELISA methods.

Results: Circulating levels of apelin were found to be significantly lower in the acromegalic subjects as compared to that in the controls. Moreover, active acromegalic subjects showed decreased apelin levels as compared to the controlled acromegalic subjects. Apelin demonstrated a negative correlation with insulin resistance, carotid intima-media thickness, epicardial fat thickness, body mass index as well as growth hormone and insulin-like growth factor-1 in subjects with acromegaly. Furthermore, an independent negative correlation was found between apelin levels and carotid intima-media thickness & epicardial fat thickness by the multivariate regression analysis.

Conclusion: Decrease in circulating levels of apelin is associated with cardiovascular risk markers like carotid intima-media thickness and epicardial fat thickness and also has a negative correlation with growth hormone and insulin-like growth factor-1 in subjects with acromegaly.

Keywords: Acromegaly; apelin; carotid intima-media thickness; epicardial fat thickness; growth hormone; insulin-like growth hormone-1; insulin resistance

Özet

Amaç: Akromegali, nadir görülen bir hastalıktır, aşırı büyüme hormonu üretiminden kaynaklanmaktadır. Akromegali hastaları vasküler hastalık ve metabolik disfonksiyon riski altında olma eğilimindedir. Adipoz dokudan türetilmiş bir peptit olan apelin, insülin direnci, metabolik ve kardiyovasküler bozukluklarla ilişkilidir. Bu çalışmanın amacı aktif akromegali, kontrollü akromegalisi olan bireylerde ve akromegali olmayan bireylerde apelin düzeylerinin değişimini araştırmak ve apelin ile hormonal, kardiyo-metabolik parametreler arasında olası bir ilişkinin varlığını saptamaktır.

Gereç ve Yöntemler: Bu kesitsel çalışmaya 42 aktif ve 32 kontrollü akromegalili ve 40 yaş ve beden kitle indeksi uyumlu akromegali olmayan kontrol grubu alındı. Olguların karotis intima media kalınlığı ve epikardiyal yağ kalınlığı ile birlikte hormonal ve metabolik özelliklerinin değerlendirilmesi yapıldı. Apelin seviyeleri, ELISA yöntemleri kullanılarak ölçüldü.

Bulgular: Dolaşımdaki apelin seviyeleri akromegalisi olanlarda kontrollere göre anlamlı olarak düşüktü. Ayrıca, aktif akromegali olanlarda, kontrollü akromegal hastalara göre apelin seviyelerinde bir azalma olduğunu gösterdi. Apelin, akromegali hastalarında insülin direnci, karotis intima media kalınlığı, epikardiyal yağ kalınlığı ve beden kitle indeksi yanı sıra büyüme hormonu ve insülin benzeri büyüme faktörü-1 ile negatif korelasyon gözlendi. Daha sonra, multivarite regresyon analizlerinde, apelin seviyelerinin bağımsız olarak karotis intima media kalınlığı ve epikardiyal yağ kalınlığı ile negatif ilişki içinde olduğu saptandı.

Sonuç: Dolaşımdaki azalmış apelin düzeyleri, karotis intima media kalınlığı ve epikardiyal yağ kalınlığı gibi kardiyovasküler risk belirteçleri ile ilişkilidir ve aynı zamanda akromegali hastalarında büyüme hormonu ve insülin benzeri büyüme faktörü-1 ile de negatif ilişkilidir.

Anahtar kelimeler: Akromegali; apelin; karotis intima media kalınlığı; epikardiyal yağ kalınlığı; büyüme hormonu; insülin benzeri büyüme hormonu-1; insülin direnci

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Introduction

Apelin is an adipocyte-derived peptide hormone, secreted from various sites including adipose tissue, lungs, kidneys, brain, and the heart. Apelin exerts its action by binding to its receptor, APJ (apelin) receptor, which is a G protein-coupled receptor. Apelin, a multifunctional molecule, participates in various pathological and physiological processes including atherosclerosis, glucose metabolism and obesity (1-4). It has been reported that apelin has the ability to decrease insulin resistance in mice by inducing glucose consumption as well as by restoring glucose tolerance (5). The literature reports studies with conflicting results on altered levels of apelin in metabolic disorders as well as the relationship between apelin and insulin resistance (6-8). The results obtained from a clinical study suggest that the circulating levels of apelin are found to be decreased in newly diagnosed and untreated patients with type 2 diabetes (T2DM) (6). On the other hand, increased apelin levels have been observed in subjects with T2DM and impaired glucose tolerance as compared to that in subjects with normal glucose tolerance (7). Another study demonstrates that the expression of apelin is up-regulated with insulin resistance and exhibits diabetes mellitus (8). Additionally, it has been reported that apelin either possesses a protective role against atherosclerosis or, has an inducing activity in the development of atherosclerosis (9-12). However, this particular area of investigation on apelin levels and metabolic disorders has not yet been completely verified and many hypotheses referring to this issue appear debatable.

