https://doi.org/10.46389/rjd-2022-1095
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2022; volume 29, issue 2, pages 214–219

Original Research

Metabolic syndrome: Correlation between main hormones and oxidative stress parameters

Prodan A. M.*, Dzhyvak V. G.

I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

*Correspondence to: Andrii Prodan, PhD, Associate Professor, Faculty of Postgraduate Education, Department of Surgery, I.Horbachevsky Ternopil National Medical University, Maidan Voli str., 1, 46001, Ternopil, Ukraine, Phone: +380677120199, E-mail id: prodan@tdmu.edu.ua

Received: 18 February 2022 / Accepted: 4 April 2022

Abstract

Background and aims: Pathogenetic aspects of metabolic syndrome are still the subject of debate due to significant differences in the results of many studies. The aim of the study was to examine the correlation between the levels of ghrelin, leptin, insulin, adiponectin, resistin, metabolic syndrome, and oxidative stress parameters in patients with obesity of varying degrees. **Materials and methods:** It was examined with 44 patients (59.10% of women and 40.90% of men) aged 31–79 years with a body mass index ▯30 kg/m² (main group) and 12 patients (66.67% – women and 33.34% – men) aged 25–67 years, with a body mass index ▯25 kg/m² which made up the control group. Plasma levels of glycated hemoglobin, glycaemia, as well as total cholesterol, low- and high-density lipoproteins, triglycerides, eptin, ghrelin general, adiponectin, oxidized low-density lipoprotein cholesterol, and resistin were measured. **Results:** The analysis confirmed the presence of an imbalance of orexigenic and anorexigenic hormones in obese patients, with a progressive increase in leptin, insulin and decreased levels of ghrelin, adiponectin with increasing obesity, as evidenced by the corresponding correlations. Metabolic syndrome is characterized by excessive accumulation of fat in adipose tissue, which leads to adipocyte hypertrophy, hypoxia, and the development of systemic oxidative stress. Because adipose tissue is responsible for the production of various vasoactive adipokines to modulate vascular function, adipose tissue dysfunction caused by obesity significantly contributes to the pathogenesis of the cardiovascular disease. Correction of metabolic parameters and oxidative stress is a potential strategy for the prevention and treatment of cardiovascular disease.

Keywords: metabolic syndrome, ghrelin, resistin, adiponectin, leptin, oxidized low-density lipoprotein cholesterol.

Background and aims

Metabolic syndrome is a worldwide pandemic [1]. It is not the cause of death, while cardiovascular disease, diabetes, which are caused by obesity, cause high mortality (up to 70%) [2].

Adipocytes synthesize adipokines and hormones, which play an important role in the development of obesity, chronic inflammation, and cardiovascular disease [3–5]. Being overweight disrupts carbohydrate metabolism, causes an imbalance of the lipid spectrum, changes the level of blood pressure, which together significantly reduces the quality of life of the patient.

Normally, insulin supports the proper storage and use of energy, leptin reduces constant energy consumption. Both play a key role in the central regulation of energy expenditure and glucose homeostasis [6]. Hyperleptinemia has been shown to be a driver of obesity and associated metabolic syndrome [7].

Adiponectin increases insulin sensitivity, both directly through the insulin receptor and indirectly, by reducing non-esterified fatty acid emissions and gluconeogenesis and by increasing

fatty acid oxidation. In muscles, adiponectin stimulates glucose utilization and fatty acid oxidation [8].

Ghrelin, in turn, has a depressant effect on the central nervous system, thereby blocking the satiety center. Due to the interaction of ghrelin and leptin, the hypothalamus can regulate hunger and satiety, which leads to energy homeostasis, and imbalance and dysregulation of these hormones can have a drastic effect on energy homeostasis and diabetes and cardiovascular disease [9].

The results of studies of the relationship between resistin and the development of obesity and insulin resistance in humans are quite contradictory. Some studies have shown that resistin levels are higher in obese, insulin-resistant, and diabetic individuals and are positively correlated with BMI and visceral adipose tissue volume, insulin resistance indices, and other markers of the so-called metabolic syndrome [10]. In other studies, such a connection was found [11].

Oxidative stress is a potential pathophysiological mechanism underlying cardiovascular dysfunction. Perivascular adipose tissue (PVAT) is an important adipose tissue that regulates the modulation of vascular contractility through the secretion of PVAT-derived relaxing factors (PVRFs) and PVAT-derived reduction factors (PVCFs) [12]. In obesity, increased oxidative stress, inflammation, and dysfunction of eNOS in PVAT may alter the balance between PVRF and PVCF. Obesity-induced PVAT dysfunction leads to a decrease in PVRF and PVCF production and therefore leads to increased vascular contraction, the development of hypertension.

