ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Apelin in obese frail elderly in out patient clinic in Zagazig University.

Nabila A. Hussien[1], Mohsen M. Eldarawy[1], Atef G. Hussien[2] Nearmeen M. Rashad[1], Khaled M. El- Gerby[3], Nermin Raafat.[2], Manal M. El- Gerby4 Azza H. Abd El-Fatah[1].

1-Internal Medicine Department, Faculty of Medicine, Zagazig University, Egypt.
2- Medical Biochemistry Department, Faculty of Medicine, Zagazig University, Egypt.
3- Radiodiagnosis Department, Faculty of Medicine, Zagazig University, Egypt.
4- Clinical Pathology Department , Faculty of Medicine, Zagazig University, Egypt.

Manuscript Info

Manuscript History:

Received: 15 November 2015
Final Accepted: 22 December 2015
Published Online: January 2016

Key words:
obesity; serum apelin; fraility; Insulin resistance

Corresponding Author

Nabila A. Hussien.

Abstract

Background :Obesity exacerbates the age-related decline in physical function and causes frailty in older individuals. Frailty in older obese individuals may be related to the insulin resistance and inflammation that often associated with obesity. Obesity characterized by excess body fat which secretes adipokines hormones. Apelin is one of the adipokines directly regulated by insulin and body mass index (BMI), the present study was aimed to estimate serum apelin in elderly and investigate possible correlations between serum apelin, obesity-related markers and frailty phenotypic criteria.

Subjects and methods: A case- control study included 74 lean elderly and 74 obese elderly patients who were stratified according to their body mass index (BMI) to three subgroup, In all studied participants we determined BMI, waist circumference, hip circumference, waist to hip ratio , mid upper arm circumference (MAC), self-reported exhaustion, low energy expenditure, chair-stand test s, 4-m walking time s, frailty index, max. grip strength (kg) for men and women, assessment of fat mass(FM) and fat free mass (FFM)using CT and Serum apelin levels were determined with an enzyme-linked immunosorbent assay,

Result: All obesity group subjects had significantly higher values of total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), BMI , fat mass , fasting blood glucose, fasting serum insulin, HOMA-IR, systolic and diastolic blood pressure ($P<0.05$) than lean subjects. diabetic obese group had significantly higher LDL-C, TC, TG, fasting blood glucose, fasting serum insulin, HOMA-IR, systolic and diastolic blood pressure than non-diabetic group, serum apelin levels were significantly higher in obese groups compared to control ($P= \leq 0.05$), and correlated positively with BMI, and fat mass, fasting insulin, HOMA-IR and triglycerides .In obese subjects, Self-reported exhaustion, low energy expenditure, Chair-stand test s, 4-m walking time s, frailty index, Max. grip strength (kg) for men and women were significantly associated with obesity and serum apelin.

Conclusion:-We found that serum apelin and phenotype parameters of frailty were higher in obese than lean elderly. In obese group, serum apelin and phenotype parameters of frailty positively correlated with HbA1c values, fasting blood glucose, fasting serum insulin, HOMA-IR, LDL ,TG, BMI and fat mass.

Introduction:-

Population ageing worldwide is rapidly accelerating from 461 million people aged over 65 years in 2004 to an estimated 2 billion people by 2050 (1, 2), which has profound implications for the planning and delivery of health and social care. The most problematic expression of population ageing is the clinical condition of frailty. Frailty is a state of increased vulnerability to poor resolution of homeostasis following a stress, which increases the risk of adverse outcomes including falls, delirium and disability. (3, 4, 5)

Prevalence of obesity is reaching epidemic all over the world. Stress is one of the major inducers of visceral fat and obesity development, underlying accelerated aging processes. In old age there are limited physical activity, poor fitness, chronic inflammation and hormonal change (6). Age related reduction in glucose elimination accompanied by reduced glucose effectiveness and increased hepatic insulin extraction all these are risk factors for obesity and abdominal obesity. (6)

Adipose tissue is at present considered as an active endocrine organ producing important mediators involved in metabolism regulation as well as in inflammatory mechanisms (7).Different kinds of stressors, including life stressful events, on the other hand, have been particularly linked to development of visceral obesity (8).

The hypothalamic-pituitary adrenal axis and the central and peripheral components of the autonomic nervous system constitute the two main vital stress-system functions (5). States of over- or under nutrition may impair the crosstalk between metabolic and immune system, leading to the activation of the immune response and the development of a "low-grade systemic inflammation," as confirmed by increasing circulating levels of proinflammatory cytokines, adipokines and other inflammatory markers detected in obese subjects. Activation of the immune response in obesity is mediated by specific signaling pathways, with Jun N-terminal kinase and IkappaB kinase beta/nuclear factor kappa-light-chain-enhancer of activated B cells being the most studied. It is known that the above events modify insulin signaling and result in the development of insulin resistance (8). Adipose tissue stores triglycerides and also secretes polypeptides, adipocyte-produced hormones called adipokines (or adipocytokines), including leptin, visfatin, vaspin, apelin, adiponectin and resistin, which all play important roles in metabolism and energy homeostasis (9).

Apelin, identified by Tatemoto et al. (9), is a novel bioactive peptide expressed in adipocytes of humans; it is encoded by the APLN gene and is the endogenous ligand of the orphan G protein-coupled receptor, APJ, now known as apelin receptor, APLNR. The gene encodes a 77-amino acid polypeptide (9). Active forms of apelin are expressed in many peripheral tissues (heart, lung, kidney, liver, adipose tissue, gastrointestinal tract, and endothelium) and brain regions (hypothalamus). (10)

The synthesis of apelin in adipocytes is triggered by insulin and its plasma levels are reported to increase in association with insulin resistance and hyperinsulinemia. (11) Expression of apelin in adipocytes is shown to be increased in mouse models of obesity associated with hyperinsulinemia, and apelin levels paralleled plasma insulin levels during fasting and refeeding of mice. (12) Tasci et al. (13) have also found that plasma apelin-12 was lower in patients with elevated LDL-C. While Erdem et al. (14) found significantly reduced plasma apelin levels in obese subjects with untreated type 2 diabetes compared to non-diabetic subjects, Li et al. (15) have found elevated plasma apelin levels in people with impaired glucose tolerance and type 2 diabetes mellitus.

Subjects and Methods:-

These studies include 148 elderly subjects divided into two equal groups; Group I include 74 lean subjects (34 males & 40 females) their ages ranged from 65 to 86 years group II include 74 obese subjects (40 males & 34 females) their ages ranged from 65 to 84 years, studied subjects were matched by age and sex and this groups subdivided according to BMI into 3subgroup; grade I: 30-34.9 ,grade II: 35-39.9,grade III: ≥ 40 recruited from diabetes and endocrinology outpatient clinic of Internal Medicine Department of Zagazig University Hospitals in the period from November, 2013 to April , 2015. Obese patients were stratified into three subgroups according to their fasting blood glucose based on the American Diabetes Association criteria, reported in 2015(American Diabetes Association, 2015), those non diabetic patients (n = 27),impaired fasting blood glucose(n=14) and 33 patients with type 2 diabetes.

