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Original Research Article

Comparison of Serum Visfatin Level in Normal Weight,Overweight and Obese Individuals

Sudipta Kumari Onkar[1], Rasmita Kumari Padhy[2], Nirupama Devi[3], Bishnupriya Panda[4], Sucheta Panda[5], Manaswini Das[6], Subrat Kumar Pradhan[7]

[1]Senior Resident, Department of Biochemistry, MKCG MCH, Berhampur, Odisha, India
[2]Professor & Head, Department of Biochemistry, SRM MCH, Odisha, India
[3]Professor & Head, Department of Biochemistry, MKCG MCH, Berhampur, Odisha, India
[4]Senior Resident, Department of Biochemistry, MKCG MCH, Odisha, India
[5]Associate Professor, Department of Biochemistry, MKCG MCH, Berhampur, Odisha, India
[6]Senior Resident, Department of Biochemistry, MKCG MCH, Berhampur, Odisha, India
[7]Post Graduate Trainee, Department of Biochemistry, MKCG MCH, Berhampur, Odisha, India

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Corresponding Author: Nirupama Devi
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Abstract:

Introduction: Adipose Tissue Is Not Only The Storehouse Of Fats And Energy But Also A Major Endocrine Organ Secreting Adipocytokines. Adipocytokines Play An Important Role In Regulation Of Food Intake & Body Weight, Insulin Sensitivity,Inflammation And Vascular Haemostasis. Visfatin Is A Novel Adipokine (52kda) Predominantly Secreted From Visceral Fat. It Shows Insulin-Mimetic Effects Which Correlate With Overweight & Obesity.Our Aim Is To Compare Serum Visfatin Level In Normal Weight,Overweight &Obese Individuals.

Materials & Methods: This Is A Cross-Sectional Comparative Study Of Total 162 Adults Between Age Group Of 20-60yrs Including Both The Sexes. All The Individuals Are Divided Into 3 Groups With 54 Subjects Each As Per Bmi. 3ml Of Whole Blood Is Collected With Informed Consent From All Individuals.Serum Visfatin Level Is Analyzed By Elisa. All Data Are Presented As Mean (M)& Standard Deviation (±Sd).Statistical Analysis To Be Done Using Spss Version 20. The Values Are Considered Significant At P <0.05.

Results: Fasting Serum Visfatin Level Is Increased In Overweight (100.8+29.9pg/Ml) & Obese Individuals (129.1+21.4 Pg/ Ml) As Compared To Normal Weight Subjects (82.4+26.5pg/ Ml) Which Is Statistically Significant (P<0.012).

Conclusion: Serum Visfatin Is Elevated In Overweight & Obese Individuals Compared To Normal Individuals.The Significant Increase Level Of Visfatin In Obese May Be Used As A Diagnostic And Prognostic Biomarker For Insulin Resistance And Metabolic Syndrome.

Keywords: Adipokines, Visfatin, Bmi &, % Bfm.

Introduction

Obesity is defined as an abnormal growth of adipose tissue due to an enlargement of fat cell size (hypertrophic obesity) or an increase in fat cell number (hyperplasic obesity) or a combination of both [1]. Moreover, obesity is one of the most prevalent non-communicable disease with major health concerns. This is a condition characterised by the excessive accumulation of fat in the visceral and subcutaneous regions. The excess
weight gain occurs when energy intake exceeds the energy consumption. This excess weight is stored in adipose tissue consisting of adipose cells or adipocytes with an incredible capacity to store the excess energy in the form of lipids.

Obesity is one of the most common & neglected, public health problem in both developed and developing cou tries as per World Health Organization (WHO) [2].Obese individuals have higher rates of mortality and morbidity as compared to non-obese individuals [3]. Obesity is the leading preventable cause of death worldwide and serious public health concern of the 21st century.

In India, more than 135 million individuals were affected by obesity.

