EVALUATION OF SERUM OSTEOPONTIN LEVEL IN OBESE IRAQI POSTMENOPAUSAL WOMEN WITH PRIMARY OSTEOPOROSIS [+]

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Abstract:

Background: Osteoporosis (OP) is a chronic and progressive disease characterized by decreased bone mass and micro architectural deterioration of bone tissue, resulting in an increased risk of fracture **.**Body mass index (BMI) has been found to be related to the risk of osteoporotic fractures in women, regardless of bone mineral density (BMD). Very few studies have investigated the comparison of fracture risk among BMI categories, classified according to the WHO criteria, despite the potential usefulness of such information for clinical purposes. Osteopontin was described as a major component protein in bone and named bone sialoprotein1 it's produced by osteoblasts and osteoclasts. It has been found to be associated with bone strength and bone remodeling. OPN influences bone homeostasis both by inhibiting mineral deposition, by promoting differentiation of osteoclasts and by enhancing osteoclast activity.

Aim of the study: This Study was planned to evaluate serum level of Osteopontin in postmenopausal women with and without primary osteoporosis, and the correlation between serum osteopontin level with women's body mass index.

Subjects and methods:

This study was performed during March 2012 to September 2012, Eighty (80) postmenopausal women were included in this study with age range (50-77 years). Subjects were divided into two groups: group A: forty four (44) women with vertebral osteoporotic fractures and group B: thirty six (36) women without osteoporosis and without fractures (serve as controls). Lateral X- ray of the thoracic and lumbar spine were taken for all women of both groups which scored according to kleerkoper method for diagnosis of fractures. Also Patients diagnosed as osteoporosis and controls as normal by measuring bone mineral density (BMD) by using dual energy X-ray absorptiometry (DXA) . Serum calcium, phosphorous and alkaline phosphatase measured by spectrophotometer, and serum osteopontin OPN measured by enzyme linked immuno sorbent assay (ELISA) techinque. All women were not drink alcohol, non smoker, had no diseases known to affect bone metabolism and they were not taking any drug known to affect bone turnover.

Results:

Mean serum osteopontin level in the postmenopausal women with primary osteoporosis was significantly higher than controls ($p<0.0001$). The mean serum osteopontin level in the postmenopausal women with primary osteoporotic is a significant difference (Mean±SD) (22.56±4.57 kg/m^2 as compare to controls (15.83±0.52 kg/m^2) ($P<0.0001$) within normal weight. Osteopontin for patients (26.75±5.17) kg/m^2 and controls (15.72±0.69) kg/m^2) ($p<0.0001$) showed significant difference within the mean of overweight . Osteopontin for patients and controls showed significant difference within the mean of

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Obesity: (Mean ± SD) (26.20±5.91kg/m²) for patients and (16.07±0.66kg/m²) for controls, (P<0.0001).Moreover a positive significant correlation between serum osteopontin level and body mass index (BMI) for patients (r= 0.4)(p< 0.05) and positive non significant correlation between serum osteopontin level and body mass index (BMI) for controls (r= 0.12)(p> 0.05) .

Conclusion:

Increasing serum OPN level in postmenopausal women with primary osteoporosis provide evidence that determining serum OPN level can be used as reliable markers in diagnostic criteria of primary osteoporosis . Data obtained in this study showed that the obesity which is a risk factor of osteoporosis, the plasma OPN level is high.

Key words: Osteopororosis, Osteopontin , postmenopausal women, obesity

تقييم مستوى مصل الاوستيوبونين في مصل دم النساء العراقيات مابعد سن الياس المصابات بهشاشة العظام الاولي

منال كمال رشيد

المستخلص :

مرض هشاشة العظام من الامراض المزمنة والمتفاقمة ويتميز بأنخفاض كثافة العظم وتدهور الشكل الهندسي للنسيج العظمي ،مما ينتج عنه زيادة خطورة الاصابة بالكسر. مقياس كتلة الجسم (BMI) وجد ان له علاقة بخطورة هشاشة العظام عند النساء، بغض النظر لكثافة العظام المعدنية (BMD.هناك دراسات قليلة شخصت العلاقة بين خطورة الكسر العظام ومقياس كتلة الجسم ، والذي صنف أنسجاما مع معيار WHO.