Acromegaly, an uncommon chronic disease, is caused by the excessive secretion of GH which is commonly due to a pituitary adenoma. Accordingly, overproduction of GH results in the induction of insulin-like growth factor (IGF-1), which is secreted by the liver. The mechanism of action of this disorder may cause many clinical signs like increased atherosclerosis, insulin resistance and metabolic disturbances, including glucose intolerance. Acromegaly has been proposed to be related to increased premature mortality and morbidity due to cardiovascular diseases (13-15). Moreover, it has been recognized that the amount of adipose tissue in acromegalic subjects is decreased due to the lipolytic effect of GH. Acromegalic patients have also demonstrated an alteration in adiponectin-derived peptides such as adiponectin and leptin (16,17). Although conflicting results on the relationship of apelin in metabolic diseases are known, there is only little evidence regarding apelin levels in acromegalic subjects. In a small sample-sized population study, apelin levels were found to be elevated in both active and controlled acromegalic subjects, when correlated with GH (18). In the same study, apelin levels were found to be decreased in active acromegalic subjects as compared to the controlled acromegalic subjects although the difference was not statistically significant. This paper aims to add a considerable certainty to the data already available on the altered levels of apelin and its association with hormonal, cardio-metabolic parameters in acromegalic subjects.

Materials and Methods

Ethics statement

The study was approved by the local ethics committee of Tepecik Training and Research Hospital. The subjects were included in the study only after a written informed consent was obtained from them. The study obeyed the Declaration of Helsinki rules (revised form, 2013) throughout the course.

Subjects and study design

This study was a cross-sectional study carried out from June 2015 to June 2016 in the Department of Endocrinology, Tepecik and Bozyaka Hospital, Izmir, Turkey. The study was carried out on 42 subjects with active acromegaly, 32 subjects with controlled acromegaly and 40 age, gender, and BMI-matched subjects, as the control group.

Acromegaly group

Acromegalic subjects were identified based on the clinical and laboratory features of the disorder which included failure of suppression of serum GH levels (lower than 1 ng/mL after a 75-g oral glucose tolerance test (OGTT)), fasting serum IGF-1 concentrations above the normal ranges as per age and gender, along with the typical clinical characteristic of

acromegaly (19). Acromegalic subjects were divided into two subgroups as an active acromegaly and controlled acromegaly group according to their GH levels following OGTT. Controlled acromegaly was described as having GH concentration lower than 1 ng/mL following a 75-g OGTT or patients undergoing treatment with somatostatin analogs, wherein random GH was lower than 1 ng/mL and IGF-1 values were in the reference arrays for age and gender. Before being recruited into the study, subjects having controlled acromegaly were in remission for at least six months (20). Out of the 42 active acromegalic subjects included in the study, 6 were newly diagnosed while the other subjects were treated using somatostatin analogs; however, of them, 16 subjects were also treated using cabergoline. None of the acromegalic study subjects received radiotherapy treatment.

Control group

Control subjects were selected among the patients who visited the endocrinology clinics of Tepecik and Bozyaka Training Hospital for a routine checkup of some disorders including hypertension, diabetes, and hyperlipidemia. Additionally, they had also applied for the routine annual checkup. Subjects in the control group were chosen such that they were of the same age, gender, BMI as acromegalic subjects and also had diabetes and hypertension. Out of 32 subjects in the control acromegaly group, 20 subjects were treated only by surgery.

Exclusion criteria

Subjects having the active infectious disease, those under the age of 18 years, those suffering from chronic inflammatory disorders or diagnosed with either cardiovascular disease or malignant disorders were excluded from the study. None of the enrolled subjects consumed tobacco or alcohol.

