Recent studies have confirmed the association between vitamin D supply and insulin resistance (IR) [1], as well as type 2 diabetes mellitus occurrence (T2DM) [2]. The significant role of vitamin D in the development of IR and T2DM among overweight and obese individuals was determined [3].

Obtained data indicate that vitamin D levels influence the regulation of adipogenesis and energy metabolism [4]. Studies conducted in vitro demonstrate leptin secretion suppression in the culture of adipocytes upon the addition of 1,25(OH)D3 [5]. It is also emphasized that elevated levels of leptin correlate with vitamin D deficiency [6—8].

Leptin resistance is characterized by increasing circulating leptin levels and is considered one of the IR mediators and carbohydrate metabolism disorders in obese individuals [9]. Leptin affects insulin secretion through a direct impact on pancreatic beta cells [10]. However, the relationship between leptin and insulin sensitivity has been insufficiently studied.

The majority of studies directed to reveal the complex bond between vitamin D and leptin levels have been conducted in healthy individuals or T2DM obese patients. [7, 11]. There is a limited amount of studies that investigated a correlation between vitamin D and leptin in insulin-resistant individuals.

Objective: to evaluate the correlation between the level of 25(OH)D3 and leptin in patients with and without insulin resistance.

MATERIALS AND METHODS

Totally, 76 patients aged 31 to 45 were included in the study. Participants were randomized into two groups (38 patients in each group): Group I — individuals with IR (according to the HOMA IR index > 2.7); Group II — participants without IR (HOMA IR < 2.7).

Inclusion criteria: normal glucose tolerance, newly diagnosed T2DM, known T2DM.

Exclusion criteria: type 1 diabetes, T2DM for over 5 years, insulin therapy, chronic comorbidities, hypercalcemia or hypocalcemia, women in the postmenopausal period, additional vitamin D medications or supplements intake.

Blood samples were collected for biochemical testing after an overnight fast. Anthropometry, fasting and 2-hour postprandial plasma glucose (after breakfast for individuals with known T2DM), glycated hemoglobin (HbA1c), fasting insulin, lipids, leptin, and 25(OH)D were tested in all study participants.

The diagnosis of T2DM was established following the Unified Clinical Protocol of Primary and Secondary (Specialized) Medical Care «Type 2 Diabetes Mellitus» and Order of the Ministry of Health of Ukraine No. 1118 dated 12.21.2012 «On Approval and Implementation of Medical and Technological Documents on the Standardization of Medical Care for Type 2 Diabetes Mellitus» (valid at https://zakonline.com.ua/documents/show/8047____730659).

The index of insulin resistance (HOMA-IR) was used to assess IR, which is based on the ratio of fasting
insulin concentrations and glycemia in fasting blood serum. The HOMA-IR was calculated by the formula:

\[ \text{HOMA-IR} = \frac{\text{serum insulin (\(\mu\)IU/mL)} \cdot \text{plasma glucose (mmol/L)}}{22.5}. \]

The glucose rate in the blood plasma was tested by the glucose-oxidizing method, and HbA1c was determined with high-speed column liquid chromatography on a Bio-Rad D-10 analyzer, USA. The vitamin D (25(OH)D) blood levels were measured by chemiluminescence immunoassay using a set of reagents for the determination of total 25(OH)D «Access 25OH Vitamin D Total» by Beckman Coulter Access Immunoassay Systems. Leptin levels were tested using an ELISA kit (BioVendor, Czech Republic). Inc, USA).

The study was conducted following the basic bioethical principles of the Council of Europe Convention on Human Rights and Biomedicine (April 4, 1997), and the Helsinki Declaration of the World Health Association on ethical principles of medical research involving human subjects (1964—2013). The Biomedical Ethics Committee of the Ukrainian Scientific and Practical Center of Endocrine Surgery, Transplantation of Endocrine Organs and Tissues of the Ministry of Health of Ukraine approved the study.

**Statistical analysis.** Clinical and biochemical variables were compared between the two groups using the unpaired t-test. The correlation between the studied variables was established by the Pearson correlation test. Data were analyzed by SPSS software (version 21.0), and differences were considered significantly different if the p value was < 0.05.

**RESULTS**

The characteristic of the clinical and biochemical parameters in the studied groups are presented in Table 1. Study participants with IR had significantly lower levels of 25(OH)D and significantly higher levels of leptin compared to those without IR (Table 2). Female individuals had significantly lower 25(OH)D levels and significantly higher leptin levels compared to males (p < 0.05).