According to World Health Organisation Statistics Report 2016, 1.9 billion adults, 18yrs and older, were overweight, of these over 650 million were obese [4]. In 2019, more than 38.2million children under 5 years of age were overweight or

obese. [5]India with 1.2 billion people is now the second-most populous country in the world and is currently experiencing rapid epidemiological transition from under nutrition to obese. [6] According to, ICMR-INDIAB study 2015, prevalence rate of obesity varies from 11.8% - 31.3%. The prevalence of overweight will more than double among Indian adults aged 20-69 years between 2010 and 2040, while the prevalence of obesity will triple. Specifically, the prevalence of overweight and obesity will reach 30.5% (27.4-34.4%) and 9.5% (5.4-
13.3%) among men and 27.4% (24.5-30.6%) and 13.9% (10.1-16.9%) among women respectively by 2040. [7]

Who Classification of Adults According to Bmi

Classification	BMI	Risk of Comorbidites
Underweight	<18.50	Low
Normal range	18.5-24.99	Average
Overweight	>25.00	High
Pre-obese	25.00-29.99	Increased
Obese class I	30.00-34.99	Moderate
Obese class II	35.00-39.99	Severe
Obese class III	>40.00	Very Severe

Visfatin is a novel adipocytokine predominantly secreted from visceral fat and found in Liver, muscle, bone marrow, lung, heart, placenta, kidney tissue and peripheral lymphocytes.[8]Visfatin corresponds to a protein identified previously as pre- B cell colony enhancing factor (PBEF), a 52-kilodalton cytokine expressed in lymphocytes. [9]. Visfatin/ PBEF gene consists of 11 exons & 10 introns spanning 34.7kb, is located on chromosome 7q22. [10] Visfatin (NAMPT), with its two isoforms, the extracellular(eNAMPT) and intracellular (iNAMPT) forms, is crucial for NADbiosynthesis, with the higher activity of the extracellular form. NAD isrequired for various processes, including metabolic processes, glucosestimulated insulin secretion, cell survival, cell cycle control, and apoptosis [11].

Circulating visfatin levels are closely correlated with WAT (White Adipose Tissue) accumulation, visfatin mRNA levels increase in the course of adipocyte differentiation, and visfatin synthesis is regulated by several factors, including glucocorticoids, TNF-α, IL 6, and GH6. Visfatin plasma concentrations and visceral visfatin mRNA expression correlated with measures of obesity.

Visfatin were shown to be increasedin females with visceral obesity. Decrease in circulating visfatinwas found in morbidly obese women who lost more than 20% of theirBMI [12], also increased plasma Visfatin concentrations in morbidly obesesubjects are reduced after gastric banding [13]. These studies show thatmore the BMI (obesity) more the visfatin levels and levels decrease afterweight loss.

Visfatin binds to &activates the insulin receptor exerting Insulin-mimetic effects which correlatewith ove weight and Obesity.[14]Increased visfatin levels in Obesity may indicate a regulatory response to keep blood glucose levels stable.Plasma levels of visfatin associated to a greater extent with amount of visceral fat than that of adipose tissue. [15] Recent research has demonstrated that visfatin reduces insulin-stimulated glucose metabolism in skeletal muscle causing Insulin resistance. [16]

Studies have shown that an increased level of visfatin is associated withIR and T2DM [16,17], metabolic syndrome (MetS) [18], polycystic ovarysyndrome[19] and T2DM-associated complications such ascardiovascular [20,21], cerebrovascular [22, 23], and renal [24, 25, 26]diseases.

Materials & Methods

Place of Study: The study was conducted in department of Biochemistry, M.K.C.G.Medical College and Hospital, Berhampur, Odisha. Samples werecollected from medical staffs and colleagues, attendant of patients, MBBSand nursing students with approval of Institutional ethical committeeand informed consent was taken.

Study Period: February 2021- November 2022.

Selection of Study Population: A total of 162 individuals were included in this study in the agegroup of 20-50years, including both sexes. All the individuals wereclassified into three category according to body mass index (BMI) –Normal weight, Overweight, and Obese in accordance withrecommendations of World Health Organizations and waistcircumference (WCF) ≥ 90cms in men and ≥80cms in women and waisthip ratio (WHR) ≥1.0 in men and ≥0.85 in women. [27]

Healthy Individuals Group I (GP-I): Age, sex and BMI matched apparently healthy individual recruited as controlled with normal body weight, BMI-(18.0-24.99)

Overweight Individuals Group II (GP-II); Individuals with BMI –(25.00-29.99)

Obese Individuals Group III (GP-III): Individuals with BMI - ≥30.00

Inclusion Criteria

1. Age group between 20- 50years
2. Both the sex
3. Normal weight, Overweight & Obese individuals.

Exclusion Criteria

1. Age below < 20yrs & ≥50 years.
2. Diabetes mellitus.
3. Hypertension.
4. Thyroid disease.
5. Drug history like steroid, OCP.
6. Any chronic illness like T.B., Liver, kidney and heart diseases.
7. Pregnancy.
8. Smoking and alcoholism.
9. PCOS.