الاوستيوبيوتين هو البروتين المكون الاساسي للعظام ويسمى سيالوبروتين وهو ينتج من خلايا المكونة والهادمة للعظم. ويوجد أيضا في خلايا تقوية العظام وأعادة بناء العظام. والاستيوبونتين يؤثر في توازن العظام من خلال تثبيط ترسيب المعادن او من خلال زيادة المفاضلة بين عملية الانحلال وارتشاف النسيج العظمي وزيادة فعالية انحلال العظم.

هدف الدراسة:

هذه الدراسة تضمنت تقييم مستوى مصل أوستيوبونتين عند النساء مابعد سن الياس المصابات بهشاشة العظام (بنخر العظام) والغير مصابات بهشاشة العظام من النوع الاولي ومقارنة النتائج بالنساء غير المصابات بهشاشة العظام كمجموعة ضابطة لغرض المقارنة . وهذه الدراسة تضمنت العلاقة مابين مستوى مصل أوستيوبونتين ومقياس كتلة الجسم عند النساء.

الاشخاص وطرق العمل:

انجزت الدراسة من الفترة اذار الى ايلول من سنة 2012 .تضمنت الدراسة ثمانون امرأة بعمر يتراوح بين(50-77)سنة . النساء قسموا الى مجموعتين : الاولى أ وعددها اربعة واربعون(44) امراة مصابات بهشاشة العظام الاولي ومصابة بكسر والمجموعة الثانية ب وعددها ست وثلاثون (36) امراة غير مصابات بهشاشة العظام ولايعانين من كسر

(كمجموعة ضابطة)لغرض المقارنة. حيث تم تشخيص جميع النساء في المجموعتين ا و ب للتاكد من وجود كسر من عدمه عن طريق اشعة السنية(x–ray)(طريقة كلير كوبر)وكذلك تم تشخيص مرض هشاشة العظام من عدمه عن طريق قياس كثافة العظم بجهاز DXA (GE machine)

بالاضافة الى ذلك تم قياس مستوى كل من كالسيوم ، فسفور والاكلاين فوسفاتيس في مصل الدم بواسطة المطياف الضوئيSpectrophotometer)وتم قياس الاستيوبونتين في مصل الدم بواسطة الاليزة(ELISA) .

وتم التاكد من ان جميع النساء لم يتناولن الكحول ، غير مدخنات ، غيرمصابات بمرض يؤثر على ايض العظام وكذلك لم يتناولن دواء له تاثير على بناء الهيكل العظمي.

النتائج:

أن متوسط قيم مستوى الاوستيوبونتين كان مرتفعا بشكل معنوي في مصل دم النساء مابعد سن الياس المصابات بهشاشة العظام ويعانين من كسر مقارنة بالنساء الغير مصابات بهشاشة العظام ولايعانين من كسر (المجموعة الظابطة) $p<0.0001$. أن متوسط مصل الاوستيوبيونتين عند النساء مابعد سن اليأس مصابات بهشاشة العظام كان مرتفعا بشكل معنوي(22.56 ± 4.57 kg/m^2) مقارنة بالنساء غير المصابات بهشاشة العظام ولايعانين من كسر العظام كمجموعة ظابطة ($P<0.0001$) (15.83 ± 0.52 kg/m^2) ذو الوزن الطبيعي . وجد ايضا هناك زيادة معنوية في متوسط قيم مستوى الاوستيوبونتين عند المرضى (26.75 ± 5.17) kg/m^2 مقارنة بالمجموعة الظابطة ((15.72 ± 0.69) kg/m^2 ($p<0.0001$)ذو الوزن الزائد . هناك زيادة معنوية في متوسط قيم مستوى الاوستيوبونتين عند المرضى (26.20 ± 5.91kg/m²) مقارنة بالمجموعة الضابطة ($p<0.0001$) (16.07 ± 0.66kg/m²) المصابون بالبدانة. وجد ايضا انا هناك علاقة معنوية موجبة مابين مستوى مصل الاوستيوبونتين ومقياس كتلة الجسم عند المرضى ($p< 0.05$)($r= 0.4$) اوعلاقة غيرمعنوية موجبة مابين مستوى مصل الاوستيوبونتين ومقياس كتلة الجسم عند المجموعة الضابطة ($p> 0.05$)($r= 0.12$) .

