Study of serum apelin and its relation to obesity-associated hypertension

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Introduction
Over the past few decades obesity has become a major burden on health worldwide. The prevalence of hypertension has increased with a significant increase in the prevalence of overweight and obesity. Recent studies indicate an important role of adipose tissue hormones called adipokines in obesity-associated complications. Apelin has recently been added to the family of adipokines. One of the physiologic functions of the apelin/APJ system is regulation of the cardiovascular function. The aim of this study was to determine the relation of serum apelin to obesity-associated hypertension as well as to myocardial performance.

Patients and methods
The study included 30 obese hypertensive patients, 30 obese nonhypertensive patients, and 25 age-matched and sex-matched controls. In all studied participants we determined the lipid profile, serum insulin, fasting blood glucose level, HOMA-IR, serum apelin, and echocardiographic results of left ventricular systolic and diastolic function.

Results
Higher levels of fasting blood glucose, fasting serum insulin, HOMA-IR, triglycerides, total cholesterol, and low-density lipoprotein were detected in obese hypertensive and nonhypertensive patients. Left ventricular mass index (LVMI) was increased in both obese hypertensive and nonhypertensive patients in comparison with healthy individuals. Left ventricular ejection fraction and E/A ratio were significantly lower in hypertensive obese versus nonhypertensive obese individuals ($P = 0.004$ and <0.001, respectively), whereas LVMI was higher in hypertensive versus nonhypertensive patients ($P < 0.001$). Apelin levels were significantly equally higher in obese hypertensive and nonhypertensive patients (6.10 ± 1.88 and 6.40 ± 1.60 ng/ml) compared with controls (4.22 ± 0.86 ng/ml, $P < 0.001$). In hypertensive obese individuals, serum apelin correlated negatively with left ventricular ejection fraction ($P = 0.02$) and directly with E/A ratio ($P = 0.03$).

Conclusion
Apelin levels are significantly higher in obese hypertensive and nonhypertensive patients. This increase might be a compensatory mechanism against myocardial dysfunction with obesity.

Keywords:
adipokines, apelin, hypertension, obesity

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Introduction

Over the past few decades obesity has become a major burden on health worldwide. Its prevalence in developing countries that have adopted a western lifestyle has tripled in 20 years. At present, more than 1.1 billion adults worldwide are overweight (BMI >25 kg/m^2) and 312 million of them are obese (BMI >30 kg/m^2) [1].

Obesity is a multifactorial disease; several factors including genetic predisposition, misregulation of energy balance, and environmental and social factors contribute to its development. Excess energy intake and decreased energy consumption due to a sedentary lifestyle are the main contributors to the obesity epidemic. Energy balance is regulated by a complex network of neurons in the central and lateral hypothalamus. In obesity, these regulatory mechanisms fail to inhibit excess food intake and storage of energy [2].

Obesity is a major factor in the development of the metabolic syndrome, a state characterized by overweight, insulin resistance, hypertension, and impaired lipid metabolism and body fat distribution. Individuals with metabolic syndrome have marked risks for the development of type 2 diabetes mellitus and they possess high cardiovascular mortality [3]. Because of these adverse consequences, obesity has been estimated to decrease life expectancy by 7 years at the age of 40. In addition, obesity predisposes to the development of cancer, asthma, osteoarthritis, sleep apnea, pregnancy complications, and depression, leading to overall decrease in the quality of life [4].

In obesity, the size and number of adipocytes are increased, and this is accompanied by changes in the gene expression profile in large adipocytes [5]. Adipose tissue is infiltrated with macrophages and the macrophage quantity has been correlated with measures of insulin resistance. In addition to stimulation of low-grade inflammation, the secretion of adipokines regulating food intake, insulin sensitivity, blood pressure, and inflammation is altered in obesity [6].