All patients were subjected to thorough history taking and full clinical assessment including blood pressure and anthropometric variables. BMI, height, and waist (measured at a level midway between the lowest rib and the iliac

crest) and hip circumferences (widest diameter over the greater trochanters) to calculate obesity indices as follows: BMI = weight/height2 (kg/m2),waist-to-hip ratio(WHR) = waist circumference (cm)/hip circumference (cm), Mid upper arm circumference (MAC) taken midway between the olecranon process of the ulna and the acromion process of the scapula in cm (16). Exclusion criteria for all subjects included history of stroke, respiratory disease, heart failure , cancer, severe hepatic , renal diseases, thyroid, any active inflammatory diseases and abdominal surgery that could have an impact on abdominal fat distribution, as well as receiving medications that affect glucose metabolism or for weight reduction or participating in a dietary or exercise programs were excluded from the study. The ethical committee of Faculty of Medicine, Zagazig University approved our study protocol, and all participants assigned written informed consent.

Physical function included: maximal grip strength (kilograms) in the dominant hand (three measures averaged), using a Jamar_ hand-held dynamometer (Sammons Preston Rolyan, Bolingbrook, Illinois, USA), assessment of time to walk 4 m at usual walking speed and assessment of five times sit-to-stand test (FTSTS).

Fried Frailty Score

Subjects were tested for frailty using the Fried (17) criteria. A patient was identified as a frail subject when at least three Fried criteria were met, while a subject was considered non-frail when none of the criteria were met, in order to discriminate sufficiently between the subgroups. A phenotype of frailty was identified by the presence of three or more of the following determinants of frailty:

1-Unintentional weight loss of ≥5 kg in the prior year or, at follow-up, of ≥5 % of body weight in the prior year.
2-Hand grip strength of <30 kg (men) or <18 kg (women).
3-Normal walking speed of less than 0.76 m/s (more than 6 s for 4 m)
4-Poor energy expenditure in the last 3 months, reflected in more than 4 h sitting a day, less than 1 stroll/month, and no cycling or joggin.
5-The presence of self-reported exhaustion, identified by a positive answer to two questions from the Center for Epidemiologic Studies Depression scale.(18)

Do you struggle to get going? or Does everything you do take effort?

Abdominal computed Tomography (CT):-

Zigzag university hospital, Radiodiagnosis department GE (Atchiva II) medical system (USA).

CT scans were done for assessment of Subcutaneous and visceral fat Areas and to measure fat mass (FM) ,fat free mass (FFM), FFMI (kg/m2) and FMI (kg/m2). CT images extending from the third lumbar vertebrae (L3) in the inferior direction were assessed the foremost image being the one in which both transverse processes were first clearly visible. Images were analyzed with SliceOmatic V4.3 software (Tomovision), which enables specific tissue demarcation using Hounsfield unit (HU) thresholds. Skeletal muscle was identified and quantified by HU thresholds of −29 to +150 (19).

The muscles in the L3 region contain *psoas, erector spinae, quadratus lumborum, transversus abdominus,* external and internal obliques, and *rectus abdominus.* The following HU thresholds were used for adipose tissues: −190 to −30 for s.c. and i.m. adipose and −150 to −50 for visceral adipose. Tissue boundaries were manually corrected as needed. Cross-sectional areas (cm2) were computed automatically by summing tissue pixels and multiplying by pixel surface area. All CT images were analyzed by a single trained observer. Cross-sectional area for muscle and adipose tissue was normalized for stature (cm2/m2) and reported.(20)

Blood samples

Blood samples were drawn from all subjects after an overnight fasting and divided into 3 portions: and HbA1c; 1 ml of whole blood was collected into evacuated tubes containing fluoride for fasting blood glucose. Serum was separated immediately from remaining part of the sample and stored at −20 °C until analysis.

Biochemical measurements

We determined fasting blood glucose using the glucose oxidase method (Spinreact, Girona, Spain). Total cholesterol and triglycerides were measured by routine enzymatic methods (Spinreact, Girona, Spain). HDL cholesterol was determined after precipitation of the apoB-containing lipoproteins. LDL cholesterol was calculated using the Friedewald formula (21) Fasting serum insulin concentrations was measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA) The homeostasis model assessment of IR (HOMA-IR) index was calculated as follows: HOMA-IR = fasting insulin (mIU/l) × glucose (mg/dl)/405.(22) . Serum apelin (Phoenix Pharmaceuticals, Burlingame, Calif) was determined by using the apelin-12 microplate enzyme-linked immunosorbant assay (ELISA) kit

2.3. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 22.0; SPSS Inc., Chicago, IL, USA). Data were expressed using descriptive statistic (mean ± standard deviation) and were analyzed using "t" test. One-way analysis of variance (ANOVA) test was done to compare different parameters between more than two groups. Pearson correlation coefficient was used to assess the association between apelin, obesity indices, frailty phenotype parameters and the other studied metabolic parameters in lean and obese elderly. a multiple linear regression analysis was performed to detect the main predictors of serum apelin concentration in obese patients with and without type 2 diabetes mellitus P-values were considered significant if <0.05. The statistical significances of differences in the frequencies of variants between the groups were tested using the X2 test.

Results:-

Anthropometric and biochemical characteristics of the study subjects are summarized in Table 1.

Obese patients had significantly higher values of fasting blood glucose, HbA1c values, fasting serum insulin, HOMA-IR, LDL, TC and TG compared to controls. Moreover, serum apelin levels and all anthropometric indices and parameters (waist circumference WC, hip circumference HC,BMI,FM,FMI, , FFM, FFMI waist/hip ratio, Mid upper arm circumference (MAC), were significantly higher in obese patients compared to controls. On the contrary, obese patients had significantly lower levels of HDL.C compared with controls.