الاستنتاجات:

ان ارتفاع مستوى مصل الاوستيونونتين في مصل دم النساءمابعد سن الياس المصابات بنخر العظام ويعانين من كسر يعطي دليلا على ان قياس مستوى الاوستيونونتين في مصل الدم يمكن الاستفادة منه في تشخيص الاصابة بهشاشة العظام الاولي . ومن النتائج التي ظهرت من هذا البحث ان السمنة هي عامل خطر ومؤثر على هشاشة العظام والذي يؤدي الى زيادة الاوستيوبونتين.

Introduction:

Osteoporosis (OP) is a chronic and progressive disease characterized by decreased bone mass and microarchitectural deterioration, leading to increased bone fragility and a consequential increased risk of fracture[1] . Osteoporosis occurs three times in women than in men because women have a lower peak bone mass and hormonal changes that occur at the menopause. Estrogens have an important function in preserving bone mass during adulthood, and bone loss occurs as levels decline [2].Fracture consequences can be grouped into three categories: pain, physical changes and impairment and psychosocial declines[3] .

Bone mineral density (BMD) has been found to be the main predictor of fragility fractures[4]. Among other predictors of fragility fracture the body mass index (BMI), a height

standardized measure of body weight, has also been found to be related to the risk of osteoporotic fractures[5] , thus representing a factor to be considered in their prevention. The role of BMI as a risk factor for fragility fracture is mediated mainly by its relationship with the BMD, of which BMI is one of the main determinants. Nevertheless, BMI may also be a risk factor for fragility fracture regardless of BMD by increasing the propensity to fall due to muscle weakness in lean people, or due to increased postural instability of obese people. In fact, BMI has been found to be inversely related with the risk of osteoporotic hip fracture[6].In fact, advice on lifestyle habits to optimize BMI for fracture prevention should be given to patients, also taking into account that BMI is implicated in the prevention of metabolic and diseases[7]. Osteopontin was first described as a major component protein in bone and named bone sialoprotein1[8]**.** OPN is known to be involved in bone resorption, wound repair, immune function, angiogenesis, cell survival and cancer biology[9] and named bone sialoprotein1[8],[10]. Osteopontin was originally identified as a major component of the non-collagenous bone matrix produced by osteoblasts and osteoclasts. It has been found to be associated with bone strength and bone remodeling[11]. OPN influences bone homeostasis both by inhibiting mineral deposition, by promoting differentiation of osteoclasts and by enhancing osteoclast activity [9]**.** Osteopontin was also shown to suppress proliferation and differentiation in a certain type of osteoblastic cells [12]**.**

Subjects and methods:

Eighty (80) postmenopausal women were included in this study with age range (50-77 years); Menopausal status was defined by the absence of menses for more than one year in a woman 50 years of age and over. All women were attended to Osteoporosis Clinic in Baghdad Teaching Hospital during the period from March 2012 to September 2012. Subjects were divided into two groups: Group A: forty four (44) women with osteoporotic fractures (Patients) and group B: thirty six (36) women without osteoporosis and without fractures (serve as controls). The complete case history was taken from each women and lateral X- ray of the thoracic and lumbar spine were taken for all women of both groups which scored according to kleerkoper method for diagnosis of fracture. Patients diagnosed as osteoporosis and controls as normal by measuring bone mineral density (BMD) by using Dual energy X-ray absorptiometry (DXA). All women were not drink alcohol, non smoker, had no diseases known to affect bone metabolism and they were not taking any medication known to affect bone turnover. Serum level of ALP, calcium and phosphate were determined by calorimetric method .

Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Women were classified according to BMI such as obese $\geq$ 30 kg/m^2, overweight (25-29.9)kg/m^2 and normal (18.5-24.9) kg/m^2[13]. Serum Osteopontin was determined by enzyme immune sorbent assay (ELIZA)for the in vetro quantitative measurement of human OPN in serum using kit manufactured by (Ray Bio,USA) the optical density was measured for each well and then converted to concentration by using standard curve[14] **.**

Statistical analysis:

All continuous variables are expressed as mean ± standard deviation of the mean. The unpaired t-test was performed to test hypotheses about means of different groups. Significance of difference was assessed using Student-t test for two independent

means used for. Correlation and regression was applied for the relationship between two quantitative variables, taking $P \leq 0.05$ lowest limit of significance[15].