A statistically significant negative correlation was determined between 25(OH)D and leptin levels in blood serum. A significant negative correlation was also noted between the 25(OH)D and leptin rates in females, in opposite to males (r = −0.4; p = 0.04 vs. r = −0.042; p = 0.8, respectively). The HOMA-IR was statistically significantly negatively correlated with 25(OH) D levels only in individuals with IR and a positively — with serum leptin level (r = +0.27; p = 0.015) (Table 3).
DISCUSSION

Our study revealed higher levels of leptin and lower levels of 25(OH)D in individuals with IR compared to those without IR. A reliable negative relationship between 25(OH)D and leptin levels in blood serum was assessed. Although the HOMA-IR was positively correlated with leptin levels in all study participants and was significantly negatively correlated with 25(OH)D only in individuals with insulin resistance.

Females and males with different body mass and glucose tolerance participated in this study. Body mass index ranged from non-obese to overweight and obese, and glucose tolerance categories included normal glucose tolerance, newly diagnosed diabetes, and known T2DM.

Published results of recent studies that attempted to analyze the relationship between vitamin D and leptin should not be generalized, because were conducted with the inclusion of both healthy individuals and those with T2DM with and without obesity [12, 13].

A significant negative relationship between serum 25(OH)D and leptin levels in our study confirms the results obtained by other researchers, who also reported corresponding results [6, 12].

The inverse correlation between vitamin D and leptin levels may be explained by a direct inhibitory effect of vitamin D on leptin secretion from adipose tissue. Consequently, vitamin D deficiency promotes leptin resistance [14, 15] with the development of hyperleptinemia and IR. In addition, obesity-induced hyperleptinemia may lead to decreased 25(OH)D levels [16].

The effect of the additional vitamin D intake on the leptin level has not been confirmed, considering the controversial results of the conducted studies [17, 18]. Some authors suggest that vitamin D-mediated inhibition of leptin secretion by adipocytes may play an important role only in early-staged obesity, on the other hand in advanced and morbid obesity other mechanisms are involved in this process [19]. Most of the participants in our study were overweight or obese. Therefore, at this stage, vitamin D deficiency and concomitant leptin oversecretion probably will cause hyperleptinemia. Apparently, in the case of obesity upstaging, the effect of obesity-related hyperleptinemia may become more important, leading to a decrease in vitamin D levels [19].

At the same time, the exact mechanism of vitamin D’s interaction with leptin secretion and its role in the regulation of leptin levels in vivo is insufficiently studied and needs to be clarified. Whether low vitamin D levels lead to hyperleptinemia or hyperleptinemia leads to low vitamin D levels cannot be answered with certainty in this study. Therefore, additional research is needed to clarify this complex relationship.

We also found higher levels of leptin in individuals with IR and a reliable relationship between leptin and the HOMA IR index. A similar connection was also reported earlier [20], which can be explained by resistance to leptin in the hypothalamus with subsequent development of IR in various peripheral tissues, in particular in the liver and skeletal muscles [21].

Data from our study suggest decreased 25(OH)D levels in individuals with IR and a significant inverse relationship of serum 25(OH)D levels with HOMA-IR also in individuals with IR. Thus, the effect of 25(OH)D levels on insulin sensitivity is significant only at higher rates of insulin resistance. It may also mean that IR is secondary to hypovitaminosis D. Therefore, further studies are needed to resolve this issue.

Our study has several limitations. This was a cross-sectional study with one measurement of serum vitamin D and leptin levels, so causality cannot be established. The level of parathyroid hormone was not measured, which could provide additional information about the value of vitamin D deficiency. The inclusion of individuals with obesity of II—III degrees could further clarify the relationship between leptin and vitamin D across the spectrum of weight categories.

CONCLUSIONS

This study revealed a reliable negative relationship between serum 25(OH)D and leptin levels. Leptin levels were higher and 25(OH)D levels were lower in individuals with insulin resistance.

Significantly lower 25(OH)D levels were found in individuals with IR and a significant inverse relationship of serum 25(OH)D levels with HOMA IR only in individuals with IR. These data suggest that the effect of 25(OH)D levels on insulin sensitivity is significant only at higher rates of insulin resistance.

Conflicts of interest: none.