Anthropometric & Biochemical evaluation

A detailed history of all the study subjects was taken. Physical examination and anthropometric measurements (weight, age, waist circumference, blood pressure) of all the subjects were also accomplished. BMI was measured using the following formula: weight (kg)/square meter of height (m^2).

Venous blood samples were collected from the antecubital veins of the subjects in the morning (between 08:00-09:00) after a minimum of 12 h of the fasting period. The samples were centrifuged for 30 min at room temperature at 2000 ×g for clotting. The clotted samples were then stored in aliquots at -80 °C prior to the analysis of apelin.

Some other tests including fasting blood glucose (FBG), measurement of serum insulin, glycated hemoglobin $A1_C$ ($HbA1_C$), total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), liver function tests (ALT (alanine aminotransferase), AST (aspartate aminotransferase), kidney function tests (BUN i.e. blood urea nitrogen, creatinine) and apelin levels was also performed.

An auto-analyzer (Abbott Architect C 16000, IL, USA) and its exclusive kits (Abbott Diagnostics, Wiesbaden, Germany) were used to measure the routine parameters including FBG, total cholesterol, insulin, total HDL-C, triglyceride, AST, ALT, BUN, and creatinine levels. Circulating LDL-C levels were measured via indirect method (Friedewald equation) as follows: LDL-C=total cholesterol-(HDL-C+Triglyceride/5). $HbA1_C$ levels were measured using high-performance liquid chromatography (ADAMS $A1_C$ HA-8160; Arkray Inc., Firenze, Italy) method. A chemiluminescent immunometric assay (Immulite XPi, Siemens, Germany) was employed for the measurement of serum GH and IGF-1 levels. Each subject was controlled for insulin resistance using the homeostasis model assessment of insulin resistance (HOMA-IR): HOMA-IR=fasting insulin (μU/mL) × fasting glucose (mg/dL)/405.

cIMT Measurement

The carotid ultrasound examinations were performed by an experienced radiologist, blinded to all the clinical information, by ultrasonography using a linear probe 8-13 MHz (Toshiba Aplio 300, Tokyo, Japan). This technique measured cIMT (21).

Pericardial fat thickness measurements

The pericardial fat thickness of all the subjects was measured using the two-dimensional transthoracic echocardiography with

a Philips IE-33 system and S5-1 transducer (1-5 MHz, Philips, Bothell, WA, USA). EFT was measured as described earlier (22).

Apelin ELISA

Serum apelin levels were measured using the commercially available ELISA kits (Sunred Biological Technology, Shanghai, China) in accordance with the instructions of the manufacturer. The intra-assay and the inter-assay coefficient of variability (CV) were observed to be <6% and <8%, respectively. All samples were analyzed in duplicate.

Statistical analysis

Continuous variables are represented as the mean±standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the demographic and laboratory characteristics of the enrolled subjects in three groups. As per the results of ANOVA test, in case of any difference in variables in the groups, a post hoc test, namely Bonferroni multiple comparison methods, was considered to determine the particular group or groups causing the difference. Kolmogorov-Smirnov test was used to demonstrate whether the apelin levels were distributed normally; the results of the test showed that the apelin levels exhibited a normal distribution. Circulating apelin levels were measured and compared among the groups using a general linear model of univariate analysis; this model was adjusted for age, sex, BMI, HOMA-IR, diabetes, and hypertension. In the acromegaly groups, Pearson's correlation analysis was used to confirm the existence of any relationship between apelin and metabolic features as well as between cIMT and EFT. Further, multiple linear regression analyses were used to clarify the relationship between apelin and, cIMT, and EFT. Disease activity was incorporated in the models as a covariate. Likewise, the models were adjusted for blood pressure, BMI, HOMA-IR, lipids, and age. The chi-square test was used to compare categorical variables.

A level of 95% confidence interval (CI) was used to measure all the values. A *P* value <0.05 was considered statistically significant. All the variables were compared using Statistical Package for the Social Sciences software version 18.0 (SPSS Inc. Chicago, IL, USA).

Results

Clinical and laboratory characteristics of the study population

Active acromegaly, controlled acromegaly, and control groups were compared for the demographic and laboratory parameters (results demonstrated in Table 1).

All the participants were consistently matched with their age, gender, and BMI.