Results:

Table 1 shows postmenopausal women with osteoporotic fractures (n=44) and without osteoporosis and without fractures (controls)(n=36) were classified according to body mass index , patients with normal BMI (n=15), (34.1%) , with overweight (n=17), (38.6 %)and with obesity (n=12),(27.3%) and range (20-40.2) while controls with BMI normal (n=8), (22.2 %) ,with overweight (n=12), (33.3 %)and with obesity (n=16),(44.4%) and range (20.3-40.4).

While Table 2 , figure 2 shows a significant difference in mean value (Mean±SD) of Osteopontin between patients (22.56±4.57 kg/m^2) and controls (15.83±0.52 kg/m^2) (P<0.0001) within normal weight . Osteopontin for patients (26.75±5.17) kg/m^2 and controls (15.72±0.69) kg/m^2) (p<0.0001) showed significant difference within the mean of overweight . Osteopontin for patients and controls showed significant difference within the mean of Obesity: (Mean ± SD) (26.20±5.91kg/m²) for patients and (16.07±0.66kg/m²) for controls, (P<0.0001).

Table 1: Number and percentage of patients and controls according to Body mass index

BMI(Kg//m^2	patients		controls		P value
	No.	%	No.	%	
Normal (18.5-24.9)	15	34.1	8	22.2	0.388
Overweight(25-29.9)	17	38.6	12	33.3	
Obese(=>30)	12	27.3	16	44.4	
Mean±SD(Range)	27.48±5.23	(20.0-40.2	29.0±4.7	20.3-40.4	0.179

Table2: Mean value of BMI (kg/m2) for patients and controls

BMI(Kg/m^2	Serum Osteopontin (OP N)(ng/ml		
	Patients	**Controls**	**P Pvalue**
Normal(18.5-24.9)	22.56±4.57	15.8.83±0.52	0.0001*
Overweight(25-29.9)	26.75±5.17	5.72±0.69	0.0001*
Obese(=>30)	26.20±5.19	16.07±0.66	0.0001*
Pvalue using ANOVA	0.0001*	0.546	

***P value significant**

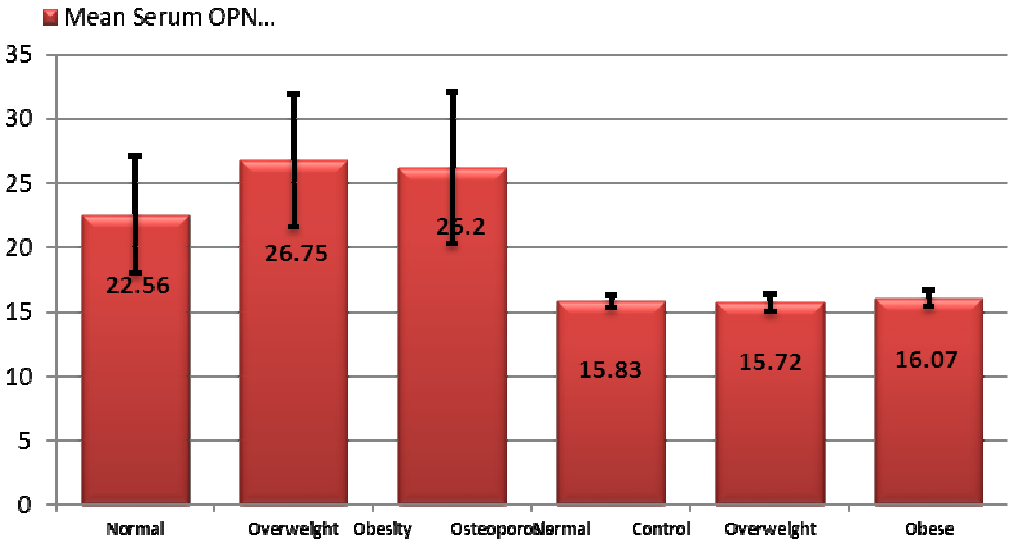

Figure 1: The mean levels of osteopontin ng/ml according to BMI for patients and controls.

The study shows that serum OPN levels were positively significant correlated with BMI in patients (r= 0.4) figure 2,and non significant correlated in controls (r=0.12) figure 3.