Obese elderly were classified as regard BMI to three groups, There were statistically significant increase of HbA1c values, fasting blood glucose, fasting serum insulin, HOMA-IR, systolic in grade III (n = 30)as compared to grade I (n = 20),and grade II (n = 24) ($p < 0.05$).in addition to, there were significantly higher values of LDL, TC and TG , WC,HC, BMI,FM,FMI ,FFM, FFMI , and MAC. **(Fig 1a, Fig1b**).There were no statistically significant different between the studied groups as respect to diastolic blood pressure and waist/hip ratio ($p > 0.05$) On the other hand, grade III obese patients had significantly lower levels of HDL.C compared with grade I and II **(Table 2).**

Table 3 shows that, Obese patients were classified to non diabetic (n=27), IFG (n=14) and diabetic group (n=33), there were statistically significant differences between the diabetic obese group ,impaired fasting group, and the non diabetic subjects in terms of HbA1c values, fasting blood glucose, fasting serum insulin, HOMA-IR, LDL ,TG, serum apelin levels, WC,HC, BMI,FM,FMI, MAC, and systolic blood pressure ($p < 0.05$). On the other hand, type 2 diabetes obese patients had significantly lower levels of FFM compared to impaired fasting group, and the non diabetic .There were no statistically significant different between the obese groups as respect to diastolic blood pressure, TC, HDL, waist/hip ratio and Max. grip strength (kg)d for men and women : $p > 0.05$) **(Table 3).**

In obese elderly patients (n=74), serum apelin levels were positively correlated with Waist ($r=.793$, $p < 0.001$), the hip ($r =.549$, $p < 0.001$), Waist /hip ratio ($r = .136$, $p < 0.05$) ,LDL ($r =.738$, $p < 0.001$), BMI ($r =.789$, $p < 0.001$), and TG ($r=.746$, $p<0.001$) HbA1c ($r =.931$, $p < 0.001$), fasting blood glucose($r = 0.738$, $p< 0.001$), fasting serum insulin($r =.877$, $p< 0.001$), HOMA-IR ($r = .877$, $p<0.001$), systolic blood pressure ($r =.555$, $p < 0.001$) and diastolic blood pressure($r = .234$, $p < 0.001$) **(Table 4).**On the other hand, serum apelin levels in obese elderly were not statistically significant correlated with the HDL ($r .179=$, $p > 0.05$) , TC ($r=.221$, $p > 0.05$) and MAC($r = 0.292$, $p > 0.05$).There were no statistically significant correlation between serum apelin levels and clinical, anthropometric or metabolic parameters in the control group (**Table 4**).

In obese group (n=74), stepwise linear regression analysis showed that, serum apelin levels were independently correlated with TC levels and 4-m walking time (s), ($p < 0.001$; (**Table 5**).

As regard phenotypic criteria of frialty

Duration of Chair-stand test (s) and 4-m walking time (s) were significant higher in obese elderly patients(27.1±7.73 vs. 16.28 ±4.65, respectively) compared with lean group(9.01±2.6 vs. 5.4±1.583, respectively). Moreover, frailty index was significant higher in obese elderly than lean group (9.01±2.6 vs. 5.4 ±1.583 , respectively). Comparing patients and controls max. grip strength (kg) for men and max. grip strength (kg) for women were lower in obese patients but not significant table 1.

In obese patients, a significantly higher duration of Chair-stand test (s) and 4-m walking time (s) were observed in grade III(35.32 ± 4.47, 21.19± 2.68 respectively) compared with grade I (19.87 ± 4.11 ,11.92 ± 2.47, respectively). and II subjects(24.43 ± 2.96 , 14.66 ± 1.77 , respectively). **table 2**. Also, frailty index was significant highest in grade III(4.33±.477) elderly compared with grade I (2.79 ±0 .412). and II subjects (3.22 ±0.296). moreover, Max. grip strength (kg) for men and Max. grip strength (kg) for women were significant lowest in grade III(27.7 ±

2.48,17.77 ±2.48 , respectively) compared with grade I (30.7±6.39, 20.7 ±6.39, respectively). and II subjects(31.03 ±.4.49,21.03 ±4.94, respectively).

Also in obese group, regarding estimation of frailty parameters in diabetic,IFG and non diabetic group: Duration of Chair-stand test (s) and 4-m walking time (s) were significant highest in non diabetic obese elderly patients(20.37 ± 4.13, 12.22 ±.2.48 , respectively) compared with IFG group(24.29 ± 3.06, 14.58 ± 1.83 respectively) and, type 2diabetic group (33.84 ±5.45 , 20.32 ±3.27 respectively). Moreover, frailty index was significant highest in diabetic obese elderly patients(4.19 ± 0.546) compared with IFG group (3.23 ± .306) and, non diabetic group(2.84 ±.413). Comparing diabetic, IFG and non diabetic max. grip strength (kg) for men and max. grip strength (kg) for women were lowest in diabetic obese patients but not significant. **table 3.**

Compared to lean controls, obese patients had significantly higher frequencies of weight loss (69% vs. 30.6%), regarding Self-reported exhaustion and Low energy expenditure, obese patients had s higher frequencies (68.9% vs.63.5 and 59.4% vs.53.6%, respectively) but these differences were not significant.

In obese groups, the highest significant frequencies of weight loss, Self-reported exhaustion and Low energy expenditure were observed in grade III (85.1%,85.3%,90,1%,respectively) compared with grade I (50%,70%,33%,respectively) and II subject (60%.61%,80%,respectively) **Fig 3**

Pearson correlation of serum apelin with frailty phenotype parameters in obese elderly patients (n = 74), serum apelin levels were positively correlated with Self-reported exhaustion($r = .819$, $p < 0.001$). Low energy expenditure($r = .819$, $p < 0.001$), duration of Chair-stand test (s) ($r = .988$, $p < 0.001$), and 4-m walking time s($r = .988$, $p < 0.001$), and frailty index ($r = .988$, $p < 0.001$), on the other hand, serum apelin levels were negatively correlated with max. grip strength (kg) for men ($r = -.238-$, $p < 0.001$) and max. grip strength (kg) for women($r = -.238$, $p < 0.001$). but serum apelin levels were not correlated with weight loss $p \geq 0.005$. **Fig.4**

Pearson correlation of serum apelin with obesity parameters in obese elderly patients (n = 74), serum apelin levels were positively correlated with BMI($r = .784$, $p < 0.001$), FM($r = .821$, $p < 0.001$), and FMI ($r = .821$, $p < 0.001$), on the other hand, serum apelin levels were negatively correlated with FFM ($r = .821$, $p < 0.001$) and FFMI($r = .821$, $p < 0.001$) but serum apelin levels were not correlated with waist/hip ratio $p \geq 0.005$. **Fig.2**

Discussion:-

The ageing process is characterized by declining functional capacity and increasing vulnerability to disease, disability, and death. This is driven by the gradual, lifelong accumulation of molecular and cellular defects (23). Individuals of a given chronological age vary, however, in how far these processes have advanced. Some enter a state of increased risk, compared with others of the same age, known as frailty (24). Obesity clearly exacerbates the age-related decline in physical function and causes frailty in older individuals. Frailty in older obese individuals may be related to the insulin resistance and inflammation that often accompany obesity (25). This is reflected by self-reported impairment in activities of daily living in the older obese individual, limitations in mobility and decreased physical performance (as detailed in the former segment), increased risk for functional decline, and a higher rate of nursing home admissions (24,26–27). Of particular significance in establishing a cause-and effect relationship between obesity and frailty is the recent report that weight loss and exercise can ameliorate frailty in older obese adults (28).