The increasing prevalence of hypertension occurred in conjunction with a dramatic increase in the prevalence of overweight and obesity. Data from the NHANES show a strong linear relationship between BMI and systolic and diastolic blood pressure. The Framingham Heart Study showed that a 5% weight gain increases hypertension risk by 30% in 4 years [7–9]. Not only is obesity linked with hypertension, but weight loss in obese individuals is associated with a decline in blood pressure [10].

Recent studies indicate an important role of adipose tissue hormones called adipokines in obesity-associated complications. A number of adipokines such as leptin, adiponectin, and resistin have been identified, and apelin has recently been added to the family of adipokines; expression of both apelin and apelin receptor (APJ) has been described in adipocytes [11,12].

In 1998, apelin-36 was isolated from bovine stomach extracts and used to sequence the cDNA of human and bovine apelin. It was subsequently identified as the endogenous ligand of the APJ receptor, one of the G protein-coupled receptors (GPCRs), whose function had been unknown until the discovery of its ligand. The APJ receptor was first cloned by homology to another known receptor (GPCRs), and the human APJ amino acid sequence is 31% identical to that of the angiotensin II type-1A receptor [13,14].

Biologically active apelin is found in the epithelial cells of the gastric mucosa, the myocardium, and endocardial endothelium, in the lungs, kidneys, and adrenals, and in the endothelium of large and small blood vessels. In the heart, it is found in endothelial cells in both small and large coronary vessels, in the endocardial endothelium, and in smaller quantities in cardiomyocytes and vascular smooth muscle cells [15,16].

One of the physiologic functions of the apelin/APJ system is regulation of cardiovascular function. Although apelin is widely distributed, the main target of its action appears to be the cardiovascular system. It induces temporary falls in systolic and diastolic blood pressure, mediated by endothelium-dependent vasodilation, accompanied by a slight rise in heart rate and contractility following intravenous injection [17].

Apelin peptides mediate three major actions in tissues from patients with cardiovascular disease: endothelium-dependent vasodilatation, direct vasoconstriction, and increased cardiac contractility [18].

Insulin directly regulates apelin production in adipocytes, demonstrating the potential link with obesity-associated variations of insulin sensitivity status [11]. It was shown that apelin inhibited insulin secretion in mice, suggesting a link between apelin and glucose homeostasis [19].

In humans, higher plasma apelin levels were found in obese compared with leaner individuals [11]. In patients with essential hypertension, circulating apelin levels are reduced, and lower plasma apelin is independently associated with more profound left ventricular systolic and diastolic function impairment [20].

The aim of this study was to determine the relation of serum apelin to obesity-associated hypertension as well as to myocardial performance.

Patients and methods

Patients

The study was carried out on 60 obese patients (BMI ≥30 kg/m^2) of both male and female sex, aged between 20 and 50 years, attending the Internal Medicine and the Endocrinology Outpatient Clinics in Alexandria Main University Hospital. The patients were categorized into two groups: group I included 30 obese hypertensive patients (blood pressure >140/90) and group II included 30 obese nonhypertensive individuals (blood pressure <140/90). In addition, the study included 25 healthy individuals of matched age and sex as a control group. All participants signed a written informed consent form after being explained the nature and aim of the study.

Exclusion criteria

Patients with a history of cardiac disease [coronary artery disease, rheumatic heart disease, diabetes mellitus, left ventricular systolic dysfunction (ejection fraction <40%), renal diseases, hepatic diseases, other endocrinal dysfunctions] and patients receiving antihypertensive drugs affecting the renin–angiotensin–aldosterone system were excluded from the study.

Methods

History and examination

History of hypertension, cardiovascular disease, coronary artery disease, and diabetes mellitus was

taken. Complete physical examination was performed, including blood pressure measurement. Height, weight, and waist and hip circumference were measured and BMI was calculated using the formula weight in kg/height in m^2. Body fat mass percentage was calculated using the Deurenberg equation as follows: body fat percentage = 1.2 (BMI)+0.23 (age)−10.8 (sex)-5.4, with age being in years and sex being designated as 1 for male and 0 for female patients [21].