Circulating levels of apelin were found to be remarkably reduced in subjects with active acromegaly as compared to both, subjects of controlled acromegaly group and controls. Subsequently, controlled acromegalic subjects displayed significantly higher levels of apelin as compared to subjects of active acromegaly group (65.59±14.50 ng/mL in active acromegaly, 88.25±17.47 ng/mL in controlled acromegaly, 98.06±18.93 ng/mL in controls) (Figure 1).

The levels of cIMT were observed to be significantly higher in both the acromegaly group subjects than that in the controls. In addition, no difference was observed in cIMT levels between the two acromegaly groups. The EFT levels were found to be elevated in subjects with active acromegaly and controlled acromegaly as compared to that in the controls. No significant difference in EFT levels was observed between the active and controlled acromegaly groups.

Active acromegalic subjects illustrated higher levels of insulin as well as insulin resistance marker-HOMA-IR as compared to both, controlled acromegaly and control groups.

Correlation of apelin with clinical parameters

Table 2 presents the relationship between circulating apelin levels and other parameters in acromegalic subjects.

Circulating apelin levels were found to be negatively associated with insulin, HOMA-IR, FBG, BMI, and waist circumference. Moreover, a remarkably negative correlation was observed between apelin and cIMT & EFT. It was also found that apelin levels were negatively correlated to GH and IGF-1 in the acromegalic subjects. No correlation between apelin levels and lipid parameters could be found in the acromegalic subjects.

Table 1. Comparison of the demographic and laboratory characteristics of the subjects.

Variables	Active acromegaly n=42	Controlled acromegaly n=32	Control n=40	P[a]
Age, years	50.26±9.84	52.37±10.12	51.50±14.48	0.739
Female/male	22/20	14/18	21/19	0.706
BMI, kg/m^2	30.46±5.01	31.33±6.46	29.77±5.85	0.488
Waist circumference, cm	97.22±11.02	98.93±10.60	97.40±14.39	0.814
SBP, mmHg	121.66±13.89	121.47±11.44	124.93±16.23	0.597
DBP, mmHg	77.86±8.05	80.52±12.06	80.69±9.58	0.463
Duration of the disease, month median (min-max)	66 (1-276)	168 (12-468)	-	<0.001*
Diabetes, -/+	21/21	17/15	24/16	0.531
Hypertension, -/+	24/18	23/9	27/13	0.384
FBG, mg/dL	119.85±42.74	107.34±19.22	115.92±43.05	0.366
Insulin, µIU/mL	15.93±13.13	9.18±7.22	11.64±6.41	0.011*
HOMA-IR	5.31±6.59	2.52±2.22	3.39±2.59	0.023*
GH, ng/mL	7.30±11.10	0.51±0.49	0.49±0.84	<0.001*
IGF-1, ng/mL	578.00±331.63	159.64±30.11	137.51±51.17	<0.001*
A1C,%	7.94±2.22	7.41±2.23	7.39±1.77	0.408
Total cholesterol, mg/dL	233.13±52.53	238.03±46.75	235.53±46.32	0.912
LDL-C, mg/dL	139.73±44.24	143.59±33.22	141.52±42.58	0.922
HDL-C, mg/dL	48.83±14.59	53.68±18.66	52.05±13.85	0.392
Triglycerides, mg/dL	222.80±95.50	203.75±89.36	209.77±117.23	0.709
BUN, mg/dL	30.90±14.83	34.50±10.15	29.47±9.53	0.198
Creatinine, mg/dL	0.89±0.21	0.94±0.17	0.95±0.24	0.369
ALT, IU/L	30.42±18.47	32.18±18.34	34.95±18.68	0.541
AST, IU/L	26.85±14.57	27.93±14.17	28.52±10.41	0.844
Epicardial fat thickness, mm	7.16±2.85	6.90±2.95	4.05±2.48	<0.001*
CIMT, mm	1.21±0.72	1.13±0.73	0.65±0.67	<0.001*

Results are given in mean±SD. a One-way analysis of variance (ANOVA) test was used. A P value of <0.05 was considered significant (*). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; BUN: Blood urea nitrogen; DBP: Diastolic blood pressure; FBG: Fasting blood glucose; GH: Growth hormone; HDL-C: High density lipoprotein cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL-C: Low density lipoprotein cholesterol; IGF-1: Insulin-like growth factor 1; SBP: Systolic blood pressure.

Multiple linear regression analysis

Two linear regression models were considered in order to clarify the existence of any relationship between apelin and cIMT or EFT (Table 3). Both the models were adjusted for lipid profiles, blood pressure, GH, and IGF-1. Both the models showed an independent negative correlation between apelin and cIMT or EFT.