The incidence of the metabolic syndrome rises with increasing BMI, and waist circumference is more common in men older than 65 years than in younger age-groups (29). The occurrence of the metabolic syndrome reaches peak levels in the 6th decade for men and the 7th decade for women, and a decline is noted only in the 8th decade for men and for some women in different ethnic groups (29). As recently outlined by the American Heart Association Professional Education Committee of the Council for High Blood Pressure Research, older age and obesity are two of the most powerful risk factors for uncontrolled hypertension (30)

Both mouse and human adipocytes express and secrete apelin (30). They have important physiological roles and are involved in the regulation of cardiovascular and fluid homeostasis, food intake, cell proliferation and angiogenesis (32). It has been shown that apelin regulates glucose-stimulated insulin secretion and is involved in glucose homeostasis (32, 34, 35). Apelin injection can improve glucose tolerance and glucose utilization in insulin-resistant mice (34, 36). It has also been reported that insulin sensitivity was diminished in apelin-knockout mice, but could be

restored by the injection of apelin (37). Ma et al (38) found that apelin expression and circulating apelin concentrations are increased in obese, insulin-resistant animals and humans and suggested plasma apelin to be a novel biomarker for predicting type 2 diabetes in men(38).

BMI has clear limitations, and more detailed evaluation of body composition clearly revealed wasting of the lean tissues, with a majority of patients below or well below benchmark levels of muscularity known to be associated with mortality and functional disability. The estimated lean body mass of patients classified as sarcopenic was within the range described for a variety of wasted/emaciated patient populations with and without malignant disease. In the current literature it is becoming increasingly evident that concurrent sarcopenia and high fat mass is a worst case scenario and this was clearly apparent in our study group (albeit small), in which sarcopenic overweight/obese patients had the worst prognosis overall, even compared with patients who were sarcopenic and had a lower body weight. (39)

There is ongoing controversy as to what the best definition for sarcopenia is. Mourtzakis et al. (41) have previously shown that CT cross-sectional area at L3 is strongly related to appendicular skeletal mass, measured by dual-energy X-ray densitometry, used commonly in the definition of sarcopenia. Subsequent derived cutoffs for sarcopenia, based on CT, used in this study are in fact close to that described by Baumgartner et al. i.e., appendicular skeletal mass >2 SDs below a young healthy adult population). Equally, the optimal regression for conversion of CT image data to conventional units of whole-body composition measures has yet to be resolved in large population studies.(40)

In the present study, case and control group were matched by age and sex, our results provided evidence that in obese patients, irrespective to age, serum profile of apelin is different from lean ones.

To best our knowledge for the first time we have demonstrated that apelin-serum levels are increased in obese elderly and investigate possible correlations between serum apelin, obesity-related markers; including lipids, insulin sensitivity and insulin resistance index (HOMA-IR) and frailty phenotype parameters.

In the present study we observed that BMI,FM,FMI, waist ,hip circumference and waist to hip ratio of obese elderly patients were significantly higher than that of lean individuals, these findings are in concordance with Assaad et al, (38) who reported that, waist circumference of obese hypertensive patients was significantly higher than that of obese normotensive individuals as the intra abdominal fat accumulation itself may play an important role in the pathogenesis of hypertension in obesity.

In the present study, we observed that, fasting blood glucose, serum insulin, HbA1c and HOMA-IR were higher in obese group and within the obese group highly significant in diabetic subgroup patients. The same findings were observed by Assaad et al (38) and Heinonen et al (42) 2005, 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

In our study we found that obese elderly had higher serum triglycerides, LDL-C levels, and serum cholesterol levels when compared with lean individuals, moreover obese diabetic individuals had significantly higher serum triglycerides, LDL-C levels, and serum cholesterol levels when compared with the non diabetic group.
This was in agreement with Assaad et al, (38) who found that, obese hypertensive and normotensive patients had significantly higher serum triglycerides, LDL-C levels, and serum cholesterol levels when compared with non obese individuals, whereas obese non hypertensive individuals had significantly lower HDL-C when compared with the control group.

The main finding of the present study is that, Serum concentrations of apelin are significantly increased in obese patients, moreover serum apelin concentrations increase in diabetic obese elderly than nondiabetic obese subjects

This was in line with the previous studies, Boucher et al. (16) reported increased plasma apelin levels in obese adult males and suggested further that, since adipose tissue is an important source of apelin production, and the expression of apelin and APJ both increase in adipose tissue in obese individuals, elevated serum apelin of obese subjects might be attributable to augmented adipose tissue. Minor changes in plasma apelin were associated with changes in BMI in obese subjects during 8-weeks of a very-low-calorie diet.

Similar results confirmed by Li et al (43). Heinonen et al 2005 (42) they found that apelin plasma concentration was significantly higher in morbidly obese patients compared to normal-weight controls.

According to our study, in obese elderly patients, apelin levels were positively correlated with Waist, the hip, LDL, BMI, TG, HbA1c, fasting blood glucose, fasting serum insulin, HOMA-IR, FM, FMI, systolic blood pressure and diastolic blood pressure.

Correlations between apelin and insulin resistance, a major characteristic of obesity and type 2 diabetes, have been demonstrated by several authors. Erdem et al 40 demonstrated a negative correlation with HOMA-IR in newly diagnosed type 2 diabetes mellitus. Tasci et al 4(44)1. have reported a mild to moderate negative correlation between apelin and HOMA-IR in patients with elevated LDL-C. In contrast, Li et al. (42) found a positive correlation with HOMA-IR in patients with impaired glucose tolerance and type 2 diabetic subjects, and Hosoya et al.(46) suggested that plasma apelin levels increased markedly in insulin resistance and hyperinsulinemia.

Baet al et (47) found that, HOMA-IR was significantly higher in all obese subjects compared to that in non-obese controls. Taken together, our results and those reported previously indicate that different associations between apelin and insulin resistance may depend on the extent of insulin resistance. Insulin resistance is common to both obesity and type 2 diabetes and apelin is linked with obesity-associated variations of insulin sensitivity status.

Similar results observed by Castan-Laurell et al. (48) who suggested that apelin may act as an insulin sensitizing agent and may be a potential target for diabetes treatment that is, given its potent activity in energy metabolism and ability to improve insulin sensitivity. In contrast, Reinehr and colleagues (2011) (49) evaluated apelin concentration, weight status, body fat, insulin resistance, leptin and obesity-related cardiovascular risk factors before and after oneyear lifestyle intervention, demonstrating that weight loss in obese children was not associated with changes in apelin concentration as the authors have hypothesized, and no significant relationships were found between apelin, insulin resistance, cardiovascular risk factors and obesity in children

In contrast, Heinonen et al 2009 (42) Those authors have suggested that apelin may not correlate as strongly with fat mass as with more abundant adipokines such as leptin and adiponectin.

Assaad et al (38) did not observe significant correlation between apelin concentration and BMI, waist circumference, and HOMA-IR, they explained that due to 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).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.

To support our results, we further analyzed which clinical, metabolic and anthropometric parameters independent correlated with serum apeline in obese women, we found that, serum apelin-2 levels were independently correlated with HbA1cvalues and visceral CT.