Biochemical assays

Venous sampling was carried out in the morning (8.00–10.00 a.m.) after an overnight fast of 8–10 h. The blood was drawn into an empty tube and then centrifuged for 10 min. The separated serum was used for the following assays: lipid profile [triglycerides, cholesterol, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C)], fasting serum insulin by ELISA [22], and fasting glucose level. Homeostasis Model Assessment 2 (HOMA2) calculator was used to estimate insulin resistance (HOMA-IR) [23]: fasting insulin (mIU/l) ×fasting glucose (mg/dl)/405. Plasma apelin levels were quantified by an enzyme immunoassay [Apelin-36 (Human) EIA kit (EK-057-15); Phoenix Pharmaceuticals Inc., Burlingame, California, USA] with intra-assay and interassay coefficients of variation less than 10% and less than 15%, respectively. The kit can identify the different active isoforms of apelin, including apelin-36 and apelin-13, as it targets the C terminus peptide present in all active forms of apelin with a normal range of 0–100 ng/ml [11].

Echocardiography

Echocardiographic imaging was performed. Cardiac dimensions and wall thicknesses were measured according to standard recommendations. Left ventricular mass was calculated by the following formula: LVMI (Penn) = 1.04[(LVIDD+PWTD+IVSTD)^3-LVIDD^3]−13.6 g. Left ventricular ejection fraction (LVEF) and left ventricular fractional shortening were estimated. Left ventricular diastolic function was assessed by measuring mitral flow E wave, A wave, E/A ratio, and mitral annulus tissue velocities. Pulmonary artery pressure was derived from pulmonary flow acceleration time.

Results

All studied groups were matched for age and sex. A significant increase in BMI, waist circumference, hip circumference, and body fat percentage was detected among obese patients with or without hypertension in relation to normal individuals. Waist circumference was significantly higher in obese hypertensive versus obese nonhypertensive patients (Table 1).

There were statistically significantly higher levels of fasting blood glucose, fasting serum insulin, HOMA-IR, triglycerides, total cholesterol, and LDL in obese hypertensive and nonhypertensive patients. In contrast, the level of HDL was statistically significantly lower in obese nonhypertensive patients than in healthy controls (Table 1 and Fig. 1).

LVEF was statistically significantly lower in obese hypertensive patients versus obese nonhypertensive patients. LVMI was increased in both group I and group II in comparison with healthy individuals. A significantly higher level was noticed in group I versus group II. E/A ratio and pulmonary acceleration time (PAT) were significantly lower in group I in comparison with groups II and III (Table 2).

Table 1 Clinical and laboratory characteristics of the studied groups and control

Variable	Group I	Group II	Group III	*P*
Age (years)	42.50 ± 7.49	39.13 ± 9.59	37.08 ± 8.93	0.070
BMI	45.39 ± 7.22	42.89 ± 8.86	25.65 ± 2.30	<0.001*
Waist circumference (WC) (cm)	125.93 ± 20.61	111.97 ± 16.19	88.84 ± 4.60	<0.001*,Δ
Hip circumference (HC) (cm)	131.0 ± 18.92	122.50 ± 16.44	100.92 ± 9.2	<0.001*
Body fat (BF)%	54.62 ± 8.13	53.27 ± 10.34	31.89 ± 4.40	<0.001*
FSG (mg/dl)	91.60 ± 10.62	90.73 ± 9.07	77.88 ± 6.45	<0.001*
Fasting serum insulin (μIU/ml)	14.32 ± 8.06	12.65 ± 5.28	6.43 ± 2.80	<0.001*
HOMA-IR	3.22 ± 1.88	2.83 ± 1.16	1.23 ± 0.43	<0.001*
Triglycerides (TG) (mg/dl)	163.80 ± 43.1	166.63 ± 42.25	88.12 ± 20.03	<0.001*
Total cholesterol (TC) (mg/dl)	190.10 ± 29.2	194.07 ± 28.86	125.44 ± 23.7	<0.001*
LDL (mg/dl)	113.17 ± 27.2	115.97 ± 23.99	82.04 ± 12.61	<0.001*
HDL (mg/dl)	43.63 ± 5.09	40.77 ± 5.20	46.56 ± 4.21	<0.001*
Fasting serum apelin (ng/ml)	6.10 ± 1.88	6.40 ± 1.60	4.22 ± 0.86	<0.001

