



Assessment of Leptin and Nesfatin-1 with Antioxidant Capacity in Obese Iraqi Patients

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ABSTRACT

This study was involved the exploration of nesfatin-1 and leptin association with the total antioxidant capacity (TAC) in obese males. The experimental part of the study has involved the analyze of nesfatin-1, leptin, TAC, triacylglycerol (TAG), total cholesterol (T.Ch), high-density lipoprotein-cholesterol (HDL-Ch), low-density lipoprotein-cholesterol (LDL-Ch), very low-density lipoprotein-cholesterol (VLDL-Ch), urea, and creatinine in 60 obese males, and controlled with 60 lean males of comparable ages. Anthropometrics for obesity were calculated for all of the selected subjects including body mass index (BMI), waist circumference (WC), waist to hip ratio (WHpR), and waist to height ratio (WHtR). The results have expressed a significant ($P<0.05$) higher level of leptin with significant ($P<0.05$) lower levels of nesfatin-1 and TAC in obese males compared to lean males. Additionally, TAG, T.Ch, LDL-Ch, VLDL-Ch, and urea were significantly ($P<0.05$) higher in obese males than lean males, while HDL-Ch was significantly ($P<0.05$) lower. The differences in creatinine were non-significant ($P>0.05$). Leptin was associated directly with BMI and inversely with nesfatin-1 in obese males. On the other hand, nesfatin-1 was associated directly with TAC, and both were associated negatively with BMI in obese males. In conclusion, nesfatin-1 has important role in obesity condition, and it involved in the oxidative stress that developed in obesity.

Keywords: Obesity, Leptin, Nesfatin-1, Lipid profile



تقييم هرمون اللبتين والنسفاتين-1 مع القدرة المضادة للأكسدة في المرضى العراقيين الذين يعانون من السمنة المفرطة

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المستخلص

تشتمل هذه الدراسة علي معرفة ارتباط النسفاتين -1 واللبتين مع القدرة الكلية لمضادات الاكسدة (TAC) لدى الذكور الذين يعانون من السمنة المفرطة. يشمل الجزء التجريبي من الدراسة على تحليل النسفاتين-1، اللبتين، TAC، ثلاثي الجلسرين (TAG)، الكوليسترول الكلي (T.Ch)، البروتين الدهني مرتفع الكثافة (HDL-Ch)، البروتين الدهني منخفض الكثافة (LDL-Ch)، الكوليسترول الدهني منخفض الكثافة للغاية (VLDL-Ch)، اليوريا، والكرياتينين في 60 من الذكور الذين يعانون من السمنة المفرطة، وتم التحكم بها من خلال 60 من الذكور النحيفين من الاعمار المماثلة.

تم احتساب القياسات البشرية للسمنة لجميع العينات المختارة بما في ذلك مؤشر كتلة الجسم (BMI)، ومحيط الخصر (WC)، ونسبة الخصر الى الورك (WHpR)، ونسبة الخصر الى الطول (WHtR).

اظهرت النتائج ارتفاع ($P > 0.05$) في مستوى اللبتين مع انخفاض ($P > 0.05$) في النسفاتين -1 و TAC في الذكور البدناء مقارنة بالذكور النحيفين. فضلا عن ان نسبة TAG، T.Ch، LDL-Ch و VLDL-Ch واليوريا اعلى بشكل ملحوظ ($P < 0.05$) في الذكور الذين يعانون من السمنة المفرطة مقارنة بالذكور النحيفين، في حين كان HDL-Ch اقل بشكل ملحوظ ($P > 0.05$). كانت الاختلافات في الكرياتينين غير هامة.

وارتبط اللبتين مباشرة مع مؤشر كتلة الجسم وعكسيا مع نسفاتين -1 في الذكور الذين يعانون من السمنة المفرطة، ومن ناحية اخرى ارتبط النسفاتين -1 مباشرة مع TAC، وكلاهما ارتبط سلبا مع مؤشر كتلة الجسم لدى الذكور الذين يعانون من السمنة المفرطة.

نستنتج من ذلك ان للنسفاتين -1 دور مهم في حالات السمنة، كما انه يشارك في الاجهاد التأكسدي الذي ينشأ بسبب السمنة.

