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VITAMIN D AUTO-/PARACRINE SYSTEM ACTIVITY IN RAT LIVER DEPENDING ON VITAMIN D STATUS

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The liver plays a central role in vitamin D metabolism, facilitating its primary activation and systemic transport. However, the molecular mechanisms of vitamin D hydroxylation and reception involving the auto-/paracrine vitamin D system in the liver remain insufficiently understood. The aim of the study was to evaluate cytochrome P450 (CYP) enzymes and vitamin D receptor (VDR) expression in the liver of rats with nutritional vitamin D_3 deficiency and after therapeutic treatment. Vitamin D deficiency was modeled by maintaining female Wistar rats on vitamin D-free diet for 60 days. The treatment was performed by cholecalciferol (1000 IU/kg bw) oral administration for 30 days. Serum 25(OH)D level was determined by ELISA, 25-hydroxylase activity in hepatocytes was evaluated using [^{3}H]cholecalciferol as a substrate. The level of CYP2R1, CYP27A1, CYP27B1, CYP24A1 and VDR proteins in the liver was studied via Western blot analysis. A 60-day vitamin D-deficiency led to a critical decrease in 25(OH)D level in the serum, significant increase in 25-hydroxylase activity, CYP2R1 and VDR expression, against the background of reduced CYP24A1 level, in the liver. Vitamin D_3 administration ensured the restoration of serum 25(OH)D, as well as the level of the molecular markers of vitamin D transformation and reception in the liver, except for CYP27B1, which maintained moderately elevated expression. Thus, the metabolic adaptation under vitamin deficiency was manifested through compensatory enhancement in vitamin D synthesis and increased receptor sensitivity against the background of suppressed catabolic pathways. Nutritional correction effectively restored the balance in the auto-/paracrine vitamin D system, ensuring stable homeostasis.

K e y w o r d s: vitamin D_3 (cholecalciferol), D-hypovitaminosis, liver, 25-hydroxylase, CYP2R1, CYP27A1, CYP27B1, CYP24A1, VDR.

Vitamin D refers to a group of fat-soluble compounds, primarily cholecalciferol (D_3) and ergocalciferol (D_2) [1]. In the current clinical paradigm, its role has been fundamentally redefined: no longer viewed strictly as a nutrient for bone health, it is now recognized as the precursor to calcitriol (1α,25$(OH)_2D_3$), a multipotent hormone with pleiotropic effects across nearly every physiological system. As the central effector of the D-endocrine system, this hormonally active form regulates genetic stability, metabolic health, and systemic resistance to pathogens [2]. This shift in understanding has moved the medical focus from simply preventing deficiency to optimizing the endocrine activity of the ligand for the management of chronic diseases [1-3].

The biological potency of the active metabolite is realized through the vitamin D receptor (VDR), a ligand-activated transcription factor. Through the genomic pathway, the VDR-RXR complex modulates the expression of 500 to 1,000 genes – representing approximately 3–5% of the human genome [1, 4]. This allows the hormone to orchestrate cellular proliferation, differentiation, and apoptosis, establishing it as a pivotal component in oncoprotection and regenerative medicine [5]. Concurrently,

non-genomic effects mediated by membrane-associated receptors (e.g., Membrane-Associated Rapid Response, Steroid-binding – MARRS) trigger rapid protein kinase cascades (PKC, MAPK, and PKA) and modulate ion channel functionality, ensuring instantaneous cellular adaptation to environmental stressors and acute inflammatory triggers [6].

Particular scientific attention is currently directed toward the epigenetic role of vitamin D, as it regulates gene expression without altering the DNA sequence [7]. Calcitriol possesses a profound capacity to influence DNA methylation, histone modifications, and the profiling of non-coding RNA, thereby shaping the epigenetic landscape of genes governing vital physiological processes and adaptive metabolic pathways. Furthermore, vitamin D acts as a potent immunomodulator, utilizing the mechanisms of nutriepigenetics to mitigate autoimmune risks and the "cytokine storm" phenomenon [8]. By switching the pro-inflammatory Th1 and Th17 response to an anti-inflammatory Th2 and Treg profile, it effectively modulates the course of complex inflammatory and autoimmune disorders [9].

The classical metabolic activation of vitamin D involves two sequential enzymatic hydroxylations. The primary stage occurs in the liver, forming the major transport form, 25-hydroxyvitamin D [25(OH)D], while the secondary stage takes place predominantly in the kidneys via the enzyme 25-hydroxyvitamin D-1 alpha hydroxylase (CYP27B1) to synthesize the active hormone, calcitriol [1]. However, a conceptual breakthrough has highlighted the critical importance of extrarenal calcitriol synthesis [1-3]. The enzyme CYP27B1 is expressed in a wide range of tissues, including immune cells, the intestinal epithelium, vascular endothelium, and nerve cells (neurons and glial cells). Within the nervous system, vitamin D acts as a neurosteroid, modulating neurotrophin synthesis and protecting the blood-brain barrier [10]. Unlike strictly regulated renal synthesis, extrarenal hormone production is governed by local stimuli and performs autocrine and paracrine functions to ensure localized immune responses directly within the foci of pathological processes [11].

Such systemic and local efficacy is vital given the global pandemic of vitamin D deficiency, which affects over one billion people [12]. For Ukraine, situated above the 40th parallel north, more than 90% of the population faces a high risk due to insufficient UVB radiation during winter months [13]. The resulting chronic deficiency acts as a determinant for "inflammaging" – a state of chronic, low-grade inflammation that accelerates the progression of systemic diseases [2, 3, 14]. Within this hierarchy, the liver serves as the central regulatory node. It not only facilitates primary activation via the CYP2R1 enzyme but also synthesizes the vitamin D-binding protein (VDBP), which dictates the bioavailability and transport dynamics of all metabolites [10]. Disruptions in hepatic function can severely compromise the body's ability to maintain a functional reservoir of 25(OH)D, leading to systemic "D-hormone" failure.

