Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Assessment of ghrelin and leptin receptor levels in postmenopausal women who received oral or transdermal menopausal hormonal therapy*

Barbara RUSZKOWSKA†[1], Alina SOKUP[2], Arleta KULWAS[1], Maciej W. SOCHA[3], Krzysztof GÓRALCZYK[1], Barbara GÓRALCZYK[1], Danuta ROŚĆ[1]

([1]*Department of Pathophysiology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland*)

([2]*Department of Gastroenterology, Angiology and Internal Diseases, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland*)

([3]*Department of Obstetrics, Gynecology and Oncological Gynecology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland*)

†E-mail: ruszkowska.basia@gmail.com

Received Aug. 24, 2011; Revision accepted Nov. 30, 2011; Crosschecked Dec. 14, 2011

Abstract: Objective: In postmenopausal women, an increased leptin concentration and reduced levels of ghrelin and adiponectin were observed. The aim of this study was to evaluate the concentrations of the active form of ghrelin, total ghrelin, leptin receptor, lipoprotein(a) (Lp(a)), and plasminogen activator inhibitor type 1 (PAI-1) in postmenopausal women who received oral or transdermal menopausal hormonal therapy (MHT). Methods: The study involved 76 healthy women: 46 women aged from 44 to 58 years who received oral (26) or transdermal (20) MHT; the control group consisted of 30 women aged from 44 to 54 years who did not receive MHT. The plasma concentrations of total ghrelin, the active form of ghrelin, Lp(a), and PAI-1:Ag were measured by enzyme-linked immunosorbent assay (ELISA). The concentration of the leptin receptor was measured by enzyme immunometric assay (EIA). Results: We observed a significantly higher concentration of total ghrelin and the active form of ghrelin in women who received transdermal MHT in comparison with those who took oral MHT. We also found a significantly lower concentration of total ghrelin in women who received oral MHT compared with the control group. A higher concentration of PAI-1:Ag was found in the group of women who took transdermal MHT in comparison with those who took oral MHT and with the control group. The differences were statistically significant. Additionally, we found a significant negative correlation between the concentrations of total ghrelin and PAI-1:Ag and a positive correlation between the concentrations of total ghrelin and leptin receptor in women who received transdermal MHT. Conclusions: The study showed that women who used transdermal MHT had higher levels of total ghrelin than women who took oral MHT. This indicates a beneficial effect of the transdermal route of MHT. However, transdermal therapy was associated with adverse effects with regard to the observed higher levels of PAI-1:Ag, which in turn, can lead to a reduction in fibrinolytic activity.

Key words: Menopausal hormonal therapy (MHT), Plasminogen activator inhibitor type 1 (PAI-1), Leptin receptor, Ghrelin, Menopause

doi:10.1631/jzus.B1100276 **Document code:** A **CLC number:** R335$^+$.9

1 Introduction

Leptin and ghrelin are antagonistic hormones that regulate and control body weight. Ghrelin is a peptide hormone with a chain of about 28 amino acids.

* Project supported by the Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń (No. 73/04), Poland

An octanoyl group is attached to the third position of the polypeptide chain, where serine is located. In serum there is also a ghrelin molecule without an octanoyl group, which presumably has no endocrine action. In human gastric mucosa, four ghrelin derivatives have been isolated. They were classified according to the structure of the acyl group at the serine molecule. Peptides which were distinguished either had no substituent (most numerous), or their substituent was an octanoyl (C8:0, active form), decanyl (C10:0) or decenyl (C10:1) group. Ghrelin is synthesized mainly in the stomach but also in small amounts in the placenta, pancreas, kidney, pituitary, and hypothalamus. Its production is also considered as a sign of starvation before having a meal (Meier and Gressner, 2004). Biological activity of ghrelin is possible through two types of receptors, G-protein-coupled receptors types 1 and 1b (growth hormone secretagogue receptor (GHS-R)), which are located in the pituitary, hypothalamus, kidney or adipose tissue (Konturek *et al*., 2004).

Ghrelin significantly affects the functioning of the digestive tract. Through the vagus nerve it stimulates the motility and secretion of hydrochloric acid by the stomach and inhibits the secretion of pepsin. It plays an important role in the regulation of appetite, energy balance, and glucose homeostasis. The serum ghrelin level increases during starvation, weight loss, and with an increase in blood insulin levels, while it decreases in a period of satiety (Toshinai *et al*., 2001; Nishi *et al*. 2005). Furthermore, a circadian rhythm of ghrelin secretion has been observed. Its level increases during fasting, before meals and at night, but decreases after meals, especially in those who are rich in fats and carbohydrates (Weigle *et al*., 2003).