Group I, obese hypertensive patients; group II, obese nonhypertensive patients; group III, control; HDL, high-density lipoprotein-cholesterol; HOMA-IR, Homeostasis Model Assessment – Insulin Resistance; LDL, low-density lipoprotein; *Group I and II versus group III; ΔGroup I versus group II.

In the studied obese hypertensive patients (n = 30), there was a statistically significant direct correlation between BMI and LVMI (r = 0.44, P < 0.015) (Fig. 2).

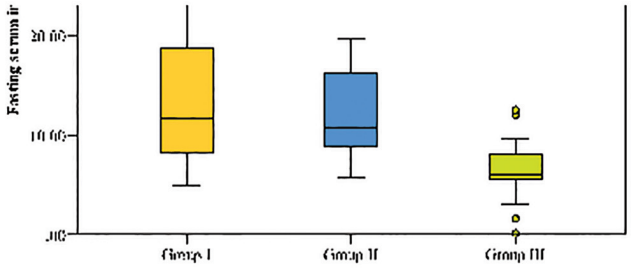
The mean serum apelin level was statistically significantly higher in obese patients (groups I and II) in comparison with healthy controls (group III) (Fig. 3). In the studied obese hypertensive patients (n = 30), there was a statistically significant inverse correlation between serum apelin and LVEF (r = –0.423, P = 0.020) and there was a statistically significant direct correlation between serum apelin and E/A ratio (Figs. 4 and 5). In the studied obese nonhypertensive patients (n = 30), there was a statistically significant inverse correlation between serum apelin and LVMI (r = 0.44, P < 0.015) (Fig. 6).

Table 2 Echocardiographic characteristics of the studied groups and control

Variable	Group I	Group II	Group III	*P*
LVEF	65.60 ± 6.44	71.20 ± 6.77	68.12 ± 5.26	0.004*
LVMI	244.5 ± 104.4	166.1 ± 70.2	106.3 ± 11.13	<0.001*,Δ
E/A ratio	0.95 ± 0.38	1.26 ± 0.35	1.40 ± 0.10	<0.001*
PAT	110.7 ± 12.4	129.3 ± 22.9	117.3 ± 7.5	<0.001*

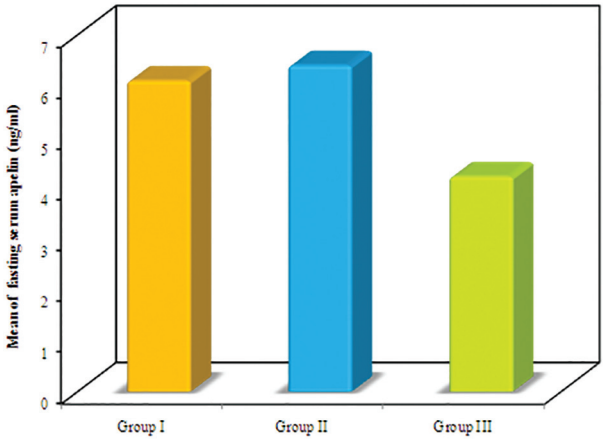
Group I, obese hypertensive patients; group II, obese nonhypertensive patients; group III, control; LVEF, left ventricular ejection fraction; *Group I versus group II; ΔGroup I and group II versus group III.