Association of frailty phenotypic criteria with serum apelin, obesity-related markers:

Frailty is the most problematic expression of population ageing. It is a state of vulnerability to poor resolution of homeostasis following a stress and is a consequence of cumulative decline in multiple physiological systems over a lifespan.The brain, endocrine system, immune system and skeletal muscle are intrinsically inter-related and are currently the organ systems best studied in the development of frailty (50). Frailty has also been associated with loss of physiological reserve in the respiratory (51), cardiovascular (52), renal (53) and haemopoietic and clotting systems (52,53) and that nutritional status can also be a mediating factor (56, 57-58).

Large waist circumference, greater body mass index (BMI) and weight-gain in middle age are all associated with higher mortality or lower healthy survival (59,60). In addition, low skeletal muscle mass is associated with increased likelihood of functional impairment and disability.

Blood pressure (BP) (61) and blood lipids (63) are currently the strongest predictors of CV morbidity and mortality. Increases in diastolic BP and systolic BP are associated with increased risk of CV mortality (60) and high BP in midlife with cognitive decline in later life. Ageing is associated with reduced metabolic capacity exemplified by diminished glucose homeostasis. Raised fasting blood glucose and glycated haemoglobin (HbA1C) are associated with age, CV events and mortality, cognitive impairment, and dementia, in non-diabetics(62).

Frailty and comorbidity (defined as two or more of the following nine diseases: myocardial infarction; angina; congestive heart failure; claudication; arthritis; cancer; diabetes; hypertension; chronic obstructive pulmonary disease) was present in 46.2% of the population, frailty and disability (defined as the presence of restriction in at least one activity of daily living) was present in 5.7%, and the combination of frailty, disability and comorbidity was present in 21.5% of the study group. Importantly, frailty was present without comorbidity or disability in 26.6% of the study group. This finding provides support for frailty as an independent concept, distinct from comorbidity and disability. However, more recent work suggests that the overlap is more frequent and increases with greater frailty (64)

The main finding of our study, Regarding separate phenotype of frailty:Self-reported exhaustion, Low energy expenditure, duration of Chair-stand test s, and 4-m walking time s, frailty index, weight loss, Max. grip strength (kg) for men and Max. grip strength (kg) for women are significantly associated with obesity especially grade III obesity, moreover, these phenotype parameter of frailty increase in diabetic obese elderly than nondiabetic obese subjects on the other hand, serum apelin levels were positive correlated with Self-reported exhaustion, Low energy expenditure, duration of Chair-stand test s, and 4-m walking time s, frailty index and negatively correlated with Max. grip strength (kg) for men and Max. grip strength (kg) for women but serum apelin levels were not correlated with weight loss.

Osher and Stern(65) found that, obese, or overweight, older subjects with such presumed unimpaired longevity are nevertheless more likely to have hypertension and diabetes; develop coronary artery disease and possibly stroke; experience erectile dysfunction; suffer from accelerated loss of cognitive function, incontinence, frailty, osteoarthritis, and functional disability; and are dependent on others. The clustering of so many well-defined ailments resulting from, or associated with, obesity, particularly in older subjects, is impressive enough to view obesity as a real primary disease that requires attention and medical care.

Limitation of our study, we used the Fried criteria to assess frailty in our subjects. Although this phenotype model has been validated and generally accepted, other important factors such as cognitive impairment, a highly prevalent condition associated with functional decline and disability, have not been included in the Fried criteria so we need new phenotype models include cognitive impairment and therefore future studies to confirm this results.

Fig (1): CT scan analysis with Hounsfield units used to measure area of skeletal muscle, subcutaneous fat and visceral fat (a) of mild obesity, (b) marked obesity.

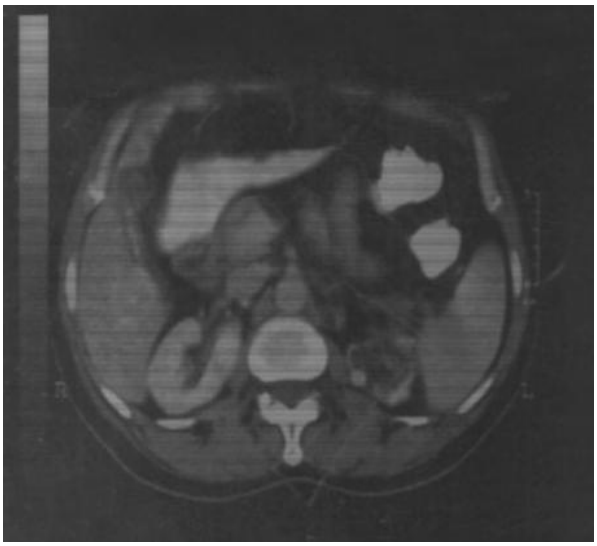

Fig (1a)

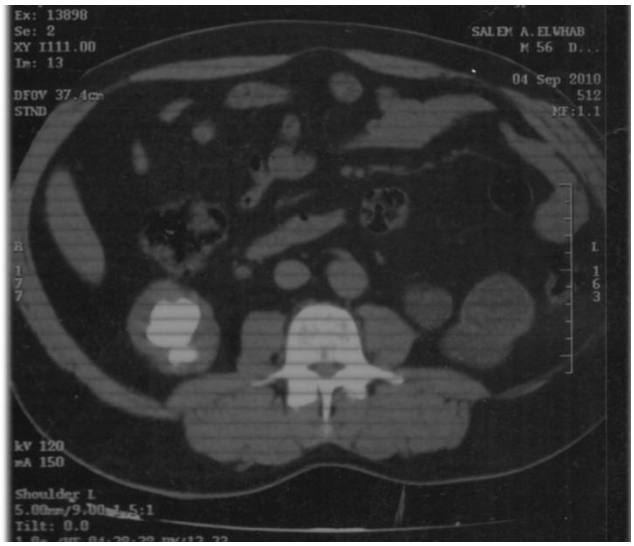

Fig (2b)

Fig.(2): correlation of serum apelin with FM, FMI, FFM, FFMI, and BMI in obese group