Leptin is synthesized primarily by adipocytes of white fatty tissue under the skin, including the muscles, placenta, and stomach. Women have higher leptin levels than men due to their higher content of subcutaneous tissue. Biological action of leptin is possible via membrane receptors that belong to the class I cytokine family. The receptors are located in the hypothalamus (where there are appetite control centers), as well as in the thyroid, adrenal glands, and ovaries (Meier and Gressner, 2004; Szumiło *et al*., 2007). Several isoforms of leptin receptors (Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, and Ob-Re) have been identified. It is believed that the Ob-Ra form is the conveyor of leptin, and Ob-Re is a soluble form of the leptin receptor transmembrane. The Ob-Rb form is the only receptor isoform that contains an active intracellular domain (Meier and Gressner, 2004).

Leptin activates processes aimed at reducing energy stores by reducing the demand for food. It affects the body's energy balance, the maturation of the reproductive system in women, and the process of angiogenesis in men (Meier and Gressner, 2004; Nishi *et al*., 2005; Szumiło *et al*., 2007). This protein stimulates the proliferation of colonocytes and belongs to a group of factors that regulate intestinal absorption (Nishi *et al*., 2005). Leptin is known as a satiety hormone because it suppresses appetite (Szumiło *et al*., 2007). In most obese people, resistance to leptin has been observed, which may affect the disorder in energy demand (Toshinai *et al*., 2001; Meier and Gressner, 2004).

After menopause, the incidence of polymetabolic syndrome increases due to the reorganization of adipose tissue and its appearance in increased amounts in the abdominal area. Intra-adipose tissue adipocytes synthesize factors that influence the inflammatory process and increase insulin resistance. These factors include leptin, ghrelin, adiponectin, and plasminogen activator inhibitor type 1 (PAI-1). In postmenopausal women, leptin and PAI-1 concentrations increase whereas ghrelin and adiponectin concentrations decrease (Cagnacci *et al*., 2002; Stachowiak *et al*., 2009).

The symptoms of menopausal syndrome are the most common reason for the application of menopausal hormonal therapy (MHT) in perimenopausal women. MHT aims to replace the natural ovarian hormonal activity through the administration of estrogen and/or progesterone in the minimum effective doses. The biological effects of hormone therapy depend on the types of estrogen and progesterone, and the doses of hormones. It also depends on the route of administration, with oral and transdermal MHT having significantly different effects (Pertyński and Stachowiak, 2006).

Transdermal MHT acts more favorably than oral MHT, because it affects the reduction of factor VII activity, reduces the levels of PAI-1 and E-selectin, and alters the lipid profile and carbohydrate balance. This results in a reduced risk of atherosclerosis and

coronary heart disease (Menon and Vongpatanasin, 2006). Oral MHT significantly affects the digestive system in comparison to transdermal MHT, causing bloating, nausea, and vomiting. It also increases the cholesterol saturation of bile and contributes to the development of gallstones (Mueck and Seeger, 2005).

A review of the literature shows that there are few studies on the effects of MHT on parameters such as ghrelin or leptin levels in postmenopausal women. Results obtained by Kellokoski *et al*. (2005) and Lambrinoudaki *et al*. (2008) were divergent. The aim of this study was to evaluate the concentrations of the total and active form of ghrelin, and the leptin receptor, as well as lipoprotein(a) (Lp(a)) and PAI-1:Ag in postmenopausal women who received oral or transdermal MHT.

2 Materials and methods

2.1 Subjects

Women from all study groups were selected in the Outpatient Gynecology Centre of the University Hospital in Bydgoszcz, Poland (Ruszkowska *et al*., 2010a; 2010b; 2011). Written informed consent was obtained from each participant before entering the study. The study was permitted by the Bioethics Committee of the Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Toruń, Poland (No. KB/305/2004).

The study was conducted on 76 healthy, non-smoking women, who were 1–2 years postmenopausal. Forty-six women aged from 44 to 58 years (mean age 52 years) used oral (26) or transdermal (20) MHT. MHT was taken daily during continuous treatment in the form of a composite preparation of estrogen and progesterone in different combinations. Twenty-six women from the study group used oral MHT [2 mg 17β-estradiol (E2) and 1 mg norethisterone acetate (NETA) (Kliogest, Novo Nordisk Pharma, Poland)] and 20 women used transdermal MHT [50 μg E2 and 170 μg NETA (SYSTEN® Conti, Janssen-Cilag, Warsaw, Poland)]. The subjects used MHT for 6–14 months. Reported climacteric symptoms, such as heavy and regular hot flushes with drenching sweat, were the main indicators for MHT (Ruszkowska *et al.*, 2010a; 2010b; 2011).