The aim of the study was to examine the correlation between the levels of ghrelin, leptin, insulin, adiponectin, resistin, metabolic syndrome, and oxidative stress parameters in patients with obesity of varying degrees.

Materials and methods

Clinical data

It was examined 44 patients (59.10% of women and 40.90% of men) aged 31–79 years with a body mass index >30 kg/m^2 (main group) and 12 patients (66.67% – women and 33.34% – men) aged 25–67 years, with a body mass index <25 kg/m^2 which made up the control group. Informed consent was obtained for the proposed examination in all patients.

Clinical data (sex, age, height, body weight, BMI, waist circumference and mean blood pressure) were analyzed. Blood pressure (BP) was measured three times on the right arm in a sitting position for 10 minutes and was considered elevated if it exceeded the level of 139/89 mm Hg. Determination of the type of fat distribution was assessed by the ratio of waist volume to hip volume (WV / HV). A value greater than 1 corresponded to the abdominal type of obesity.

Laboratory data

Blood samples taken for the study were obtained after patients fasted the night before the study. Plasma levels of glycated hemoglobin (HbA_{1c}), glycaemia, as well as total cholesterol, low- and high-density lipoproteins (LDL, HDL), and triglycerides were measured using commercial kits (Roche Diagnostics) using Hitachi automatic analyzer. The insulin resistance index was calculated by the Caro method as the value of the ratio of glucose to insulin measured in the subjects on an empty stomach.

Malondialdehyde (MDA) was measured using a TBARS assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) for assaying lipid peroxidation in plasma [13].

For measuring the plasma levels of leptin, ghrelin general, adiponectin, oxidized low-density lipoprotein cholesterol (Ox-LDL), resistin 10 ml of blood was collected in vacuum tubes. The samples were then kept at room temperature for 30 minutes and then centrifuged at 1670 *g* for 10 minutes. Isolated serum samples were stored in a freezer at –20°C.

Determination of serum levels was performed using Leptin ELISA (LDN Labor Diagnostics Nord GmbH & Co. KG, Germany), Human Ghrelin ELISA Kit (Thermo Fisher Scientific, USA), Human Adiponectin ELISA Kit (Thermo Fisher Scientific, USA), Ox-LDL (Thermo Fisher Scientific,

USA), Resistin Human ELISA Kit (Thermo Fisher Scientific, USA) on Multiskan FC analyzer (Skanlt Software version 4.1 for Microplate Readers RE, ver. 4.1.0.43) at a wavelength of 620 nm.

Statistical analysis

Statistical processing of the obtained research data was processed using the software Excel ("Microsoft", USA) and Statistica.10.1 (Statsoft, USA) with variation and correlation analysis. Data are expressed as mean ± S.D. p-Value <0.05 signified statistical significance.

Results

Analysis of the data in Table 1 showed a significant difference between the two groups in the following indicators: BMI, mean blood pressure, insulin, Caro index, total ghrelin, leptin, resistin, and adiponectin, indicating a significant metabolic imbalance in patients with metabolic syndrome. Significant differences in obese patients and the control group were not found for the following parameters: glycaemia, HbA_{1c}, cholesterol, LDL, HDL, triglycerides, and atherogenic index.

Correlation analysis in groups of patients with varying degrees of obesity revealed a relationship between BMI and leptin (r = +0.99), ghrelin (r = −0.86), adiponectin (r = −0.92), resistin (r = +0.98) HbA_{1c} (r = +0.60), total cholesterol (r = +0.99), LDL (r = +0.98), HDL (r = −0.99), atherogenic index (r = +0.96), insulin (r = +1.00), glycaemia (r = +0.84).

A comparison of leptin and ghrelin concentrations at different degrees of obesity showed strong feedback (r = −0.92), when serum ghrelin levels decreased with increasing BMI and leptin concentration.

At the same time, after analyzing the interdependence of leptin and insulin

Table 1: Comparative evaluation of metabolic disorders in obese patients and the control group.