Previous studies have demonstrated a connection between vitamin D levels and insulin resistance (IR) as well as type 2 diabetes mellitus (T2DM). The importance of vitamin D in the development of IR and T2DM in patients with overweight and obesity is confirmed. Previous studies have established a high frequency of deficiency and insufficiency of vitamin D in patients with T2DM. At the same time, there is no information on the connection between hypovitaminosis D and hyperleptinemia. Furthermore, whether this association differs between individuals with and without insulin resistance has not been established.

**Objective** — to evaluate the correlation between the level of 25(OH)D3 and leptin in patients with and without insulin resistance.

**Materials and methods.** Totally, 76 patients aged 31 to 45 were included in the study. Participants were randomized into two groups (38 patients in each group):
Group I — individuals with IR (HOMA IR index > 2.7); Group II — participants without IR (HOMA IR < 2.7). The 25(OH)D, leptin levels, anthropometric, and other biochemical parameters were compared between the two groups, and the correlation between 25(OH)D, leptin, and the HOMA-IR index was assessed.

Results. Study participants with IR had significantly lower levels of 25(OH)D and significantly higher levels of leptin compared to subjects without IR. Women were found to have significantly lower 25(OH) D levels and significantly higher leptin levels compared to men. A significant negative correlation was observed between the content of 25(OH)D in blood serum and leptin levels. A significant negative correlation was also noted between the indicators of 25(OH)D and leptin in examined women, in contrast to men (r = –0.4; p = 0.04 and r = –0.042; p = 0.8, respectively). The value of the HOMA IR index indicated a significant negative correlation with 25(OH)D levels only in individuals with IR. A significant positive correlation of the HOMA-IR index was also found with serum leptin level (r = 0.27; p = 0.015).

Conclusions. The study found higher levels of circulating leptin and lower levels of 25(OH)D in individuals with insulin resistance. Levels of 25(OH)D were inversely related to leptin levels, especially in women.

Keywords: vitamin D, leptin, diabetes, obesity, insulin resistance.

РЕЗЮМЕ
Взаємозв’язок між рівнями вітаміну D та лептину в осіб з інсулінорезистентністю

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За даними досліджень установлено, що рівень вітаміну D пов’язаний з інсулінорезистентністю (IP) та наявністю цукрового діабету (ЩД) 2 типу. Підтверджено значення вітаміну D у розвитку IP та ЩД 2 типу в осіб із надмірною масою тіла та ожирінням. У попередніх дослідженнях виявлено високу частоту дефіциту та недостатності вітаміну D (25(OH)D) у хворих на ЩД 2 типу. Відсутня інформація про зв’язок між гіповітамінозом D і гіперлептинемією. Не встановлено, чи відрізняється ця асоціація в осіб з IP та без неї.

Мета роботи — дослідити взаємозв’язок між (25(OH)D) і лептином в осіб з IP та без неї.

Матеріалі та методи. Під спостереженням перебували 76 осіб, яких розподілили на дві групи дослідження (по 38 пацієнтів у кожній групі). До першої групи запишучи осіб з IP (індекс HOMA IR > 2,7), до другої — пацієнтів без IP (індекс HOMA IR < 2,7). Проведено порівняння вмісту 25(OH)D та лептину, антропометричних і біохімічних параметрів у групах та вивчене кореляційний зв’язок між 25(OH)D, лептином та індексом HOMA-IR.

Результати. В осіб з IP зафіксовано статистично значущо нижчий рівень 25(OH)D і статистично значущо більший вміст лептину порівняно з пацієнтами без IP. У жінок концентрація 25(OH)D була статистично значущо нижчою, вміст лептину — статистично значущо більшим порівняно з показниками у чоловіків. Виявлено статистично значущий обернено пропорційний зв’язок між рівнями 25(OH)D у сироватці крові та лептину. У жінок на відміну від чоловіків цей зв’язок був статистично значущим (відповідно r = –0,4; p = 0,04 і r = –0,042; p = 0,8). Установлено сильний обернено пропорційний зв’язок між індексами HOMA IR та рівнем 25(OH)D у сироватці крові (r = +0,27; p = 0,015).

Висновки. Виявлено вищий вміст циркулюючого лептину та нижчий рівень 25(OH)D у осіб з IP. Концентрація 25(OH)D обернено пропорційно пов’язана з рівнем лептину, особливо у жінок.

Ключові слова: вітамін D, лептин, цукровий діабет, ожиріння, інсулінорезистентність.