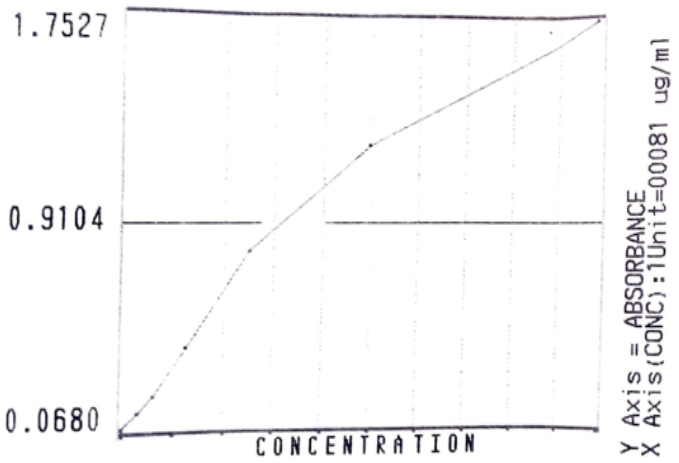
Collection of Blood Sample: 5ml ofwhole blood was collected.Two ml of blood was kept for estimation of fasting plasma glucose Three ml of blood was kept in serum separator tube for two hours at room temperature and then centrifuged at approximately 2000 to 3000 rpm for 15 to 20 minutes for Visfatin assay. by ELISA kit. The serumsamples were brought to room temperature before starting the assay.

Estimation of Human Serum Visfatiin: Visfatin is a recently described adipokine preferentially secreted by visceral adipose tissue (VAT) with insulin mimetic properties.Serum Visfatin was estimated using the commercially availableELISA kit marketed by Abbkine, Inc. with the Catalog numberKTE62221.

Assay principle: Human serum Visfatin ELISA Kit employs a two-site sandwich ELISA to quantitative Visfatin in samples. An antibody specific for Human Visfatin has been pre-coated onto a micro plate. Standards and samples are pipetted into the wells and any Visfatin present is bound by the immobilized antibody. After removing any unbound substances, HRPConjugatedVisfatin detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and colour develops in proportion to the amount of Visfatin bound in the initial step. The colour development is stopped and the intensity of the colour is measured.

Absorbance of Visfatin Standard Solutions and Their Concentration

Standard	Concentration (pg/ml)	Absorbance in nm
SD1	0	0.0680
SD2	25	0.1278
SD3	50	0.1972
SD4	100	0.3925
SD5	200	0.7919
SD6	400	1.2302
SD7	800	1.7527



Standard Curve for Visfatin

Result:

Table 1: Age and sex distribution in study groups

Age group	Normal weight			Overweight			obese		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
20-35	10	14	24	7	21	28	8	13	21
36-50	12	18	30	12	14	26	16	17	33
total	22	32	54	19	35	54	24	30	54

This table shows age and sex distribution of all three groups. All the groups were divided into two groups according to age i.e. 20-35 years and 36-50 years. Maximum cases were within the range of 36- 50 years. Percentage of females was more in comparison to males.

Table-2: Comparison of anthropometric data in study groups

Parameters	Normal Weight (n=54) MEAN+SD	Overweight (n=54) MEAN +SD	Obese (n=54) MEAN +SD	'P' Value
Age	37.6±10.8	36.4±9.3	36.8±9.2	0.862
Height	157.7±8.4	157.4±6.7	157.5±8.9	0.980
Weight	59.2±10.3	69.5±7.2	81.7±11.3	**0.000****
BMI	22.8±1.6	27.6±1.3	32.8±2.7	**0.000****
BFM	24.7±8.7	34.5±8.5	43.4±11.4	**0.000****
WC	82±10.7	90.03±7.01	106.4±11.4	**0.000****
HC	93.9±7.10	102.6±6.1	110.3±11.3	**0.000****
WHR	0.87±0.04	0.88±0.06	0.96±0.10	**0.000****