Indicator	Obese patients (n=44)	Control (n=12)	p-Value
BMI, kg/m²	41.52±6.78	20.89±2.06	0.009
Mean blood pressure, mm Hg	137.77±8.09	120.17±3.48	0.050
Glucose, mmol/l	6.01±0.54	5.58±0.37	0.379
HbA_{1c}, %	6.08±1.42	5.43±0.25	0.734
Insulin, μU/ml	24.56±4.56	8.61±2.78	0.004
Caro index	0.24	0.64	0.011
Ghrelin general, ng/ml	747.54±338.65	1380.48±146.34	0.042
Leptin, ng/ml	42.14±6.77	5.02±3.79	0.000014
Adiponectin, μg/ml	6.45±0.17	10.78±0.21	0.005
Adiponectin/leptin ratio	0.01	2.15	0.0001
Resistin, ng/ml	8.12±1.02	3.98±0.81	0.002
Adiponectin/resistin ratio	0.79	2.71	0.00087
MDA, μM	6.92±0.41	4.21±0.35	0.000006
Ox-LDL, U/l	105.41±2.78	70.34±1.89	0.0004
Total cholesterol, mmol/l	6.21±1.43	5.83±1.12	0.835
LDL, mmol/l	3.68±1.04	3.47±1.09	0.889
HDL, mmol/l	1.18±1.02	1.42±0.89	0.859
Triglycerides, mmol/l	1.76±0.64	1.54±0.75	0.824
Atherogenic index	4.78±1.34	3.21±1.27	0.398

https://doi.org/10.46389/rjd-2022-1095

concentrations, a strong direct relationship was found (r = +0.99), during which the concentration of both indicators increased with increasing BMI. Excess leptin significantly enhances the manifestations of insulin resistance in patients, according to the results of a certain level of insulin and Caro index (Table 2).

The concentration of insulin in patients was significantly higher even in the first degree of obesity compared with the control group (p = 0.009) and increased with adipose tissue mass gain. The severity of insulin resistance increased in direct proportion to the degree of obesity, as evidenced by the decrease in the Caro index relative to BMI (r = −0.95). In addition, an inverse correlation was found between the level of adiponectin and the degree of insulin resistance (according to the level of the Caro index) (r = −0.93). However, the ratio of adiponectin/leptin to the degree of insulin resistance showed a direct relationship (r = +0.70).

In patients with varying degrees of obesity, there is a pronounced imbalance of triglycerides, cholesterol, LDL and HDL due to leptin resistance, which initiates and exacerbates pathological atherogenesis and atherosclerosis. Correlation analysis of the dependence of leptin levels and lipid fractions established the following relationships: HDL (r = −0.99), triglycerides (r = +0.97), ansd atherogenic factor (r = +0.99).

According to the results of the study, it was found that the concentration of ghrelin decreased with an increasing degree of obesity, reaching the minimum value of obesity of III degrees compared with the control group (p = 0.02). Glycaemia between subgroups did not change significantly (p = 0.11). However, a significant relationship was found between BMI and mean blood pressure (r = +0.88).

The following relationships were found between the level of resistin and BMI (r= +0.95), leptin (r= +0.98), ghrelin (r= −0.97), adiponectin

Table 2: Comparative evaluation of metabolic disorders in patients with obesity of varying degrees.

Indicator	Obesity I (n=8)	Obesity II (n=15)	Obesity III (n=21)	Control (n=12)
BMI, kg/m^2	33.52±0.83	37.68±1.46	47.11±5.49	20.89±2.06
Mean blood pressure, mm Hg	128.57±6.32	137.53±6.81	140.66±7.43	120.17±3.48
Glucose, mmol/l	5.68±0.59	6.01±0.54	6.09±0.49	5.58±0.37
HbA_{1c}, %	5.59±0.42	6.22±1.33	6.10±1.70	5.43±0.25
Insulin, μU/ml	21.44±3.89	23.56±4.67	27.89±5.21	8.61±2.78
Caro index	0.26	0.26	0.22	0.64
Ghrelin general, ng/ml	1153.86±324.05	733.29±274.58	613.35±289.30	1380.48±146.34
Leptin, ng/ml	18.05±8.87	33.99±7.47	55.24±6.19	5.02±3.79
Adiponectin, μg/ml	7.01±0.83	6.21±0.72	5.76±0.91	10.78±0.21
Adiponectin/leptin ratio	0.39	0.18	0.10	2.15
Resistin, ng/ml	7.81±0.67	8.45±0.56	8.92±0.69	3.98±0.81
Adiponectin/resistin ratio	0.89	0.73	0.65	2.71
Ox-LDL, U/l	95.64±0.73	103.71±0.45	112.94±0.51	70.34±0.34
MDA, μM	5.74±0.32	6.09±0.28	7.58±0.17	4.21±0.35
Total cholesterol, mmol/l	6.40±1.24	6.56±1.46	6.78±1.78	5.83±1.12
LDL, mmol/l	3.61±1.04	3.79±1.02	4.02±0.86	3.47 ±1.09
HDL, mmol/l	1.23 ±1.04	1.16 ±1.01	1.04 ±0.98	1.42 ±0.89
Triglycerides, mmol/l	1.71 ±0.38	1.86 ±0.42	1.95 ±0.74	1.54±0.75
Atherogenic index	4.12 ±1.56	4.47 ±1.18	4.78 ±1.40	3.21±1.27