The control group consisted of 30 healthy, nonsmoking, post-menopausal women, aged from 44 to 54 years (mean age 49 years), who did not use MHT.

Blood pressure (BP) and body mass index (BMI) were measured at the beginning of the study in all groups. The systolic blood pressure (SBP) was (120±19) mmHg, diastolic blood pressure (DBP) was (74±15) mmHg, and the BMI was (21.0±3.5) kg/m^2.

Women in all study groups had neither diabetes mellitus nor glucose intolerance. They had no prior thrombosis or systemic illnesses. None of them took any other medication that might have interfered with the coagulation system. All women included in the study had a complete gynecological examination, cytology smear, breast examination, and mammography (Ruszkowska *et al*., 2010a; 2010b; 2011).

Venous blood (4.5 ml) for tests of the leptin receptor, PAI-1:Ag, and Lp(a) was collected into cooled tubes (Becton Dickinson Vacutainer® System, Plymouth, UK) containing 0.13 mol/L trisodium citrate (the final blood-anticoagulant ratio was 9:1) after 30 min of rest between 7:30 and 9:30 am and after a 12-h overnight fast. The blood samples were immediately mixed and centrifuged at 3000×*g* at 4 °C for 20 min. The obtained platelet-poor plasma was divided into 200 μl Eppendorf-type tubes and then samples were frozen at −20 °C (according to the manufacturer's procedures) until assayed, but for no longer than three months. Some precautions were required in order to determine the concentration of the active form of ghrelin, because it was very unstable and labile in plasma. To prepare plasma samples, whole blood was drawn directly into a centrifuge tube that contained 500 U of aprotinin and 1.25 mg of ethylenediaminetetraacetic acid (EDTA)-2Na per 1 ml of whole blood. The tubes were mixed gently and then the blood samples were immediately centrifuged at 1500×*g* for 15 min at 4 °C. Then, 100 μl of 1 mol/L HCl per 1 ml of collected plasma were added immediately. The obtained plasma was divided into 200 μl Eppendorf-type tubes and then the samples were frozen at ≤−40 °C until assayed. Blood for tests of total ghrelin was collected in a tube containing no anticoagulant (Becton Dickinson Vacutainer® 17490, Plymouth, UK). 4-(2-Aminoethyl)benzenesulfonyl fluoride (AEBSF) was then added to give a final concentration of 1 mg/ml, and the blood was allowed to clot at room temperature for 30 min. The serum

was separated by centrifuging at 2500×*g* for 15 min and kept at 4 °C. The serum was transferred to separate tubes and acidified with HCl to a final concentration of 0.05 mol/L and then the tubes were frozen at ≤−20 °C until assayed.

2.2 Metabolism and hemostasis assays

The concentrations of the active form of ghrelin and total ghrelin were measured by enzyme-linked immunosorbent assay (ELISA)—human ghrelin (active) ELISA and human ghrelin (total) ELISA (LINCO Research, St. Charles, Missouri, USA). The concentration of Lp(a) was measured by ELISA—lipoprotein(a) ELISA (ALPCO Diagnostics, Salem, USA); PAI-1:Ag was determined by ELISA—ASSERACHROM® PAI-1 (Diagnostica Stago, Asnieres, France). The concentration of the leptin receptor was measured by enzyme immunometric assay (EIA)—Leptin Receptor EIA (ALPCO Diagnostics, Salem, USA).

2.3 Statistical analysis

Statistical analysis was performed using the statistical program STATISTICA 9.1 StatSoft® software (StatSoft®, Cracow, Poland). The Shapiro-Wilk test was used to assess the normality of distributions. For variables with normal distribution, arithmetic means (X) and standard deviations (SD) were determined. The median (Me), lower quartile (Q_1), and upper quartile (Q_3) were used for variables that were not normally distributed. The significance of differences between groups of normally distributed variables was analyzed using the Fisher-Snedecor analysis of variance (ANOVA), and for other variables the Kruskal-Wallis ANOVA test was used. P-values of <0.05 were considered statistically significant.