الكلمات المفتاحية: السمنة، اللبتين، نسفاتين-1، ملف الدهون

1. INTRODUCTION

Obesity is a medical situation in which the store of body fats is increased due to many factors (Beets et al. 2019). Primarily, obesity results from the increase of intake calories that corresponds to insufficient expenditure of calories. This imbalance in the energy input and output is termed as behavioral factor in the etiology of obesity (Bray, 2004, Racette et al. 2003). White adipose tissue is the primary source of leptin secretion, and concentrations are directly associated with body fat content. Leptin secretion is



pulsatile, like that of several similar hormones, and it varies significantly during the day, peaking in the early evening and early morning. Leptin levels in the blood predominantly represent the number of energy that is stored as fat, with acute changes in calorie intake coming in second (Kelesidis et al. 2010).

The initial peptide nucleobindin 2 (NUCB2) is converted into the 82-amino acid polypeptide nesfatin-1, as well as the two proteins nesfatin-2 and nesfatin-3, all of which have unidentified roles (Oh-I et al. 2006). Nesfatin-1 may improve insulin sensitivity while also improving glucose and lipid homeostasis, according to molecular and animal studies. Through its main anorexigenic impact and reduced body weight impact, it controls internal environment. Importantly, some research showed that nesfatin-1 regulates adipogenesis. The peripheral injection of nesfatin-1 may have an insulin-dependent, time-dependent anti-hyperglycemic impact (Matta et al. 2022).

Antioxidants are defined as substances when present at low concentration eliminate the effects of free radicals (Fahad and Mohammed 2020). The imbalance between oxidants and antioxidants leads to the apparent of oxidative stress and its deteriorated effects, in which either ROS is elevated or antioxidants are depleted or both (Ruiz-Ojeda et al. 2018).

2. Material and Methods

2.1. Specimens Collection

The study has included 60 obese males that have been registered from different locations in Baghdad city. Also, another 60 males, who were lean and completely healthy were chosen as control for the study. All males were informed about the work and accepted to be subjects for the test and submitted for written form.

2.2. Anthropometric Measurements

The weight and the height of the males who were applied for the study were measured to calculate the BMI according to the formula that stated the divided of the weight in kg over the square of height in meter (Freedman and Sherry 2009). Additionally, the waist circumference and the hip circumference were measured by using non-elastic measuring tape from the widest point and in a way that the tape was parallel to the ground. The waist, hip and height measurements were used for the calculation of WHpR and WHtR for each male.

2.3. Total Antioxidant Capacity Assay

The assay of TAC was performed in a spectrophotometric approach according to Erel method (Erel 2004), with modification. The principal of the assay relied on the reaction of the Fe^{2+} with O-dianisidine in a Fenton reaction, yielding hydroxyl radical. This ROS material cause oxidation to the colorless O-dianisidine to dianisidyl radical and yellow color is developed under acidic conditions. The presence of



antioxidant materials would reduce the intensity of the yellow color as they will interact with the oxidizing reaction. Accordingly, the capacity of antioxidants from the serum samples will disproportionate to the intensity of the color

2.4. Determination of Leptin and Nesfatin-1

Leptin and nesfatin-1 were determined in the serum samples by using enzyme linked immuno-sorbent assay (ELISA) methodology. The principle of the method was based on the interactions of two antibodies on the antigen from the serum. The wells of the plate were pre-coated with antibodies of leptin or nesfatin-1, when the serum samples are added to the wells, the antigens of leptin or nesfatin-1 are bound to the antibodies on the well. Then, second antibodies of leptin or nesfatin-1 are added to the wells, which were labelled with horse radish peroxidase enzyme through biotin-avidin interactions. The second antibodies bound to the antigens in a sandwich like figure through an incubation step. When the wells are washed, the unbounded antibodies are removed from the wells while only bounded antibodies are remained. Then, a substrate solution of the enzyme added to the wells to develop the blue color, and the reaction stopped by the addition of an acidic solution that converts the color from blue to yellow. The intensity of yellow color is proportionate to the concentration of leptin or nesfatin-

3. Results

3.1. Characteristics of Males.

The demographic presentation of the male subjects that have been selected for this study is demonstrated in Table 3.1 and detailed below the Table.