Figure 1



Comparison between the three studied groups according to fasting serum insulin (μIU/ml) (group I: obese hypertensive patients; group II: obese nonhypertensive patients; group III: control participants). *P < 0.001 versus group III.

Figure 3

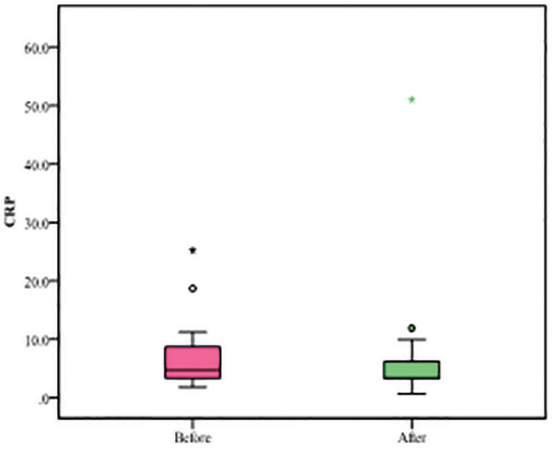


Comparison between the three studied groups according to fasting serum apelin (group I: obese hypertensive patients; group II: obese nonhypertensive patients; group III: control individuals). *P < 0.001 versus group III.

Discussion

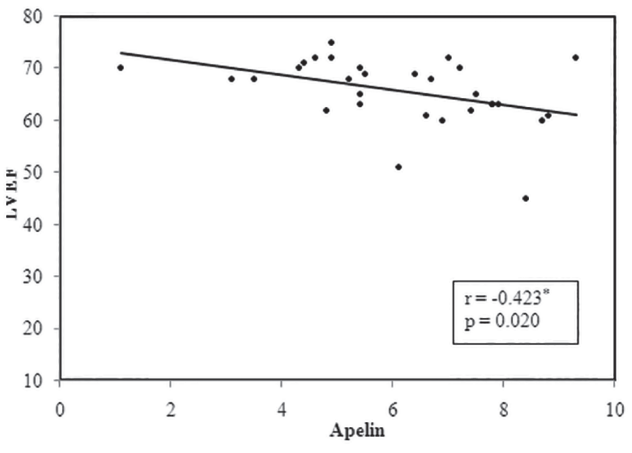
In the present study we observed that the waist circumference of obese hypertensive patients was significantly higher than that of obese normotensive individuals. Guagnano *et al.* [24] documented that waist circumference was the most important anthropometric

Figure 2



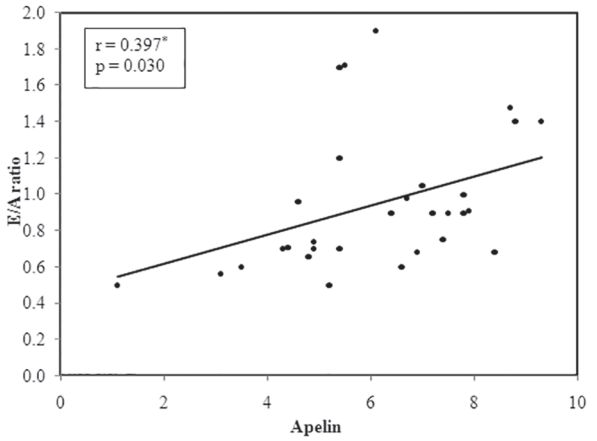
Correlation between BMI and LVMI in group I (obese hypertensive).