Discussion

Atherosclerotic vascular disorders are commonly observed in patients with acromegaly (13-15). The role of apelin has previously been investigated in the atherosclerotic process and disorders (9-12,23,24). In this study, the levels of circulating apelin in acromegalic subjects were compared to that of the controls and it was aimed to determine whether or not there was a link between apelin levels and cIMT, EFT in the

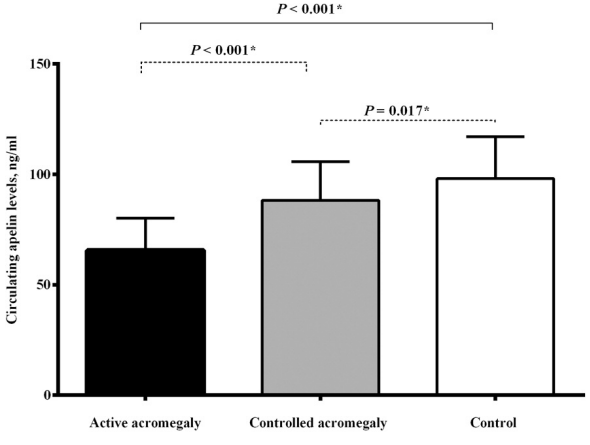

Figure 1: Circulating apelin levels in the test groups. * A P value <0.05.

Table 2. Correlation coefficient between apelin levels and clinical parameters in subjects with acromegaly.

	Apelin	
	r	P
Age	0.105	0.147
BMI	-0.125	0.038*
Waist circumference	-0.156	0.031*
Insulin	-0.232	0.025*
FBG	-0.112	0.037*
HOMA-IR	-0.202	0.031*
GH	-0.146	0.029*
IGF-1	-0.167	0.021*
cIMT	-0.232	0.011*
EFT	-0.198	0.019*
Total cholesterol	0.067	0.138
LDL-C	0.168	0.116
HDL-C	0.107	0.178
Triglycerides	-0.103	0.128

Pearson's correlation analysis was used. r: Pearson's correlation coefficient. A P value of <0.05 was considered significant (*). BMI: Body mass index; cIMT: Carotid intima-media thickness; EFT: Epicardial fat thickness; FBG: Fasting blood glucose; HDL-C: High-density lipoprotein cholesterol; HOMA-IR: Homeostasis model assessment of insulin resistance; LDL-C: Low-density lipoprotein cholesterol.

acromegalic subjects. The results of the study indicate that circulating levels of apelin were lower in subjects with active acromegaly as compared to that in controlled acromegaly group and controls. Also, the decrease in apelin levels in active acromegalic subjects was inversely associated with GH, IGF-1, insulin resistance, and BMI. Furthermore, decreased levels of apelin were also observed to be inversely and independently associated with cIMT and EFT.

Adipose tissue is an endocrine organ that secretes molecules that have local and/or systemic activity and play critical roles in many systems and processes including glucose and lipid metabolism, inflammation, and cardiovascular system (25,26). The amount of adipose tissue in acromegalic subjects tends to be modified. It has been observed that in these subjects, the visceral and subcutaneous adipose tissues are decreased while the intramuscular adipose tissue is increased (27). Another important implication suggests that the levels of secreted adipose-derived cytokines are altered in patients with acromegaly (16,17). Adipose tissue dysfunction contributes to the development of metabolic abnormality and cardiovascular risk in acromegalic subjects (28). Apelin is involved in regulation of glucose metabolism such as insulin resistance (5,29). A preclinical study detected that apelin injection resulted in stimulation of glucose consumption in both, normal and obese insulin-resistant mice (5). Another *in vivo* study on apelin knockout mice reported that apelin had the ability to activate glucose uptake and improving insulin sensitivity (29). Previous investigations reveal the lack of accurate documented results on the apelin levels and its relationship to the metabolic parameters in humans. In another study, apelin levels were found to be lower in newly diagnosed T2DM as compared to that in the controls and a negative correlation was reported between apelin levels and insulin resistance (30). A study on the association of apelin serum levels with early atherosclerosis or fat distribution in young subjects with increased risk for T2DM, apparently revealed that there was no signifi-

Table 3. Multiple regression analysis.