PARAMETERS	CONTROL MALES	OBESSE MALES	P-VALUE
Number	60	60	-
Age (year)	29.63±8.03	31.25±7.40	0.254
BMI (kg.m ⁻²)	23.07±1.41	35.71±3.64	0.0001
WC (cm)	80.20±5.33	117.22±7.62	0.0001
WHpR	0.75±0.08	0.98±0.04	0.0001
WHtR	0.46±0.03	0.66±0.06	0.0001

Table 3.1. Demographic presentation of obese and lean males

* Mean±SD; significant at $P \leq 0.05$.

The age differences were non-significant ($P > 0.05$) between the lean males (29.63±8.03 year) and the obese males (31.25±7.40 year). The BMI of obese males (35.71±3.64 kg.m⁻²) was significantly ($P < 0.05$) higher than the BMI of lean males (23.07±1.41 kg.m⁻²). The waist circumference of obese males (117.22±7.62 cm) was significantly

($P < 0.05$) higher than the waist circumference of lean males (80.20 ± 5.33 cm). The WHpR of obese males (0.98 ± 0.04) was significantly ($P < 0.05$) higher than the WHpR of lean males (0.75 ± 0.08). The WHtR of obese males (0.66 ± 0.06) was significantly ($P < 0.05$) higher than the WHtR of lean males (0.46 ± 0.03).

3.2. Leptin

The concentration of leptin in the serum of obese males (12.50 ± 4.65 ng/mL) was significantly ($P < 0.05$) elevated when compared to the concentration of leptin in the serum of lean males (3.43 ± 1.71 ng/mL). Figure 3.2.

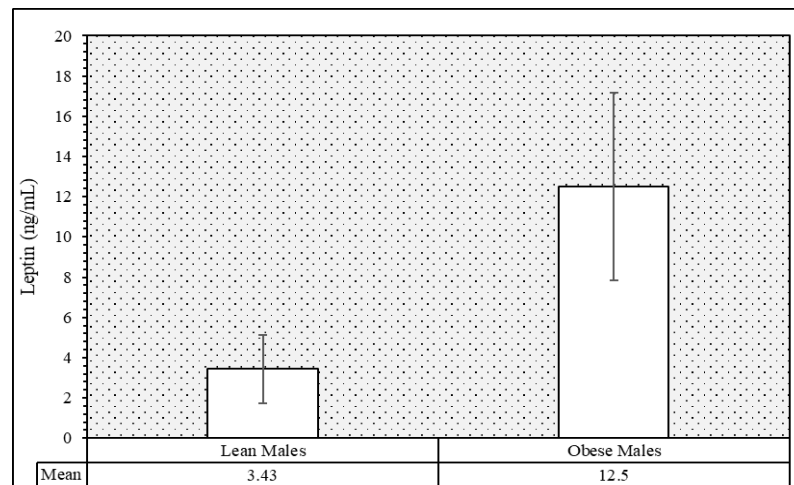


Figure 3.2. Male's (obese and lean) Leptin differences

3.3. Nesfatin-1

The concentration of nesfatin-1 in the serum of obese males (1.44 ± 0.40 ng/mL) was significantly ($P < 0.05$) reduced when compared to the concentration of nesfatin-1 in the serum of lean males (2.92 ± 1.49 ng/mL), Figure 3.3.