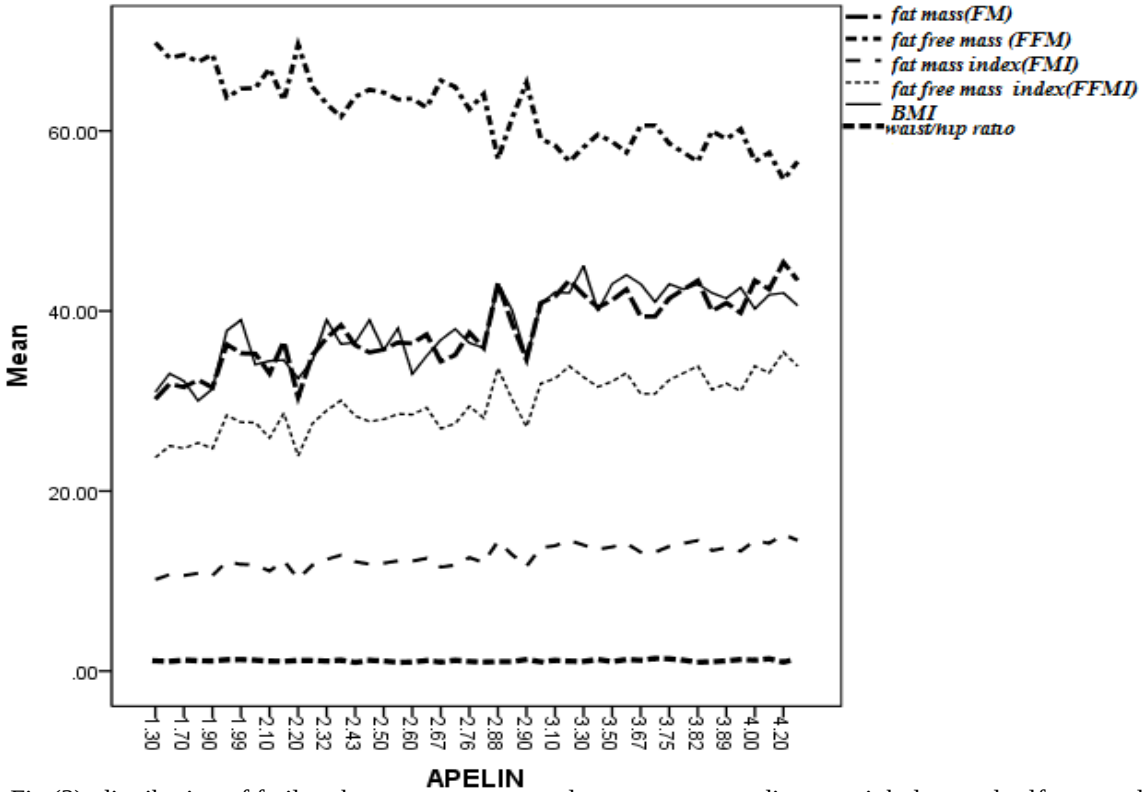

Fig (3): distribution of frailty phenotype parameters low energy expenditure, weight loss and self-reported exhaustion in all studied subject stratified according to BMI.

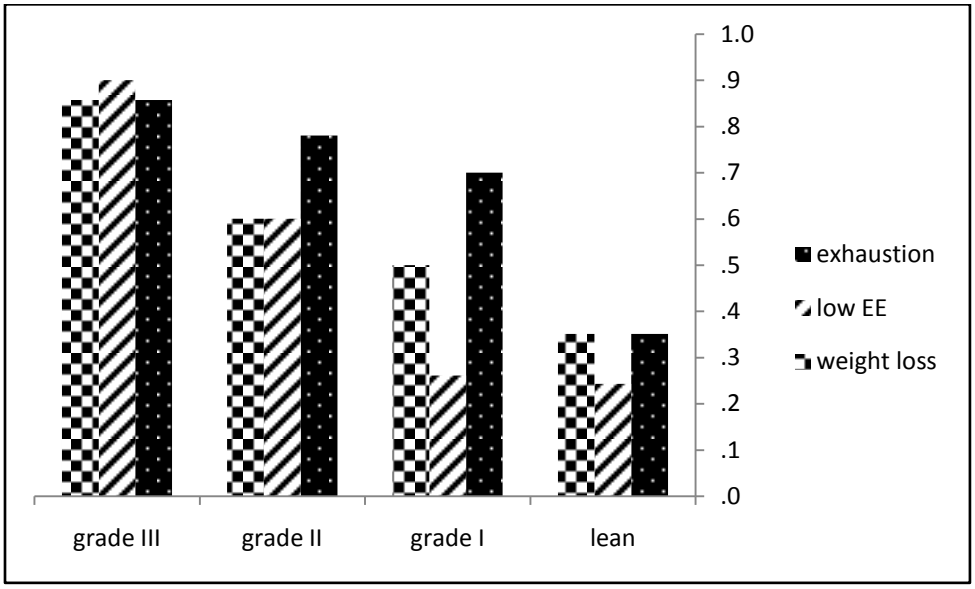

Fig.(4): Correlation of serum apelin with frailty phenotype parameters in obese elderly subject