3 Results

Table 1 shows the concentrations of total ghrelin, the active form of ghrelin, leptin receptor, Lp(a), and PAI-1:Ag in women who received oral or transdermal MHT and in the control group. We observed a significantly higher concentration of total ghrelin and the active form of ghrelin in women who received transdermal MHT in comparison with the group of women who took oral MHT (P=0.0497; P=0.0220, respectively). We found also a significantly lower concentration of total ghrelin in women who took oral MHT compared with those in the control group (P=0.0286). Moreover, a higher concentration of PAI-1:Ag was found in the group of women who received transdermal MHT in comparison with those who used oral MHT and with the control group. The differences were statistically significant (P=0.0001 and P=0.0234, respectively).

Table 2 shows correlations between selected parameters. We found a significant negative correlation between the concentrations of total ghrelin and PAI-1:Ag (P=0.015) and a significant positive correlation between the concentrations of total ghrelin and leptin receptor (P=0.04), in women who received transdermal MHT.

4 Discussion

Menopause is an independent risk factor for polymetabolic syndrome. It is estimated that the risk of the polymetabolic syndrome increases for up to 14 years after menopause and then gradually decreases. Therapeutic procedure in perimenopausal women with metabolic syndrome involves the implementation

Table 1 Concentrations of assayed parameters in women who received oral or transdermal MHT and in the control group

Group	Total ghrelin (pg/ml)	Ghrelin active form (pg/ml)	Leptin receptor (ng/ml)	Lp(a) (mg/dl)	PAI-1:Ag (ng/ml)
Transdermal MHT (n=20)	134.06±35.90	82.25 (49.20/167.30)	27.63±8.93	6.68 (4.45/12.84)	58.25±20.37
Oral MHT (n=26)	102.80±48.33	48.20 (44.00/50.15)	32.90±9.61	4.40 (3.07/9.16)	31.96±10.85
Control (n=30)	139.64±56.78	50.55 (48.10/55.90)	26.78±10.63	4.48 (3.55/8.86)	41.82±26.10
P-value	0.0497*, 0.0286#	0.0220*	0.1012	0.4113	0.0234Δ, 0.0001*

* P<0.05, transdermal MHT vs. oral MHT; # P<0.05, oral MHT vs. control group; Δ P<0.05, transdermal MHT vs. control group. Values of studied parameters are shown as mean±SD or median (Q_1/Q_3)

Table 2 Correlations between concentrations of ghrelin active form, total ghrelin and Lp(a), leptin receptor, PAI-1:Ag

Variable	Correlation coefficient			
	Ghrelin active form		Total ghrelin	
	Transdermal MHT	Oral MHT	Transdermal MHT	Oral MHT
Lp(a)	−0.2075	0.0975	−0.2564	−0.1505
	(P=0.477)	(P=0.658)	(P=0.376)	(P=0.493)
Leptin receptor	−0.1809	0.0860	0.5752	−0.2844
	(P=0.554)	(P=0.711)	(P=0.040*)	(P=0.212)
PAI-1:Ag	0.3516	−0.2356	−0.6344	−0.1393
	(P=0.218)	(P=0.268)	(P=0.015*)	(P=0.516)

* $P<0.05$

of MHT. It has an undeniable impact on the balance of carbohydrate, and thus on insulin resistance. The use of estrogen during menopause leads to a reduction in the risk of visceral obesity, insulin resistance or type 2 diabetes. An appropriate choice of progestin is extremely important because improper use can lead to the abolition of the positive impact of estrogen on the balance of carbohydrate (Stachowiak *et al*., 2009).

In the present study, we obtained significantly higher levels of total ghrelin and the active form of ghrelin in women who received transdermal MHT compared with women who took oral MHT, and lower levels of total ghrelin in women who took oral MHT compared with the control group.

The main site of ghrelin synthesis is the gastric mucosa. Isomoto *et al*. (2005) observed that in the course of acute gastritis ghrelin levels increased, a response which is associated with an occurring inflammatory process. Because ghrelin has anti-inflammatory activity and it acts as a gastroprotective agent, it causes an increase in local blood flow and an increase in the production of prostaglandins (Konturek *et al*., 2004; Isomoto *et al*., 2005; Michalski *et al*., 2008). Ghrelin concentrations are significantly higher in lean individuals and in patients with chronic liver disease, celiac disease, bulimia or anorexia nervosa (Meier and Gressner, 2004; Michalski *et al*., 2008). The use of oral contraceptives containing estrogen and progesterone increases the concentration of ghrelin in women with severe malnutrition (Grinspoon *et al*., 2004). Conversely, lowering the concentration of ghrelin is associated with obesity and insulin resistance (Lambrinoudaki *et al*., 2008).