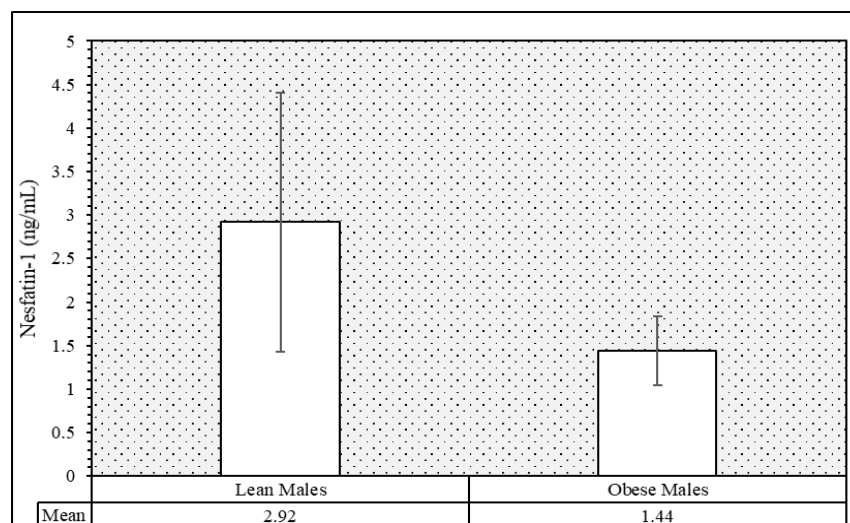




Figure 3.3. Male's (obese and lean) nesfatin-1 differences

3.4. Total Antioxidant Capacity

The level of TAC in the serum of obese males (1.53 ± 0.51 $\mu\text{mol vit. C Eq/L}$) was significantly ($P < 0.05$) reduced when compared to the level of TAC in the serum of lean males (3.74 ± 1.48 $\mu\text{mol vit. C Eq/L}$), Figure 3.4.

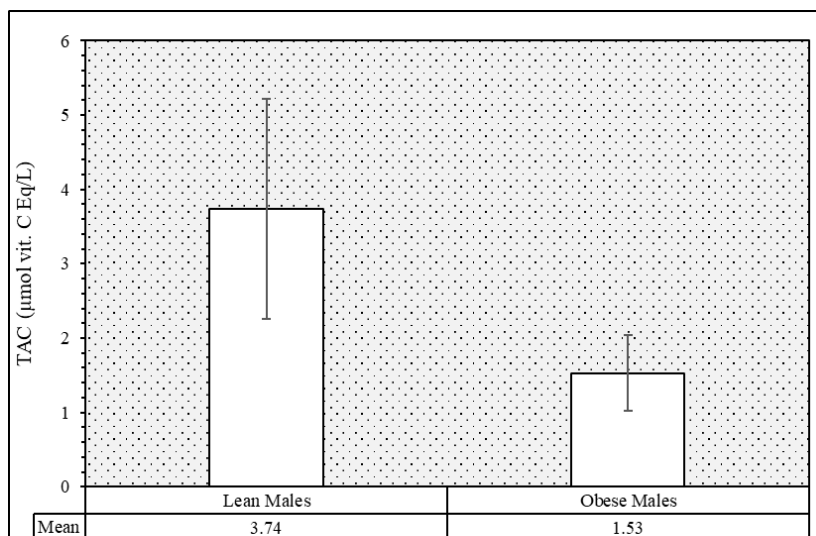


Figure 3.4. Male's (obese and lean) TAC differences

3.5. Triacylglycerol



The concentration of TAG in the serum of obese males (160.48 ± 38.25 mg/dL) was significantly ($P < 0.05$) elevated when compared to the concentration of TAG in the serum of lean males (97.85 ± 7.53 mg/dL).

3.6. Total Cholesterol

The concentration of T.Ch in the serum of obese males (193.32 ± 28.71 mg/dL) was significantly ($P < 0.05$) elevated when compared to the concentration of T.Ch in the serum of lean males (137.33 ± 13.42 mg/dL).

3.7. High-Density Lipoprotein-Cholesterol

The concentration of HDL-Ch in the serum of obese males (42.35 ± 6.91 mg/dL) was significantly ($P < 0.05$) reduced when compared to the concentration of HDL-Ch in the serum of lean males (52.69 ± 5.47 mg/dL).

3.8. Low-Density Lipoprotein-Cholesterol

The concentration of LDL-Ch in the serum of obese males (118.88 ± 28.95 mg/dL) was significantly ($P < 0.05$) elevated when compared to the concentration of LDL-Ch in the serum of lean males (65.07 ± 12.05 mg/dL),

3.9. Very Low-Density Lipoprotein-Cholesterol

The concentration of VLDL-Ch in the serum of obese males (32.10 ± 7.65 mg/dL) was significantly ($P < 0.05$) elevated when compared to the concentration of VLDL-Ch in the serum of lean males (19.57 ± 1.51 mg/dL).