(r= –0.99), insulin (r= +0.96), Caro index (r= –0.81). Comparing the ratios adiponectin/leptin, adiponectin/resistin and Caro index did not find strong correlation.

Analysis of oxidative stress indicators established increased oxidized low-density lipoprotein cholesterol according to the level of BMI (r = +0.98), the same correlation was between MDA and BMI (r = +0.99). Comparing Ox-LDL with total cholesterol we received positive strong correlation (r= +0.99), with LDL (r= +1.00), triglycerides (r= +0.98), HDL (r= –0.99).

Discussion

With a diagnosis of obesity, even in the first degree, there is resistance to leptin, possibly at the stage of its transport across the blood-brain barrier, and in some cases at the post-receptor level due to possible genetic "breakdown". That is why our study, as in many studies of other researchers found fairly high levels of serum leptin, which progressively increased with increasing BMI [14]. Another function of leptin is to protect peripheral tissues from ectopic lipid accumulation due to its antistetogenic effect and regulation of fatty acid homeostasis. Elevated leptin levels increase low-density lipoprotein levels and cholesterol levels [15].

Many studies showed, no significant difference in resistin concentration between patients who had normal BMI and those who were obese. Similarly, Kocot et al. did not find any differences in resistin concentration between BMI groups [16]. On the other hand, Mabrouk et al. found that resistin concentrations were higher in obese patients with diabetes than in obese non-diabetic participants [17]. In our study, we got the same data, the presence of a strong positive correlation between the BMI and level of resistin.

S. Zoccali et al. [18] showed that people with "high-normal" serum levels of adiponectin have a lower rate of mortality associated with cardiovascular pathology. N. Ouchi et al. [19] in a long-term study demonstrated that in individuals with coronary heart disease, the concentration of adiponectin is significantly lower than in the control group comparable in terms of BMI and age.

Serum adiponectin concentrations have been shown to be inversely correlated with the severity of insulin resistance in patients with high BMI. Consistent with the findings of previous studies [20], the present study showed that a lower level of adiponectin was present in patients with obesity II and III stages and inversely correlated with BMI, resistin, leptin, and insulin levels. Adiponectin is considered to have anti-diabetic and anti-inflammatory effects; therefore, it is reasonable to presume that patients with lower levels are more likely to develop diabetes mellitus on the background of obesity.

Previous studies have suggested that adiponectin/leptin and adiponectin/resistin ratios are more closely related to the severity of insulin resistance [21]. In our study, we did not find a significant correlation between these ratios and the Caro index.

Kopprasch et al. [22] reported that LDL cholesterol and triglycerides were the strongest predictors of circulating Ox-LDL levels, followed by HDL cholesterol. They added that the strong correlation of Ox-LDL with LDL cholesterol indicates that LDL oxidation is preferentially associated with dyslipidemia and that Ox-LDL increase may explain the high atherogenic potency of dyslipidemia in the pre-diabetic state. Our study shows a positive correlation between Ox-LDL and dyslipidemia, however, it is not strong enough to exclude the effect of oxidative stress.

Conclusion

The analysis confirmed the presence of an imbalance of orexigenic and anorexigenic hormones in obese patients, with a progressive increase in leptin, insulin, and decreased levels of ghrelin, adiponectin with increasing obesity, as evidenced by the corresponding correlations.

Obesity is characterized by excessive accumulation of fat in adipose tissue, which leads to adipocyte hypertrophy, hypoxia, and the development of systemic oxidative stress. Because adipose tissue is responsible for the production of various vasoactive adipokines to modulate

https://doi.org/10.46389/rjd-2022-1095

vascular function, adipose tissue dysfunction caused by obesity significantly contributes to the pathogenesis of cardiovascular disease.