Results concerning a potential association between ghrelin levels and the route of administration of MHT in postmenopausal women have been conflicting (Lambrinoudaki *et al*., 2008). di Carlo *et al.* (2007) reported that women who received continuous transdermal hormone therapy (E2 at a dose of 50 mg/d and nomegestrol at 5 mg/d for 12 d/month), had higher levels of ghrelin compared with women who used oral estrogen-progesterone hormone therapy. Kellokoski *et al*. (2005) found a higher concentration of the active form of ghrelin in postmenopausal women who used oral estrogen therapy. Lambrinoudaki *et al.* (2008) did not observe statistically significant differences in circulating ghrelin concentrations between women who took oral estrogen therapy and those who had complex hormone therapy. However, Cagnacci *et al*. (2002) observed that postmenopausal women with a polimetabolic syndrome who used oral estrogen hormone therapy showed an increase in the level of leptin and a decrease in ghrelin concentration. This using transdermal estrogen hormone therapy showed a decrease in ghrelin levels but without any effect on leptin levels. This indicates a favorable effect of transdermal therapy on the polymetabolic syndrome factors in postmenopausal women.

The above results suggest that gastrointestinal bypass by using transdermal MHT has beneficial effects on the cardiovascular system in postmenopausal women. This in turn, may be linked to the anti-inflammatory and anti-atherosclerotic activities of ghrelin, which does not affect the adhesion or aggregation of platelets but is essential in reducing the formation of atherosclerotic plaque and thrombotic complications.

In the current study no statistically significant differences in leptin receptor concentrations were found, independently of the study group.

The soluble form of the leptin receptor affects leptin's effect on food intake and body weight regulated ectodomain shedding of the membrane-spanning leptin receptor, which may constitute a new mechanism for regulating the biological function of leptin (Meier and Gressner, 2004). Patients with end-stage heart failure have elevated levels of leptin and its receptor. Leptin may participate in the process of catabolism and lead to cardiac cachexia in chronic heart failure (Christian Schulze *et al*., 2003).

Insulin resistance and visceral obesity are associated with low levels of soluble forms of the receptor and a low rate of binding of free leptin, independent of body fat (Meier and Gressner, 2004). Leptin reduces insulin secretion from pancreatic B-cells and participates in the inhibition of insulin-glucose synthesis in the liver. It is also a protein important in sexual maturation and maintenance of fertility, because its concentration in the blood affects the level of secreted hormones (estrogen) (Szumiło *et al*., 2007). Patients with lipodystrophy had significantly reduced levels of leptin, but hyperleptinemia is a basic feature of obesity. The BMI is the best predictor of leptin levels (Meier and Gressner, 2004). Leptin stimulates proliferation and inhibits apoptosis in normal and cancer cells (Nishi *et al*., 2005; Hoda *et al*., 2007).

The effect of MHT on leptin levels remains unclear. Several studies show no effect of MHT on leptin levels independent of the BMI (Lambrinoudaki *et al*., 2004; 2008). On the other hand, there are studies in which an increase in leptin was achieved after one month of MHT, after two months treatment with 2 mg of estradiol administered orally, and after six months of estrogen hormone therapy (Elbers *et al*., 1999; Konukoglu *et al*., 2000). These discrepancies may be explained by the indirect effect of MHT on lowering leptin levels (which reduces adipose tissue) in contrast to the direct effect of estrogen, which stimulates the secretion of leptin (Lambrinoudaki *et al*., 2008).

Analysis of the results obtained here allowed the establishment of a positive correlation between ghrelin and leptin receptor levels in women who used transdermal hormone therapy. Cummings and Foster (2003) suggested a correlation between ghrelin and leptin in the regulation of lipid changes.

A review of the literature shows that no similar correlation was found in postmenopausal women who took MHT. Bagnasco *et al*. (2002) obtained a correlation between changes in ghrelin levels and leptin levels. During fasting, plasma ghrelin levels increased whereas leptin concentrations decreased, but during food intake the situation was reversed. This observation can be explained by the fact that ghrelin, depending on the concentration, activates arcuate nucleus neurons and leptin is their inhibitor (Cummings and Foster, 2003). Meier and Gressner (2004) described a positive correlation between BMI and leptin concentrations and a negative correlation between BMI and ghrelin levels, depending on the population tested. Shiiya *et al*. (2002) found that the ghrelin concentration was negatively correlated with the BMI during fasting in groups of Japanese patients with and without diabetes. Tschöp *et al*. (2001) measured ghrelin levels in fasting adult Caucasians and Pima Indians, and obtained negative correlations between ghrelin and the amount of body fat and the concentrations of insulin and leptin.