3.10. Serum Urea

The concentration of urea in the serum of obese males (27.22 ± 6.03 mg/dL) was significantly ($P < 0.05$) elevated when compared to the concentration of urea in the serum of lean males (24.43 ± 5.43 mg/dL).

3.11. Serum Creatinine

The concentration of creatinine in the serum of obese males (0.85 ± 0.09 mg/dL) was non-significantly ($P > 0.05$) elevated when compared to the concentration of creatinine in the serum of lean males (0.82 ± 0.08 mg/dL). Table 3.2 contains a summary of the



obtained results for the clinical parameters that have been detailed previously in this chapter.

VARIABLE	CONTROL	PATIENTS	P-VALUE
Leptin	3.43±1.71	12.50±4.65	<0.001
Nesfatin-1	2.92±1.49	1.44±0.40	<0.001
TAC	3.74±1.48	1.53±0.51	<0.001
Triglycerides	97.85±7.53	160.48±38.25	<0.001
Cholesterol	137.33±13.42	193.32±28.71	<0.001
HDL	52.69±5.47	42.35±6.91	<0.001
LDL	65.07±12.05	118.88±28.95	<0.001
VLDL	19.57±1.51	32.10±7.65	<0.001
Urea	24.43±5.43	27.22±6.03	0.009
Creatinine	0.82±0.07	0.85±0.09	0.118

Table 3.2. Summary of the clinical data

3.12. Correlation

In obese males, the leptin was inversely correlated with nesfatin-1, directly correlated with BMI, directly correlated with waist circumference, directly correlated with WHpR, directly correlated with WHtR, directly correlated with TAG , and directly correlated with VLDL-Ch. In obese males, the nesfatin-1 was directly correlated with TAC , inversely correlated with BMI, inversely correlated with waist circumference, inversely correlated with WHpR, and inversely correlated with WHtR. In obese males, the level of TAC was correlated inversely with the age of the subjects. In obese males, the BMI was directly correlated with WC, directly correlated with WHpR, directly correlated with WHtR, and inversely correlated with urea .

VARIABLE	LEPTIN		NESFATIN-1		TAC		BMI	
	R	P	r	P	r	P	r	P
Nesfatin-1	-0.320*	0.013	-	-	0.417*	0.001	-0.294*	0.023
TAC	-0.038	0.776	0.417*	0.001	-	-	0.013	0.918
BMI	0.447*	<0.001	-0.294*	0.023	0.013	0.918	-	-
Age	-0.006	0.965	-0.152	0.246	-0.283*	0.029	-0.028	0.834
WC	0.415*	0.001	-0.541*	<0.001	-0.111	0.400	0.444*	<0.001
WHpR	0.334*	0.009	-0.468*	<0.001	-0.147	0.262	0.275*	0.033



WHR	0.457*	<0.001	-0.565*	<0.001	-0.145	0.269	0.520*	<0.001
TAG	0.406*	0.001	-0.005	0.970	0.092	0.486	0.184	0.160
T.Ch	0.047	0.723	-0.003	0.980	-0.119	0.364	-0.192	0.141
HDL-Ch	-0.092	0.486	-0.051	0.698	0.018	0.894	-0.051	0.699
LDL-Ch	-0.039	0.767	0.010	0.938	-0.147	0.264	-0.227	0.081
VLDL-Ch	0.406*	0.001	-0.005	0.970	0.092	0.486	0.184	0.160
Urea	-0.116	0.379	-0.005	0.969	-0.047	0.724	-0.276*	0.033
Creatinine	-0.140	0.285	0.040	0.760	-0.030	0.817	-0.183	0.162

Table .3.3 Correlation in obese males

* Significant

3.13. Receiver Operating Characteristic

The ROC curve was used to examine the usefulness of leptin, nesfatin-1, and total antioxidant capacity in the prognosis of obesity in males. In Table 3.4 the parameters of ROC curve expressed by the AUC value, the cut-off and the values of sensitivity and specificity for the three variables.