Figure 4



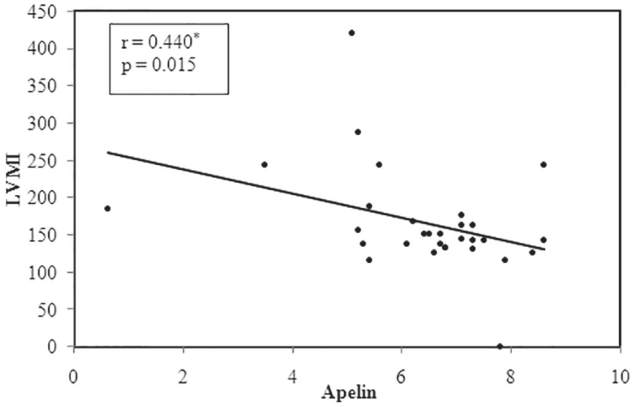
Correlation between serum apelin and left ventricular ejection fraction (LVEF) in group I (obese hypertensive).

Figure 5



Correlation between serum apelin and E/A ratio in group I (obese hypertensive).

Figure 6



Correlation between serum apelin and left ventricular ejection fraction (LVMI) in group II (obese nonhypertensive).

factor associated with hypertensive risk. The authors concluded that intra-abdominal fat accumulation itself may play an important role in the pathogenesis of hypertension in obesity.

In the present study, after observing the glycemic profile we have found that obese patients had high fasting blood glucose, serum insulin, and HOMA-IR with no difference between obese hypertensive and obese normotensive patients. These findings are in concordance with those of Mahadik [25], who found that serum insulin levels, HOMA-IR measure of IR, leptin, and hsCRP levels increased significantly and serum adiponectin levels decreased significantly in obese and obese hypertensive patients compared with nonobese control individuals; no difference was found in insulin and HOMA-IR levels between the two groups [25]. Our findings are supported by Heinonen and colleagues [26–28], who confirmed the association between hyperinsulinemia and obesity. Insulin resistance is often linked to obesity. Excess adipose tissue plays a central role in the induction of insulin resistance [29].

In the current study, it was observed that obese hypertensive and normotensive patients had significantly higher serum triglycerides, LDL-C levels, and serum cholesterol levels when compared with nonobese individuals, whereas obese nonhypertensive individuals had significantly lower HDL-C when compared with the control group. The same findings were observed by many authors [28,30–33]. Obesity is associated with increased free fatty acid resulting in impaired glucose use and increased hepatic glucose output; increased free fatty acid also affects the lipid metabolism by increasing VLDL and LDL production from the liver with lowering of HDL level; thus, atherogenic risk will be high and ischemic heart disease risk will increase, as reported by Després and Lemieux [34].

The serum apelin level was found to be equally high in obese hypertensive and normotensive patients included in our study. These findings are in concordance with most studies demonstrating that adipose tissue apelin mRNA and plasma apelin levels are increased in patients with obesity, impaired glucose tolerance, and diabetes [35–37]. In contrast to our findings, Erdem *et al.* [38] reported plasma apelin levels to be decreased in overweight patients with newly diagnosed diabetes, suggesting that factors other than adiposity may regulate apelin levels. The discrepancies of the studies are unclear, but it has been suggested that obese adults with disturbed glucose metabolism demonstrate a decrease in apelin.

In our study, we did not observe significant correlation between apelin concentration and BMI, waist circumference, and HOMA-IR. Although BMI is a good measure for overweight, one needs to be aware of its limitation as an indirect measure of fat mass. The missing relationship between plasma apelin and obesity-related parameters might be explained by different sources of apelin secretion. Adipose tissue is not the only determinant in circulating apelin levels. Other sources (i.e. central nervous system, heart, lung, testis, ovary, mammary gland, and gastrointestinal system) may also play a multifactorial role in the pathophysiological settings.

The absence of association with HOMA-IR may be explained by the observation that the strongest association is found with type 2 diabetes mellitus, in which both insulin resistance and impaired insulin secretion are present and necessary. Thus, it is possible

that HOMA-IR alone cannot determine the increase of apelin in diabetic individuals.

In our study, we were unable to find a significant correlation between apelin and insulin levels; the same results were found by Maria *et al.* [39] and Ba *et al.* [40]. Moreover, we found that, in obese patients, both plasma apelin and insulin levels are significantly higher, indicating that apelin homeostasis is impaired in the obese state and suggesting that the rise in plasma insulin could promote an increase in blood concentrations of apelin, as also suggested by Boucher *et al.* [11].