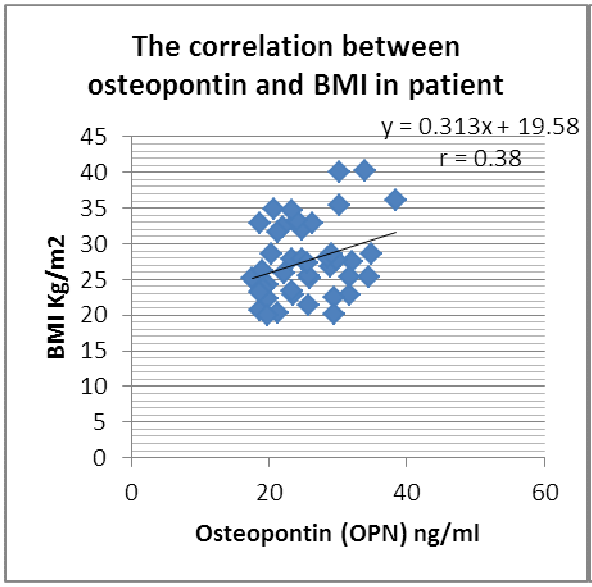

Figure 2: Correlation between serum osteopontin Levels and BMI for patients . r= 0.4 , n=44 , p< 0.05

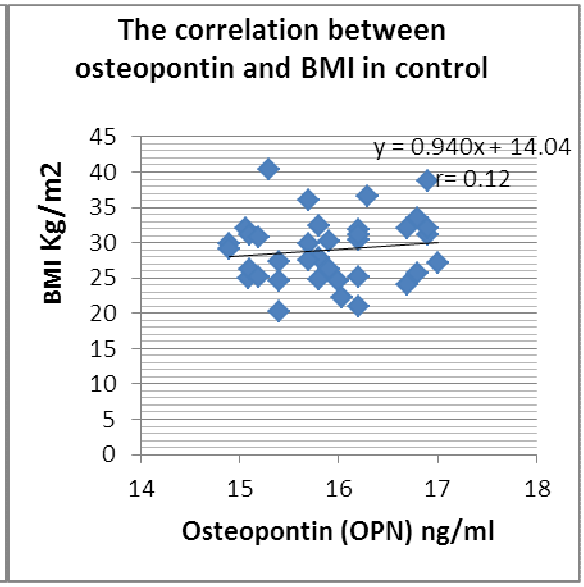

Figure 3: Correlation between serum osteopontin levels and BMI for controls . r= 0.12 , n=36, p>0.05.

Table (3) shows no significant difference in mean value of serum calcium, phosphorous and alkaline phosphatase levels between patients and controls. Mean value of serum calcium levels (Mean ± SD): (8.6±0.0.5mg/dl) for patients as compared with (8.84±0.6mg/dl) for controls (P=0.109). In addition, (Mean ± SD) of serum phosphorous levels (3.6±0.53mg/dl) for patients as compared with (3.59±0.53 mg/dl) for controls (P=0.526) and (Mean ± SD) of serum alkaline phosphatase levels (66.55±13.79 U/L) for patients comparing to (65.56±14.01 U/L) for controls (P=0.752).

Table (3): Mean value of serum calcium, phosphorous and alkaline phosphatase levels for patients and controls.

Parameters	Patients (mean±SD) n=44	Controls (mean±SD) n=36	P value
Serum calcium normal (8.5 -10.5)mg/dl	8.6±0.0.5	8.84±0.6	0.109 NS
Serum phosphorus normal (2.5-4.5)mg/dl	3.6±0.53	3.59±0.53	0.526 NS
Serum Alkaline phosphatase- normal (21-92 U/L)	66.55±13.79	65.56±14.01	0.752 NS

NS= non-significant

Discussion:

Biochemical markers represent the molecules directly connected to both the structure and function of bone tissue. The fact that changes in either the concentration or activity of these biochemical markers are reflecting dynamic status of bone metabolism is taken as advantageous. Markers of bone turnover, subdivided into markers of bone formation and bone disintegration, these markers are influenced by age, sex and menopausal status, OPN was first suggested to function as an anchor between mineral matrix of bone surfaces and osteoclast during bone resorption ,although later it was also proposed that the osteoclast itself was responsible for synthesis of OPN into resorption lacunas[16].