	cIMT				EFT			
Variables	β	95% CI		P	β	95% CI		P
Age	0.026	0.012	0.040	0.047*	0.091	0.035	0.147	0.044*
BMI	0.145	0.112	0.178	0.029*	0.187	0.104	0.270	0.031*
HOMA-IR	0.134	0.103	0.165	0.031*	0.238	0.116	0.360	0.027*
Apelin	-0.206	-0.298	-0.114	0.019*	-0.315	-0.512	-0.118	0.011*

Multiple linear regression analysis was used. β: Unstandardized regression coefficient; CI: Confidence interval; A P value of <0.05 was considered significant (*). cIMT: Carotid intima-media thickness; EFT: Epicardial fat thickness; BMI: Body mass index; HOMA-IR: Homeostasis model assessment of insulin resistance.

cant link between apelin levels and insulin resistance, fat distribution or cIMT (31). A clinical study related to apelin levels in subjects with normal glucose tolerance and type 2 diabetic patients suggested that the circulating levels of apelin were significantly elevated in subjects with type 2 diabetes as well as in those with impaired fasting glucose tolerance as compared to the controls. In the same study, it was also found that apelin was in an independent positive association with insulin resistance and BMI (7). It can be stated that providing certain information about finding the main reason of apelin level diversity and its related metabolic parameters is not completely possible in various metabolic disorders. To the best knowledge of the authors, literature reports only one study so far, which has addressed the issue of circulating levels of apelin in acromegaly. Topsakal et al. reported increased apelin levels in both active and inactive acromegalic subjects as compared to the controls, although the difference in apelin levels between both the acromegalic groups was not significant (18). They also found a significantly positive correlation between apelin and GH. Contrary to this study, a remarkable decrease in the levels of apelin in both the acromegalic groups compared to the controls has been found in the present study. Further, the levels of apelin were significantly lower in the active acromegaly group as compared to that in the controlled acromegaly group. Interestingly, a notable inverse correlation between apelin levels and both GH and IGF-1 was also observed in the present study. Although the experimental design in this study may not same as that in the previous ones, yet it can be suggested that the study could provide certain data regarding apelin levels and acromegaly. This study can also form a base for further related investigations in future.

It is implicated that cIMT and EFT, known as non-invasive methods, are surrogate markers for cardiovascular risk (32,33). In this study, it was found that cIMT and EFT both were elevated in the acromegalic subjects as compared to that in the controls. Moreover, an independent negative association was observed between apelin and cIMT or EFT. These results are in accordance with those previously obtained from a study suggesting a negative correlation between apelin and cIMT in women with previous gestational diabetes (34). According to the earlier investigations, the literature reports no studies on the relationship of apelin levels and EFT.

Yet, the current study has a number of limitations such as the lack of possibility of measurement of fat distribution in the recruited subjects, lack of availability of handling any causality in the cross-sectional design.

To sum up, it was found that the apelin levels of acromegalic subjects differed from those of the normal subjects. The results proved that the circulating levels of apelin are significantly decreased in active acromegalic subjects as compared to both, controlled acromegalic subjects and controls. It can be deduced that GH may trigger a decrease in the levels of circulating apelin in acromegalic subjects. Apart from this, decreased apelin levels have been found to be inversely associated with cardiovascular risk factors including cIMT and EFT. These data suggest that decreased apelin levels are likely to play a role in the development of cardiovascular disorders in patients with acromegaly.

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Compliance with Ethical Standards

The subjects gave their oral and written informed consent before inclusion in the study. The study adhered strictly to the principles of the Declaration of Helsinki as revised in 2013.

Source of Finance: During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest: No conflicts of interest between the authors and/or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling,

expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: Mustafa Demirpençe, Mehmet Çalan; Design: Mehmet Çalan, Mustafa Demirpençe; Control/Supervision: Mehmet Çalan, Mustafa Demirpençe; Data Collection and/or Processing: Mehmet Çalan, Mustafa Demirpençe; Analysis and/or Interpretation: Mehmet Çalan, Mustafa Demirpençe; Literature Review: Mehmet Çalan, Mustafa Demirpençe; Writing the Article: Mustafa Demirpençe, Mehmet Çalan; Critical Review: Mehmet Çalan; References and Fundings: Mustafa Demirpençe; Materials: Mustafa Demirpençe, Mehmet Çalan

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