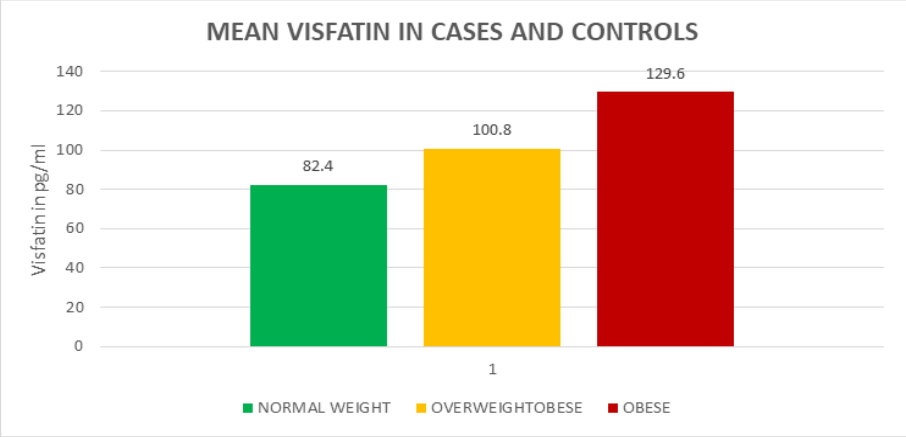
P value- <0.05* is significant

The above table depicts about comparison of demographic data of the three groups. The mean and standard deviation of AGE, HEIGHT, WEIGHT, BMI, %BFM, WHR, WC & HC has been tabulated. The data were compared between the three groups. The Mean ± SD of demographic parameters of Overweight and Obese groups are more as compared to Normal weight group which is statistically significant, p<0.05.

Table 3: Comparison of serum visfatin in study groups

Parameter	Norma Weight (n=54) MEAN+SD	Overweight (n=54) MEAN+SD	Obese (n=54) MEAN+SD	'P' Value
Serum Visfatin (pg/ ml)	82.4+26.5	100.8+29.9	129.1+21.4	**0.012****

*p<0.05 is significant.



Graph 1:

Table-3 and graph 1 showed the mean serum Visfatin values in normal weight, overweight and obese individuals. The valueswere compared in the three study groups.

Discussion

Obesity and overweight are defined as a systemic disease that shows excessive and abnormal

accumulation of body fat leading to adverse health effect. Obesity imposes devastating health and financial tolls on individuals and society [3]. Obesity has reached epidemic proportions and is a major contributor to the global burden of chronic diseases and disability [28] [4]. Obese people are at a risk of various health issues which can lead to their further morbidity and mortality [29]. In the present study, we have taken 162 healthy individuals. They were grouped into 3 categories according to BMI as normal weight, overweight and obesity. We compared serum Visfatin in cases and controls.

Age and Sex Distribution of Cases and Controls

In the study, all the individuals were again divided into two age groups 20-35yrs and 36-50years. Maximum cases and controls were within the ranges of 36-50 years. The prevalence of obesity was more in females as compared to males (Table-1).

F Garawi. et al. (2014) also had a similar finding in their case control study in which prevalence of obesity shows gendered patterning with greater prevalence and greater heterogeneity in women than in men (P<0.001)[30]

Reem T.Atawia.et al.(2019) had a similar finding in their case control study in which prevalence of obesity in females was higher than males. [31]

K. Sarvottam et al. (2020) also had a similar finding in their case –control study in Varanashi, India in which prevalence of obesity infemales was higher than in males. [32]

Shahrukh Khan. et al. (2021) showed that the prevalence of obesity was higher in females than in male. [33]

Women had higher mean value of BMI whereas men had lower and it unquestionably proposes that female gender involves in weight gain and obesity among women. [34] High prevalence of obesity in females may be due to eating food habits, stress, physical inactivity& hormonal imbalance. Women have a higher tendency to accumulate abdominal visceral fat as compared to Men. [35] The accumulation of abdominal visceral fat in women, which is a strong independent predictor of mortality, is mainly due to the higher dietary fat uptake by theirabdominal visceral fat. [36]