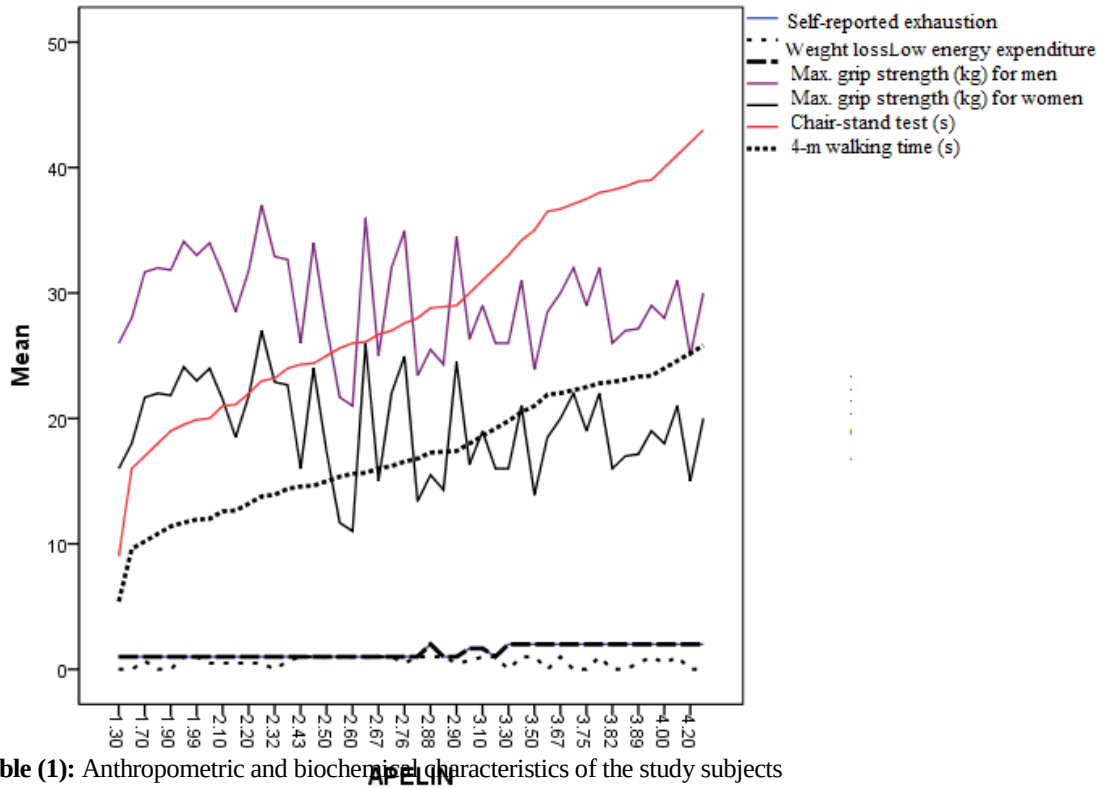

Table (1): Anthropometric and biochemical characteristics of the study subjects

	Control (n = 74)	**Obese patients (n = 74)**	**F**	**P value**
Age (years)	71.14±5.33	69.5270±4.3	4.1	.044
Systolic blood pressure (mmHg)	126.5±6.6	137.4±7.1	91.8	<0.001*
Diastolic blood pressure (mmHg	85.1±3.9	99.5±3.2	64.4	<0.001*
Body mass index (kg/m^2)	22.8±3.4	37.3±4.5	473.8	<0.001*
Waist circumference (cm)	84.2 ±7.9	117.2±12.9	348.2	<0.001*
Hip circumference (cm)	85.5±9.4	102.5±14.2	72.9	<0.001*
Waist/hip ratio	.99±.09	1.1±.12	77.9	<0.001*
FMI(kg/m2)	7.46±.809	12.40±1.44	165.5	<0.001*
FM(kg)	22±2.44	36.99±4.38	163. 5	<0.001*
FFM(kg)	17.37±1.89	63.01±4.388	155.8	<0.001*
FFMI(kg/m2)	5.4±1.58	28.94±3.37	159.5	<0.001*
Frailty score , a	1.71±.266	3.51±.773	165.5	<0.001*
Chair-stand test (s) d	9.01±2.63	27.14±7.73	162.5	<0.001*
Max. grip strength (kg)d, mean				
Men	30.2± 5.63	29.7 ±4.71	0.347	0.557
Women	20.2± 5.6	19.7 ±4.9	0.347	0.557
4-m walking time (s)	5.41±1.58	16.27±4.65	361	<0.001*
MAC(cm)	27.9±2.0	30.8±2.3	316	<0.001*
Total cholesterol (mg/dl)	167.6±20.4	202±13.7	143.4	<0.001*
Triglycerides (mg/dl)	86.9±20.1	123.2±14.9	230	<0.001*
HDL cholesterol (mg/dl)	55.4±4.2	39.4±6	155.3	<0.001*
LDL cholesterol (mg/dl)	141.3±16.8	196.6±33.3	161.6	<0.001*.
Fasting blood glucose (mg/dl)	83.9±8.6	101.9±12.8	100.4	<0.001*
Fasting serum insulin (µU/ml)	13±3.2	48±23.2	164.8	<0.001*
HbA1c (%)	5.7±.15	6±.17	69.7	<0.001*
HOMA-IR	2.7±.7	12.6±7.5	129.85	<0.001*
Serum apelin (ng/mL)	.9±.26	2.7±.7	376.4	<0.001*

a- Fried frailty score: the total sum for the presence of each item: unintentional weight loss of C5 kg in the prior year or, at follow-up, of C5 % of body weight in prior year; hand grip strength of\30 kg (men) or\18 kg (women); normal walking speed of less than 0.76 m/s (more than 6 s for 4 m); poor energy expenditure in the last 3 months reflected in more than 4 h sitting a day, less than 1 stroll/month, and no cycling or jogging; the presence of self-reported exhaustion, identified by a positive answer to two questions from the CES-D scale (Weissman et al. (18): Do you struggle to get going? Does everything you do take effort.

b- Maximal grip strength (kilograms) in the dominant hand (3 measures averaged), using a Jamar handheld dynamometer.

c- Time in seconds to walk 4 m at normal speed.

d- Time in seconds for a subject to stand up 5 times from a chair with the arms in a crossed position in front of the chest.

HOMA-IR: homeostasis model assessments of Insulin resistance; MAC, mid arm circumference; FM, fat mass; FMI, fat mass index; FFM, fat free mass ;FFMI, fat free mass index

* $P < 0.05$ when compared with control group

Table (2): Clinical & demographic data in obese group stratified according to BMI to grade I, grade II and grade III

	Obese patients		

	Grade I N=20	**Grade II N=24**	**Grade III N=30**	**F**	**P**
Systole (mmHg)	135.6 ± 4.8	132.8 ± 5.6	143.3 ± 5.7	56.40	<0.001*
Diastole(mmHg)	86.4 ± 3.5	87.8 ± 4.2	118.4± 145.8	1.96	0.12
Hip circumference (cm)	91.9 ± 9.1	102 ± 10	114 ± 14.6	68.82	<0.001*
Waist circumference (cm)	107.2 ± 6.1	112.8 ± 6.6	130.7 ± 7.3	486.87	<0.001*
Waist/hip ratio	1.16±0.104	1.12±0.118	1.16±0.144	0.775	0.464
MAC(cm)	30.4 ± 2.4	30.1 ± 2.3	32.1 ± 2.1	31.92	<0.001*
BMI(Kg/m^2)	32.2 ± 1.2	37.1 ± 1.4	42.2 ± 1.6	758.62	<0.001*
FM(kg)	32.21±2.12	36.75±1.60	41.75±1.56	184.422	<0.001*
FMI(Kg/m2)	10.82±7.701	12.32±.530	13.97±.517	184.422	<0.001*
FFM(kg)	67.78±2.12	63.24±1.60	58.24±1.56	184.422	<0.001*
FFMI(Kg/m2)	25.26±1.6	28.76±1.23	32.61±1.20	184.422	<0.001*
Max. grip strength (kg)d, mean					
Men	30.7±6.39	31.03±4.94	27.78±2.48	1.64	<0.001*
Women	20.70±6.39	21.03±4.94	17.77±2.48	1.64	<0.001*
4-m walking time (s)	11.92±2.47	14.66±1.77	21.19±2.68	66.512	<0.001*
Frailty score	2.79±.412	3.24±.296	4.33±.447	66.512	<0.001*
Chair-stand test (s)	19.87±4.11	24.43±2.96	35.32±4.47	66.512	<0.001*
Cholesterol (mg/dl)	203.7 ± 10.7	202 ± 9.6	204.1 ± 14.1	58.55	<0.001*
TG(mg/dl)	167.9 ± 13.9	189 ± 11.1	230.3 ± 29.4	263.52	<0.001*
LDL(mg/dl)	84.6 ± 12.8	76.2 ± 10.1	66.3 ± 16.8	26.25	<0.001*
HDL(mg/dl)	43.5 ± 2.2	40.8 ± 2	34.2 ± 6	199.21	<0.001*
F. glucose(mg/dl)	92.2 ± 7.3	95.1 ± 5.9	114.7 ± 9.4	99.92	<0.001*
F. insulin (mIu/L)	23.3 ± 3.1	39.8 ± 5.5	73.8 ± 13.9	543.66	<0.001*
HOMA-IR	5.3 ± 1	9.3 ± 1.4	21.1 ± 5	431.18	<0.001*
HbA1C	5.9 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	46.13	<0.001*
Serum apelin (ng/mL)	2.01±0.104	2.4±0.29	3.5±0.447	109.73	<0.001*