In the present study, we observed comparable increase in serum apelin in obese hypertensive and nonhypertensive obese individuals, and no correlation between systolic blood pressure, diastolic blood pressure, and serum apelin. The same results were reported by Rittig *et al.* [41], who examined the association of apelin serum levels with insulin sensitivity/resistance and body fat distribution as dependent cardiovascular risk factors; blood pressure was reported to be unaffected by serum apelin levels. Further, neither parameters of insulin sensitivity nor parameters of fat distribution, such as BMI and grade of adiposity, were associated with apelin serum levels [41].

In contrast, Sonmez *et al.* [42] measured the circulating apelin level in patients with essential hypertension. A total of 30 young nonobese male patients with newly established and untreated hypertension and 30 healthy controls matched for age and BMI were studied. They found that apelin levels were significantly lower in hypertensive patients compared with controls. They could only speculate on the interaction of altered apelin with high blood pressure. However, the results cannot be deemed to belong to all hypertensive populations because of the small sample size and narrow selection criteria [42].

The current study showed that obese individuals exhibited abnormalities in left ventricular structure and function, including a pattern of left ventricular hypertrophy and decreased diastolic function. Left ventricular hypertrophy was evidenced by increased LVMI in obese hypertensive and nonhypertensive patients in comparison with healthy individuals. A significantly higher level was noticed in obese hypertensive patients compared with obese nonhypertensive individuals. Our finding of low E/A ratio, which suggests a diastolic dysfunction, is similar to previous studies of Zarich *et al.* [43] and Stoddard *et al.* [44] on moderate and severely obese individuals and to a study by Peterson *et al.* [45] that showed depressed diastolic function in young obese women.

In the current study a negative significant correlation between serum apelin level and LVMI was observed and there was a significant positive correlation between serum apelin level and E/A ratio.

In agreement with our findings, Przewlocka *et al.* [20] studied 232 hypertensive patients without concomitant diseases affecting cardiovascular functions and 76 healthy controls. Patients with hypertension presented significantly higher values of left ventricular end-diastolic dimension, interventricular septal thickness, left ventricular posterior wall thickness, left atrial dimension, LVMI, and relative wall thickness compared with referents. Decreased values of E/A ratio in hypertension were indicative of left ventricular diastolic function impairment. Besides patient age, BMI, blood pressure, and LVMI, apelin was an independent determinant of left ventricular systolic and diastolic function parameters.

Experimental evidence indicates that apelin is one of the most potent endogenous inotropic agents [46–48]. An increase in myocardial contractility in response to the administration of apelin was seen both in normal and failing hearts with no accompanying left ventricular hypertrophy even during long-term observations [49–51].

The biological potential of apelin demonstrated in experimental studies makes it necessary to consider this peptide as a relevant regulator of vascular tone and cardiac function in health and disease [19]. Hypertension, with its hemodynamic alterations and cardiac complications, might be an interesting field of exploration; however, the activity of the apelinergic system in this entity has not yet been extensively investigated. These results suggest that apelin might act as an endogenous protective regulator in the heart. The relative importance of the central and peripheral actions of apelin on normal cardiovascular physiology and cardiovascular disease is also undetermined [52].

Taking into account the physiological roles of apelin in the cardiovascular system and in the control of glucose homeostasis, we can hypothesize that overproduction of apelin in the obese could be one of the last protections before the emergence of well-known obesity-related disorders such as type 2 diabetes mellitus or cardiovascular dysfunctions.

Conclusion

Apelin levels are significantly higher in obese hypertensive and nonhypertensive patients. This increase might be as a compensatory mechanism against myocardial dysfunction with obesity.

Acknowledgements

Conflicts of interest

None declared.

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