Our results showed that women who used transdermal MHT had a higher concentration of PAI-1:Ag compared with the control group and the group of women who took oral MHT.

PAI-1 is the main inhibitor of the plasminogen conversion reaction to plasmin and is synthesized by vascular endothelial cells, smooth muscle cells of vessels, megakaryocytes, hepatocytes, and adipocytes. A significant amount of PAI-1 is stored in platelet granules, from which it is released under the influence of different activating agents. The concentration of PAI-1 is higher in men and it increases with age and with obesity. High concentrations of PAI-1 pose a risk of thrombotic cardiovascular disease, including coronary heart disease (Dobrovolsky and Titaeva, 2002).

A review of the available literature indicates that MHT stimulates the fibrinolytic system by increasing the concentrations of tissue plasminogen activator (t-PA) and lowering the levels of PAI-1. It was observed that estrogen administered in MHT directly affects the biosynthesis and secretion of PAI-1. However, the progestin component may reduce the beneficial effects of estrogen on the fibrinolytic system (Stachowiak *et al*., 2005). In the present study it was observed that the route of administration of MHT had a significant effect on the concentration of PAI-1:Ag in the blood. The higher levels of PAI-1:Ag found in women who used transdermal MHT compared with those who took oral MHT were consistent with the results of Post *et al*. (2003), Stachowiak *et al*. (2005), and Ruszkowska *et al*. (2010a). They demonstrated that oral MHT significantly reduces the levels of PAI-1 (up to 50%) but did not observe such a significant change associated with the use of transdermal MHT. Post *et al*. (2003) explained this observation as being due to the stimulation by t-PA of the release of PAI-1 in these women. Oral use of

MHT results in stimulation of the synthesis of many proteins in the liver and in increased clearance in hepatocytes, not only t-PA but also PAI-1. Post *et al*. (2003) showed increased hepatic clearance in women who took oral MHT.

In the current study, a negative correlation between concentrations of PAI-1:Ag and ghrelin levels in women who used transdermal MHT was obtained. This indicates a favorable ratio between high concentrations of ghrelin and low levels of PAI-1:Ag. We can find no other study showing ghrelin's effect on lowering the concentration of PAI-1:Ag. If it was true, it would indicate another beneficial action of ghrelin as increased fibrinolytic activity would reduce the risk of heart disease or thrombosis. It is known that ghrelin is an excellent cardiometabolic marker for predicting atherosclerosis in the elderly. It has also a beneficial effect on the cardiovascular system by reducing inflammation and lowering BP.

5 Conclusions

This study showed that women who used transdermal MHT had higher levels of total ghrelin than women who took oral MHT. This indicates a beneficial effect from using the transdermal route of administration of MHT. However, transdermal MHT is also associated with adverse effects with regard to the observed higher levels of PAI-1:Ag, which in turn, leads to a reduction in fibrinolytic activity.

References

Bagnasco, M., Kalra, P.S., Kalra, S.P., 2002. Ghrelin and leptin pulse discharge in fed and fasted rats. *Endocrinology*, **143**(2):726-729. [doi:10.1210/en.143.2.726]

Cagnacci, A., Malmusi, S., Arangino, S., 2002. Influence of transdermal estradiol in the regulation of leptin levels of postmenopausal women: a double-blind, placebo-controlled study. *Menopause*, **9**(1):65-71. [doi:10.1097/00042192-200201000-00010]

Christian Schulze, P., Kratzsch, J., Linke, A., Schoene, N., Adams, V., Gielen, S., Erbs, S., Moebius-Winkler, S., Schuler, G., 2003. Elevated serum levels of leptin and soluble leptin receptor in patients with advanced chronic heart failure. *Eur. J. Heart Fail*., **5**(1):33-40. [doi:10.1016/S1388-9842(02)00177-0]