Correction of metabolic parameters and oxidative stress is a potential strategy for the prevention and treatment of cardiovascular disease.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Bluher, M. (2019). Obesity: global epidemiology and pathogenesis. *Nat Rev Endocrinol*. 15:288–298.
2. WHO (2017). Noncommunicable Diseases Progress Monitor. Available online at: https://www.who.int/publications/i/item/WHO-NMH-NVI-17.9
3. Genoux, A., Bastard, J. P., Groupe de Travail, R. A. (2020). Effects of leptin and adiponectin on the cardiovascular system. *Ann Biol Clin*. 78:253–260.
4. Chen, H., Montagnani, M., Funahashi, T., Shimomura, I., Quon, M. J. (2003). Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem*. 278:45021–45026.
5. Margaritis, M., Antonopoulos, A. S., Digby, J., et al. (2013). Interactions between vascular wall and perivascular adipose tissue reveal novel roles for adiponectin in the regulation of endothelial nitric oxide synthase function in human vessels. *Circulation*. 127:2209–2221.
6. Williams, K. W., Margatho, L. O., Lee, C. E. (2010). Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *J Neurosci*. 30:2472–2479.
7. Williams, K. W., Margatho, L. O., Lee, C. E., Choi, M., Lee, S., Scott, M.M. (2010). Segregation of acute leptin and insulin effects in distinct populations of arcuate proopiomelanocortin neurons. *J Neurosci*, 30, 2472–9.
8. Yamauchi, T., Shudo, K. et al. (2001). *Nat Med*. 7:941–946.
9. Churm, R., Davies, J. S., Stephens, J. W., Prior, S. L. (2017). Ghrelin function in human obesity and type 2 diabetes: a concise review. *Obesity Rev*. 18(2):140–148.
10. Churm, R., Davies, J. S., Stephens, J. W., & Prior, S. L. (2017). Ghrelin function in human obesity and type 2 diabetes: a concise review. *Obesity Rev*. 18(2): 140–148.
11. Lee, J. H., Mantzoros, C. S., et al. (2003). *J Clin Endocrinol Metab*. 88:4848–4856.
12. Greenstein, A. S., Khavandi, K., Withers, S. B., et al. (2009). Local inflammation and hypoxia abolish the protective anticontractile properties of perivascular fat in obese patients. *Circulation*. 119:1661.
13. Dawn-Linsley, M., Ekinci, F. J., Ortiz, D., Rogers, E., Shea, T. B. (2005). Monitoring thiobarbituric acid-reactive substances (TBARs) as an assay for oxidative damage in neuronal cultures and central nervous system. *J Neurosci Methods*. 141:219–222.
14. Myers, M. G., Cowley, M. A., Münzberg, H. (2008). Mechanisms of leptin action and leptin resistance. *Annu Rev Physiol*. 70:537–556.
15. Kumar, R., Mal, K., Razaq, M. K., et al. (2020). Association of leptin with obesity and insulin resistance. *Cureus*. 12 (12).
16. Kocot, J., Dziemidok, P., Kiełczykowska, M, et al. (2017). Adipokine profile in patients with type 2 diabetes depends on degree of obesity. *Med Sci Monit*. 23:4995–5004.
17. Mabrouk, R., Ghareeb, H., Shehab, A., et al. (2013). Serum visfatin, resistin and IL-18 in A group of Egyptian obese diabetic and non diabetic individuals. *Egypt J Immunol*. 20:1–1.
18. Zoccali, C., Mallamaci, F., Tripepi, G., et al. (2002). *J Am Soc Nephrol*. 13:134–141.
19. Ouchi, N., Matsuzawa, Y., et al. (1999). *Circulation*. 100:2473–2476.
20. Darabi, H., Raeisi, A., Kalantarhormozi, M. R., et al. (2015). Adiponectin as a protective factor against the progression toward type 2 diabetes mellitus in postmenopausal women. *Medicine. (Baltimore)* 94:e1347.
21. Frühbeck, G., Catalán, V., Rodríguez, A., et al. (2019). Adiponectin–leptin ratio: a promising index to estimate adipose tissue dysfunction. Relation with obesity-associated cardiometabolic risk. *Nutrients*. 11:pii:E454.
22. Kopprasch, S., Pietzsch, J., Kuhlisch, E., Fuecker, K., Temelkova-Kurktschiev, T., Hanefeld, M., Kuhne, H., Julius, U., Graessler, J. (2002). In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. *Diabetes*. 51:3102–3106.