Antropometric Parameters of Cases and Controls

In the present study, we observed the mean of demographic parameters like Weight(Kg), BMI(kg/m2),%BFM, Waist circumference (cm), Hip circumference (cm), and Waist Hip Ratio (WHR) in healthy overweight & obese individuals were significantly higher as compared to healthy normal weight individuals.The Mean±SD of BMI is (32.8±2.7) in obese (27.6±1.3) inoverweight and (22.8±1.6) in normal weight individuals.The Mean±SD of %BFM is (43.4±11.4) in obese, (34.5±8.5) inoverweight and (24.7±8.7) in normal weight individuals.The Mean±SD of WHR is (0.96±0.10) in obese, (0.88±0.06) inoverweight and (0.78±0.04) in normal weight individuals which werestatistically significant, $p < 0.05$. (Table-2)

The study done by Seema Dhuria. et al. (2014) carried a study on women (n=100) in Rajasthan, India. They found that the mean ± S.D of BMI (kg/m2) in obese women (29.03± 3.73),WCF (96.58 ± 9.43) and WHR (0.93 ± 0.06) were significantly higher than non-obese woman BMI (21.29± 2.61), WCF (78.37 ± 13.77) and WHR (0.84 ± 0.73)which was statistically significant, p<0.05. [37]

Study done by Fatemeh A et al. (2015)conducted upon 13 adult obese male in Tehran, found that Mean ± S.D of BMI in healthy obese individuals (31.27 ± 1.89) was significantly higher than non-obese individuals BMI (23.01 ± 0.29) with p<0.001& was similar to our findings.[38]Study done by Babu SV et al. (2017)conducted upon 80 adult obese male attending the master health checkup program at Sri Ramchandra Medical college & Research Institute, India found that the Mean±S.D of BMI (32.5±2.40) in healthy obese male was significantly higher than non-obese male BMI (21.80 ± 1.6) with p<0.001& was similar to our findings. [39]

Study conducted by R. Kutulu. et al, (2017) on age and sex matched 300 obese individuals found that the Mean ± S.D of BMI (35.39±4.38) and WCF (105.45±14.36) in obese. The Mean±S.D of BMI (25.19± 1.62) and WCF (87.56±10.78) in non-obese. They found the anthropometrical parameters like BMI and WCF in obese were elevated as compared nonobese which was statistically significant, p<0.001 & was similar to our findings. [40]

The study done by F Kabir. et al. (2018)carried a study on women (n=90) in Dhaka. They found that the Mean± S.D of BMI (Kg/m2) in obese women (28.4±3.5), WC (93.5±7.7), HC (102.2±7.3), %BFM (32.8±6.0) were significantly higher than normal weight women BMI (20.8±1.9), WC (81.0±7.3), HC (89.3±5.8), and BFM (23.5±5.0) which was statistically significant, p<0.05. [41]

Study done by Mallick A.K. et al., (2018) on 200 participants of Rohilakand, U.P found that Mean±S.D of BMI in healthy obese individuals (31.27±1.89) was significantly higher than non-obese individuals BMI (23.01±0.29) with p<0.05 which was similar to our finding.[42]Anthropometric measurements are surrogate measures of body fat and better predictors of overweight and obesity. The cause of

increased BMI, WCF, HC, % BFM and WHR might be due to sedentary lifestyle, pro-inflammatory state and genetic predisposition in obese individuals. [43][44]

Mean Serum Visfatin Level in Cases and Controls

In the present study, the (Mean ± SD) Visfatin level was recorded as (129.1±21.4) pg/ml in obese individuals, (100.8±29.9) pg/ml in overweight individuals which were increased as compared to normal weight individuals (82.4± 26.5) pg/ml which was statistically significant p<0.05. (Table4, Graph-1)

Study conducted by Jurdana Met al. (2013) includes 48 normal weight & obese individuals found that baseline levels of fasting Visfatin were significantly higher in overweight subjects compared to normal weight subjects (4.1±0.6ng/ml vs 1.8±0.6 ng/ml for males and 4.4±0.5 ng/ml vs 2.5±0.5 ng/ml) for females which was statistically significant p<0.001. [45]