PARAMETERS	LEPTIN	NESFATIN-1	TAC
AUC	0.997	0.955	0.973
SE	0.011	0.017	0.012
P-value	<0.001	<0.001	<0.001
Cut-off value	5.79 ng/mL	1.93 ng/mL	2.15 μ mol vit. C Eq/L
Sensitivity %	93.3 %	91.7 %	98.3 %
Specificity %	88.3 %	90 %	88.3 %

Table 3.4. The ROC curve parameters

***AUC: area under the curve.**

From the ROC curve, leptin has shown excellent sensitivity in the prognosis of obesity in male subjects with AUC of 0.997, and cut-off value of 5.79 with 93.3 % sensitivity and 88.3 % specificity.

From the ROC curve, nesfatin-1 has shown excellent sensitivity in the prognosis of obesity in male subjects with AUC of 0.955, and cut-off value of 1.93 with 91.7 % sensitivity and 90.0 % specificity.

From the ROC curve, TAC has shown excellent sensitivity in the prognosis of obesity in male subjects with AUC of 0.973, and cut-off value of 2.15 with 98.3 % sensitivity and 88.3 % specificity.



4. Discussion

The study has taken an interest in the researching of leptin and nesfatin-1 relationship and their effect and association with the antioxidant defense system within the biological system of obese males. The study has proceeded by selecting males with high BMI values ($>30 \text{ kg.m}^{-2}$) to confirm their obesity status. The concentration of leptin has been increased significantly in obese males of this study. In a previous study, Wali and Wali have shown that obese subjects were observed with significant higher levels of leptin compared to lean subjects (Wali and Wali 2016), which was agreed with our study. In a previous review of literature, Sengupta et al. have shown that leptin, the signaling peptide that released from the adipose tissue, is increased and overexpressed during obesity, and it may lead to (or involved in) reducing the fertility of men (Sengupta et al. 2019).

The concentration of nesfatin-1 was decreased in obese males of this study. Abaci et al. have mentioned in their study that nesfatin-1 concentration was systemically reduced in obese people compared to lean people. Moreover, they have indicating a negative relationship of nesfatin-1 with the BMI of obese people in their study (Abaci et al. 2013), this was similar to our data. Tsuchiya et al. have reported the blood concentration of nesfatin-1 was correlated inversely with the BMI of people, and they have observed that obese people have significant low concentrations of nesfatin-1 in their peripheral blood (Tsuchiya et al. 2010). The level of total antioxidant capacity was reduced significantly in obese males of this study. In the study of Amirkhizi et al. the authors have shown that obese women had significant lower plasma levels of TAC compared to lean women (Amirkhizi et al. 2010), which agreed with our data. Another agreement was found with Taay and Mohammed, who reported a reduction in the glutathione peroxidase (an antioxidant enzyme) in women with obesity compared to non-obese women (Taay and Mohammed 2020).

At last, the lipid profile parameters were changed significantly in obese males compared to lean males, while urea was significantly increased and creatinine was not changed. A previous study performed by Taay et al. have mentioned significant increase in TAG, T.Ch, LDL-Ch, VLDL-Ch, urea, and creatinine, with a decrease in HDL-Ch levels in obese women (Taay et al. 2021). The latter study has agreed with our observations, except for the creatinine which was not changed significantly in obese males.

4.1. Conclusions

- (1) The concentration of leptin was observed to be significantly higher in obese males compared to lean males.
- (2) The concentration of nesfatin-1 was observed to be significantly lower in obese males compared to lean males.



- (3) The level of TAC was observed to be significantly lower in obese males compared to lean males.
- (4) Nesfatin-1 has shown direct correlation with TAC in obese males.
- (5) Nesfatin-1 has shown inverse correlation with leptin.
- (6) Leptin has correlated positively with the anthropometric measurements of obesity in obese males, while nesfatin-1 and TAC were correlated negatively with them.
- (7) All of leptin, nesfatin-1, and TAC have shown excellent sensitivities in the prognosis of obesity in males.
- (8) Lipid profile markers were significantly changed in obese males compared to lean males.

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