This study assessed the relationship of BMI with the risk of the osteoporotic fractures, regardless of age and BMD. The main findings of the present study is that plasma OPN concentrations are increased in overweight and obese subjects, the modest diet-induced weight loss is accompanied by a significant decline in plasma OPN levels.

This is, to our knowledge, the first study describing increased plasma OPN levels in human obesity. Obese patients exhibited increase in plasma OPN concentrations compared with lean individuals. The significant positive correlation found in the present study between OPN and BMI seems to indicate that OPN levels are related to the amount of adipose tissue. However, OPN is derived from many cellular types, and the partial contribution of any other organ to circulating OPN remains unknown[17],[18]. Expression of OPN has been observed, among others, in epithelial cells, macrophages, and atherosclerotic plaques[19] . It has also been reported that OPN is expressed in adipose-derived stem cells[20].

This study revealed that serum Osteopontin (OPN) level was significantly higher in postmenopausal women with osteoporotic fractures when compared with controls, this result comes in agreement with previous study[21] which suggested that persons with high serum OPN levels had an approximately 2-fold risk of osteoporosis compared with the persons with low serum OPN levels .These findings were explained that OPN stimulates CD44 expression on the osteoclast surface, and CD44 is required for osteoclast motility[22]**.** Because inhibition of osteoclastogenesis is one of the main mechanism by which estrogen prevents bone loss, it likely that estrogen may regulate either the production of or the target cell responsiveness to receptor activator of nuclear factor kappa –B ligand (RANKL). Thus estrogen may down regulate osteoclastogenesis by differential decrease in the responsiveness of osteoclast precursor to RANKL and by directly suppressing RANKL induced osteoclast differentiation

[16]. The lack of estrogen which down regulates osteoclastogenesis and OPN up regulates osteoclast motility, which may enhance the ability of osteoclasts to absorb bone causing a high turnover in postmenopausal osteoporosis[23] .

The data showed that OPN levels of obese , overweight patients were significantly higher than those levels of obese and overweight controls. In obese group OPN levels were found to be correlated with BMI[24]. Increased systemic concentrations inflammatory markers were determined in obese animals and humans, such as adiponectin, leptin, resistin, visfatin, omentin, interleukin-6, and tumor necrosis factor-α_. These systemic inflammatory responses are mainly derived from adipose tissue .OPN is evaluated to be one of those inflammatory markers. OPN also induces the expression of other inflammatory cytokines and chemokines in peripheral blood mononuclear cells[25].

Javier, et al [20]was reported that plasma OPN concentrations are increased in overweight and obese subjects, the circulating concentrations of OPN correlate with body fat, the OPN mRNA and protein are expressed in omental adipose tissue, the expression in this fat depot is increased in obesity and further elevated in obesity associated T2DM, the modest diet-induced weight loss is accompanied by a significant decline in plasma OPN levels. We investigated the relationship of OPN and obesity in women who have a burden of osteoporosis.

The data of this study shows no significant changes in serum calcium, phosphorus and alkaline phosphatase levels between patients and controls, these results agree with previous studies which suggested that these assays do not reflect precisely the same aspects of bone metabolism as bone markers. Though osteoblasts are rich in ALP, it is also associated with the plasma membrane of the cell in the liver, intestine and placenta, all of which may contribute to the total amount of ALP. Because of multiple sources of origin, total ALP has not enjoyed wide spread use as a bone-remodeling marker. Also serum calcium and phosphorous levels are tightly regulated and homeostasis is maintained in serum regardless of their store in bone, hence these parameters did not vary significantly in patients with osteoporosis and controls[26] , another study proposed that in the bone thinning disease as osteoporosis does not affect calcium and phosphorous metabolism and a diagnosis of osteoporosis can not be inferred from any change in serum calcium, phosphorus or alkaline phosphatase levels[27].

In conclusion, data obtained in this study showed that in obesity which is a risk factor for oseoporotic . Serum OPN level is high. Although, our sample size is small, our study, besides reminding us about the measures obesity, makes us think about using OPN as a probable marker of osteoporosis and the value of determining OPN plasma levels in evaluating the results. We also speculate that targeting OPN could provide an useful approach for the treatment of obesity and related metabolic disorders.

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