Cummings, D.E., Foster, K.E., 2003. Ghrelin-leptin tango in body-weight regulation. *Gastroenterology*, **124**(5):1532-1535. [doi:10.1016/S0016-5085(03)00350-0]

di Carlo, C., Tommaselli, G.A., Gargano, V., Sammartino, A., Bifulco, G., Tauchmanova, L., Colao, A., Nappi, C., 2007. Effects of estrogen-progestin therapy on serum levels of RANKL, osteoprotegerin, osteocalcin, leptin, and ghrelin in postmenopausal women. *Menopause*, **14**(1):38-44. [doi:10.1097/01.gme.0000227855.04732.7b]

Dobrovolsky, A.B., Titaeva, E.V., 2002. The fibrinolysis system: regulation of activity and physiologic functions of its main components. *Biochemistry (Moscow)*, **67**(1):99-108. [doi:10.1023/A:1013960416302]

Elbers, J.M., de Valk-de Roo, G.W., Popp-Snijders, C., Nicolaas-Merkus, A., Westerveen, E., Joenje, B.W., Netelenbos, J.C., 1999. Effects of administration of 17β-oestradiol on serum leptin levels in healthy postmenopausal women. *Clin. Endocrinol*., **51**(4):449-454. [doi:10.1046/j.1365-2265.1999.00813.x]

Grinspoon, S., Miller, K.K., Herzog, D.B., Grieco, K.A., Klibanski, A., 2004. Effects of estrogen and recombinant human insulin-like growth factor-I on ghrelin secretion in severe under nutrition. *J. Clin. Endocrinol. Metab*., **89**(8):3988-3993. [doi:10.1210/jc.2004-0287]

Hoda, M.R., Keely, S.J., Bertelsen, L.S., 2007. Leptin acts as a mitogenic and antiapoptotic factor for colonic cancer cells. *Br. J. Surg*., **94**(3):346-354. [doi:10.1002/bjs.5530]

Isomoto, H., Ueno, H., Nishi, Y., Yasutake, T., Tanaka, K., Kawano, N., Ohnita, K., Mizuta, Y., Inoue, K., Nakazato, M., *et al*., 2005. Circulating ghrelin levels in patients with various upper gastrointestinal diseases. *Dig. Dis. Sci*., **50**(5):833-838. [doi:10.1007/s10620-005-2648-z]

Kellokoski, E., Pöykkö, S.M., Karjalainen, A.H., 2005. Estrogen replacement therapy increases plasma ghrelin levels. *J. Clin. Endocrinol. Metabol*., **90**(5):2954-2963. [doi:10.1210/jc.2004-2016]

Konturek, P.C., Brzozowski, T., Pajdo, R., 2004. Ghrelin a new gastroprotective factor in gastric mucosa. *J. Physiol. Pharmacol*., **55**(2):325-336.

Konukoglu, D., Serin, O., Ercan, M., 2000. Plasma leptin levels in obese and non-obese postmenopausal women before and after hormone replacement therapy. *Maturitas*, **36**(3):203-207. [doi:10.1016/S0378-5122(00)00153-5]

Lambrinoudaki, I.V., Christodoulakos, G.E., Panoulis, C.P., Rizos, D.A., Dendrinos, S.G., Liakakos, T., Augoulea, A.D., Creatsas, G.C., 2004. Effect of hormone replacement therapy and tibolone on serum leptin levels in postmenopausal women. *Maturitas*, **48**(2):107-113. [doi:10.1016/j.maturitas.2003.07.001]

Lambrinoudaki, I.V., Christodoulakos, G.E., Economou, E.V., Vlachou, S.A., Panoulis, C.P., Alexandrou, A.P., Kouskouni, E.E., Creatsas, G.C., 2008. Circulating leptin and ghrelin are differentially influenced by estrogen/progestin therapy and raloxifene. *Maturitas*, **59**(1):62-71. [doi:10.1016/j.maturitas.2007.10.003]

Meier, U., Gressner, A.M., 2004. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin. Chem*., **50**(9):1511-1525. [doi:10.1373/clinchem.2004.032482]

Menon, D.V., Vongpatanasin, W., 2006. Effects of transdermal estrogen replacement therapy on cardiovascular risk

factors. *Treat. Endocrinol.*, **5**(1):37-51. [doi:10.2165/00024677-200605010-00005]

Michalski, B., Pakuła-Krzemińska, M., Kasprzak, J.G., 2008. The way to the heart is through the stomach—the role of ghrelin in pathogenesis of cardiovascular disease. *Kardiol. Pol.*, **66**(5):564-568 (in Polish).