HOMA-IR: homeostasis model assessments of Insulin resistance; MAC, mid arm circumference; FM, fat mass; FMI, fat mass index; FFM, fat free mass ;FFMI, fat free mass index
* $P < 0.05$ when compared with control group

Table (3): Clinical & demographic data in obese group stratified according to ADA classification of diabetes

	Obese patients		

	NBG N=27	IBG N=14	Type 2 DM N=33	F	P
Systole (mmHg)	134.8±4.8	131±6.2	142.3±5.7	25.27	<0.001*
Diastole(mmHg)	86.7±3.9	86.2±4.2	115.5±9	1.23b	0.296
Hip circumference (cm)	94.3±12	103±8.5	109±14.6	9.8	<0.001*
Waist circumference (cm)	107±8.3	113.3±6.3	127.3±10.6	37.9	<0.001*
Waist/hip ratio	1.15±0.11	1.10±0,11	1.17±0.13	1.238	0.298
BMI(Kg/m^2)	32.4±2.5	37.2±1.1	41.3±2	134.3	<0.001*
MAC(cm)	30.4±2.1	29.5±2.8	31.7±2	5.2	<0.001*
FM(kg)	32.69±2.39	36.5±1.05	41.2±1.9	139.04	<0.001*
FMI(Kg/m2)	10.9±0.7	12.2±0.3	13.8±0.6	139.04	<0.001*
FFM(kg)	67.3±2.4	63.4±1.05	58.7±1.9	139.04	<0.001*
FFMI(Kg/m2)	25.6±1.8	28.5±0.8	32.2±1.4	139.04	<0.001*
Max. grip strength (kg)d, Men Women	 30.4±6.1 20.4±6.1	 30.9±5.6 20.9±5.6	 28.6±3.1 18.5±3	 1.645 1.645	 0.200 0.200
4-m walking time(s)	12.2± 2.4	14.5±1.8	20.3±3.2	66.512	<0.001*
Frailty score	2.8±0.4	3.2±0.3	4.1±0.5	66.512	<0.001*
Chair-stand test (s)	20.3±4.1	24.2±3.1	33.8±5.1	66.512	<0.001*
Cholesterol (mg/dl)	200.7±16.6	199.5±6.6	204±13.4	.69	<0.001*
HDL(mg/dl)	43.5±3.8	40.8±2.1	35.3±6.1	35.3	<0.001*
LDL(mg/dl)	169.1±16.8	191.7±8	221.2±31.9	.36	<0.001*
TG(mg/dl)	123.3±17.5	120.3±6.7	124.4±15.3	22	<0.001*
F. glucose(mg/dl)	93.2±6.7	95.7±5.5	111.6±12.1	31.8	<0.001*
F. insulin (mIu/L)	25±5.4	41.5±3.8	69.7±15.9	119.2	<0.001*
HOMA-IR	5.7±1.4	9.8±1	19.5±5.8	89.4	<0.001*
HbA1C	5.8±.13	5.9±.1	6.1±.14	22.9	<0.001*
Serum apelin (ng/mL)	2±.37	2.4±.3	3.3±.54	68.67	<0.001*

HOMA-IR: homeostasis model assessments of Insulin resistance; MAC, mid arm circumference; FM, fat mass; FMI, fat mass index; FFM, fat free mass ;FFMI, fat free mass index
* $P < 0.05$ when compared with control group

Table (4): Pearson Correlation Coefficient between Serum apelin (ng/mL) and clinicaland laboratory parameters in lean andobese elderly groups.

	Lean n=74		Obese n=74	
	r	P	r	P
Body mass index (kg/m^2)	.039	.744.	.789	.000
Waist circumference (cm)	-.107	.363	.793	.000
Hip circumference (cm)	-.042	.721	.549	.000
Waist/hip ratio	.128	.276	.136	.012
Hand Grip(kg)	-.095	-.095	-.202	.084
4(m/s)-meter speed	-.081	-.081	-.356	.002
MAC(cm)	.077	.906	.292	.249
Systolic blood pressure (mmHg)	.014	.795	.555	.000
Diastolic blood pressure (mmHg)	.031	.067	.234	.044
Total cholesterol (mg/dl)	.214	.153	.221	.059
Triglycerides (mg/dl)	.168	.009	.746	.000
LDL cholesterol (mg/dl)	-.023	.846	.738	.000
HDL cholesterol (mg/dl)	.172	.143	.179	.127
Fasting blood glucose (mg/dl)	-.026	.827	.726	.000
Fasting serum insulin (µU/ml)	.129	.129	.877	.000
HbA1c (%)	.031	.795	.931	.000
HOMA-IR	.102	.388	.872	.000

Table 5: Multiple linear regression analysis in obese elderly to test the influence of the main independent variables against serum apelin levels (dependent variable).

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95% Confidence Interval for B	
		B	Std. Error	Beta			Lower Bound	Upper Bound
1	(Constant)	-4.656-	2.574		-1.809-	.076	-9.809-	.497
	Hip circumference	-9.348E-5	.001	-.002-	-.154-	.878	-.001-	.001
	Waist circumference	.000	.001	-.006-	-.390-	.698	-.002-	.001
	waist/hip ratio	.001	.003	.004	.478	.634	-.004-	.007
	TC	-.002-	.000	-.029-	-3.557-	.001	-.002-	-.001-
	LDL	.000	.000	-.006-	-.481-	.632	-.001-	.000
	TG	-.002-	.002	-.016-	-1.197-	.237	-.005-	.001
	Fasting glucose	.002	.001	.034	1.495	.141	-.001-	.005
	Fasting insulin	.004	.003	.113	1.369	.177	-.002-	.009
	HOMA	-.014-	.010	-.135-	-1.355-	.181	-.034-	.007
	HBA1c	.053	.045	.012	1.166	.249	-.038-	.144
	Fat free mass	-.009-	.006	-.011-	-1.377-	.174	-.022-	.004
	4-m walking time	.159	.003	.969	57.415	.000	.153	.165
	Self-exhaustion	.036	.028	.022	1.293	.201	-.020-	.092
	Weight loss	-.016-	.014	-.010-	-1.171-	.247	-.043-	.011
	Energy expenditure.	-.007-	.017	-.004-	-.394-	.695	-.042-	.028
	Hand grip for women	-.002-	.001	-.014-	-1.417-	.162	-.005-	.001

Conclusion

We conclude that serum apelin and phenotype parameters of frailty were higher in obese than lean elderly. In obese group, serum apelin and phenotype parameters of frailty positively correlated with HbA1c values, fasting blood glucose, fasting serum insulin, HOMA-IR, LDL ,TG, BMI and fat free mass .Considering these data together makes serum apelin is a potential biomarker for obesity-related low-grade inflammation in elderly and frailty.

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