Study conducted by Nourbakhsh, Met al.(2015)includes 73 Iranian normal weight and obese individuals found that (Mean± SD) of Visfatin (5.4±1.2) ng/ml which were more in obese individuals as compared to normal weight individuals (3.9±1.0) ng/ml , which was statistically significant p<0.001.[46]

Study conducted by F Kabir. et al.(2018) includes 50 overweight subjects who had no disease condition and 42 controls found that (Mean±SD) of Visfatin (7.4±4.2) ng/ml were higher in overweight subjects in comparison to normal weight individuals (4.2±4.0)ng/ml which was statistically significant (p<0.000). [41]

Study conducted by Souvannavong-Vilivong Xet al.(2019) in Bangkok, Thailand involved 70 normal weight and obese individuals found higher value of (Mean±SD) of serum Visfatin (9.18±3.04) ng/ml in obese individuals as compared to normal weight (4.33±3.01) ng/ml,which was statistically significant (p<0.001).[47]

In the cross-sectional study conducted by Alnowihi SMet al. (2020) 83 healthy Saudi women of different body weights were recruited between 2014 and 2016, from King Abdulaziz University staff and students. The participants were divided into the following three groups according to their body mass indexes (BMIs): 35 obese women(42%) (29.0 ± 4.9 years old), 15 overweight women (18%) (23.6 ± 3.4 years old), and 33 lean women (39.76%) (22.87 ± 2.64 years old).He found that (Mean± SD) of serum Visfatin was (32.6±3.1) in obese group, (24.1±5.7) in overweight groups which was higher in comparison to (11.4±1.4) in normal weight individuals and it was statisticallysignificant, (p<0.001). [48]

Study conducted by Khanna D et al. (2022) includes 57 normal weight & obese individuals found that (Mean±SD) of Visfatin (34.2±13.4) ng/ml were higher in obese groups in comparison to normal weight groups (13.3±7.0), which was statistically significant p<0.001.[49]

Elevated levels of visfatin have been linked to increased levels of inflammatory markers such as IL-6, IL-8, C-reactive protein, and monocyte chemotactic protein-1 [50],endothelialdysfunction and increase in oxidative stress [51].These findingspoint to the existence of an average physiological level of visfatin atwhich it is properly controlled and fulfils its physiological functions, aswell as a threshold level at which its pathological consequences occur.

The significance of visfatin in the pathogenesis of IR and its associated consequences reflects the potential utility of visfatin as an early biomarker for IR in high-risk patients, especially obese adults.[52]

Obesity is closely associated with a low-grade state of inflammation, resulting from enlargement of adipocyte and increased macrophage infiltration in to the adipose tissue. [53]Obese adipose tissue is characterized by abnormal production and secretion of adipokines as well as activation of inflammatory signalling in adipocytes. [54]. The fact that Visfatin possesses both cytokine-like extrinsic activity (PBEF) and an enzymatic intrinsic activity (NAMPT) is a determinant in the physiology andpatho-physiology of obesity and metabolic disorders. [55][56]

Conclusion

Obesity has become a major worldwide health problem and is linked to a chronic low-grade inflammatory state which contributes to the development of obesity-associated disorders and metabolic dysfunction. Obesity is becoming a serious global issue due to its negative impact on health and its contributions to mortality and morbidity.

Our study finding suggests that the mean serum Visfatin level was significantly higher in obese(129.±21.4)pg/ml and overweight subjects (100.8±29.9)pg/ml incomparison to normal weight subjects (82.8±26.5)pg/ml with p valueequal to <0.012. The significant increase level of visfatin in obese may be used as a diagnostic and prognostic biomarker for Insulin Resistance and metabolic syndrome.

Visfatinand Visfatin analogue may represent a druggable target for diabetic therapy due to its insulin mimetic effect in normal physiological range.Inhibitors of Visfatin (CHS-828 and FK866) may have a role in over weight and obesity to reduce insulin resistance, inflammation, oxidative

stress and neo- vascularisation. Thus, it may be used as a targeted therapy in overweight and obese individuals to reduce morbidity and its related complications.

Factors such as antioxidant rich diet, regular exercise, lifestyle modificationsalong with inhibitors of visfatinfavours visceral fat loss migh provide an important tool toreduce the burden associated with obesity and related complications.

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