Mueck, A.O., Seeger, H., 2005. Smoking, estradiol metabolism and hormone replacement therapy. *Curr. Med. Chem. Cardiovasc. Hematol. Agents*, **3**(1):45-54. [doi:10.2174/1568016052773270]

Nishi, Y., Isomoto, H., Ueno, H., Ohnita, K., Wen, C.Y., Takeshima, F., Mishima, R., Nakazato, M., Kohno, S., 2005. Plasma leptin and ghrelin concentrations in patients with Crohn's disease. *World J. Gastroenterol.*, **11**(46):7314-7317.

Pertyński, T., Stachowiak, G., 2006. Choosing the route of drug administration for hormone therapy of the menopausal period—when is it worth to tuck for patches? *Przegl. Menopauz.*, **5**:323-328 (inPolish).

Post, M.S., van der Mooren, M.J., van Baal, W.M., Blankenstein, M.A., Merkus, H.M., Kroeks, M.V., Franke, H.R., Kenemans, P., Stehouwer, C.D., 2003. Effects of low-dose oral and transdermal estrogen replacement therapy on hemostatic factors in healthy postmenopausal women: a randomized placebo-controlled study. *Am. J. Obstet. Gynecol.*, **189**(5):1221-1227. [doi:10.1067/S0002-9378(03)00599-4]

Ruszkowska, B., Manysiak, S., Małecka, B., Dymek, G., Rość, D., Odrowąż-Sypniewska, G., 2010a. Parameters of fibrinolysis in postmenopausal women taking oral or transdermal hormone replacement therapy. *Adv. Clin. Exp. Med.*, **19**(2):203-210.

Ruszkowska, B., Manysiak, S., Bielis, L., Małecka, B., Dymek, G., Senterkiewicz, L., Rość, D., Odrowąż-Sypniewska, G., 2010b. TAFI (thrombin activatable fibrinolysis inhibitor) and parameters of its activation in postmenopausal women taking oral and transdermal hormone replacement therapy. *Med. Biol. Sci.*, **24**(3):43-49.

Ruszkowska, B., Gadomska, G., Bielis, L., Gruszka, M., Góralczyk, B., Rość, D., Odrowąż-Sypniewska, G., 2011. Risk of venous thromboembolic disease in postmenopausal women taking oral or transdermal hormone replacement therapy. *J. Zhejiang Univ.-Sci. B (Biomed. & Biotechnol.)*, **12**(1):12-17. [doi:10.1631/jzus.B1000106]

Shiiya, T., Nakazato, M., Mizuta, M., Date, Y., Mondal, M.S., Tanaka, M., Nozoe, S.I., Hosoda, H., Kangawa, K., Matsukura, S., 2002. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J. Clin. Endocrinol. Metab.*, **87**(1):240-244. [doi:10.1210/jc.87.1.240]

Stachowiak, G., Stetkiewicz, T., Połać, I., Sobczuk, A., Jędrzejczyk, S., Pertyński, T., 2005. The role of plasminogen activator inhibitor 1 (PAI-1) after menopause—the influence hormonal therapy of his period. *Przegl. Menopauz.*, **6**:17-21 (in Polish).

Stachowiak, G., Zając, A., Pertyński, T., 2009. Metabolic syndrome in women in the menopausal period. *Przegl. Menopauz.*, **1**:6-10 (in Polish).

Szumiło, M., Rahden-Staroń, I., Dubielecka, E., 2007. Adipose tissue-endocrine function in physiology and pathology. *Farm. Pol.*, **23**:1051-1065 (in Polish).

Toshinai, K., Mondal, M.S., Nakazato, M., Date, Y., Murakami, N., Kojima, M., Kangawa, K., Matsukura, S., 2001. Upregulation of ghrelin expression in the stomach upon fasting, insulin-induced hypoglycemia, and leptin administration. *Biochem. Biophys. Res. Commun.*, **281**(5):1220-1225. [doi:10.1006/bbrc.2001.4518]

Tschöp, M., Weyer, C., Tataranni, P.A., Devanarayan, V., Ravussin, E., Heiman, M.L., 2001. Circulating ghrelin levels are decreased in human obesity. *Diabetes*, **50**(4):707-709. [doi:10.2337/diabetes.50.4.707]

Weigle, D.S., Cummings, D.E., Newby, P.D., Breen, P.A., Frayo, R.S., Matthys, C.C., Callahan, H.S., Purnell, J.Q., 2003. Roles of leptin and ghrelin in the loss of body weight caused by a low fat, high carbohydrate diet. *J. Clin. Endocrinol. Metab.*, **88**(4):1577-1586. [doi:10.1210/jc.2002-021262]