The objective of this study was to conduct a comprehensive analysis of the specificities of vitamin D metabolism within hepatic tissue under conditions of varying supply levels. The research is directed toward elucidating the molecular mechanisms that determine the efficiency of hepatic hydroxylation and evaluating the role of the hepatic component in forming the reserve precursor pool necessary for the realization of systemic endocrine and local paracrine functions in both physiological and D-deficient states.

Materials and Methods

Animals and experimental design. The study was performed using 35 young female Wistar rats with an initial mean body weight of 132.33 ± 6.99 g. The animals were housed under standardized vivarium conditions, maintained at a temperature of 18-22°C and a relative humidity of 50-60%, with a natural "light-dark" cycle and provided with *ad libitum* access to both water and standard feed. The experimental subjects were allocated into three distinct groups: the control group (n = 11), consisting of rats maintained on a nutritionally complete vivarium diet; the D-deficiency group (n = 12), comprising animals subjected to a vitamin D-deficient diet for a duration of 60 days; and the correction group (n = 12), which included rats that, following the 60-day induction of deficiency, were daily administered an oral oil-based solution of vitamin D_3 (cholecalciferol dissolved in sunflower oil) at a dosage of 1000 IU/kg of body weight for the subsequent 30 days (administered per os in a precise volume of 0.1 ml).

The administered dosage of vitamin D_3 (1000 IU/kg body weight) was selected to ensure rapid normalization of serum 25(OH)D levels within the 30-day experimental timeframe, consistent with established therapeutic models [15]. According to the

FDA's dose conversion guidelines, this dosage corresponds to a supra-nutritional but sub-toxic therapeutic range for rodents. No adverse physiological effects, such as abnormal weight loss, behavioral changes, or macroscopic signs of heart and kidney calcification, were observed during the study, aligning with previous reports on the safety profile of cholecalciferol in this dosage range [16, 17].

Compliance with bioethical standards. All experimental procedures were conducted in strict adherence to international and national ethical guidelines for the humane treatment of laboratory animals. The study protocol complied with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and Directive 2010/63/EU of the European Parliament and of the Council. Furthermore, all procedures were performed in accordance with Ukrainian legislation, specifically the Law of Ukraine "On the Protection of Animals from Cruelty" (No. 3447-IV), the Order of the Ministry of Education and Science of Ukraine No. 249 (March 1, 2012), and the foundational "General Ethical Principles of Animal Experimentation" (Kyiv, 2001). To ensure the minimization of distress, all surgical and experimental manipulations were performed under ether anesthesia.

Induction of D-deficiency state. Alimentary vitamin D deficiency was induced by maintaining the rats for a period of 60 days on a specialized semi-synthetic vitamin D-deficient (VDD) diet. The specific nutritional composition of the ration was as follows: wheat (33%), barley (20%), corn (20%), dried brewer's yeast (10%), purified casein (10%), a mixture of vegetable oils (5%), and a balanced mineral premix (2%). To ensure that all other physiological requirements were met and to specifically exclude the development of multiple avitaminoses, a comprehensive vitamin complex – comprising vitamins A, E, K, C, and the B-group, but strictly devoid of cholecalciferol – was incorporated into the dietary regimen.

Collection of biological material. Following the completion of the experimental procedures, the animals were euthanized via decapitation conducted under deep ether anesthesia. Mixed blood samples were collected through direct cardiac puncture performed immediately prior to the sacrifice of the animals. Serum was subsequently separated by centrifugation at 3,000 rpm for 15 min at a temperature of +4°C. Liver tissue was promptly excised, thoroughly rinsed in an ice-cold 0.9% NaCl solution, and immediately flash-frozen in liquid nitrogen for preservation prior to subsequent biochemical and molecular analyses.

Assessment of vitamin D status and enzymatic activity. Systemic levels of 25(OH)D in the serum were quantified using an enzyme-linked immunosorbent assay (ELISA) specifically developed at the Palladin Institute of Biochemistry of the NAS of Ukraine; this method employed specific polyclonal antibodies and a biotin-streptavidin visualization system [18].

To obtain primary hepatocytes, the liver was perfused with an oxygenated buffered salt solution via the cannulated portal vein. After excision, the left lobe underwent enzymatic dissociation with 0.05% collagenase (Sigma, USA) for 1 h at 37°C. The isolated cells were then purified by centrifugation, washed, and resuspended for further analysis.

Hepatic vitamin D_3 25-hydroxylase activity was determined in vitro by measuring tritiated 25(OH)D_3 production following the incubation of hepatocytes with [^{3}H]cholecalciferol as a substrate. Unlabeled vitamin D_3 (100 nmol in 20 µl ethanol) was pre-equilibrated with albumin for 30 min to ensure protein binding. After the addition of hepatocytes, the samples were incubated with agitation (120 rpm) at 37°C for 2 h. The reaction was terminated by the addition of chloroform-methanol (2:1, v/v). Following extraction and chromatographic separation, the activity was calculated as pmol 25(OH)D_3 h^{-1} (10^6 cells)$^{-1}$ [19].

Western blot analysis. Liver tissue samples (100 mg) were homogenized in a RIPA lysis buffer (comprising 20 mM Tris-HCl, pH 7.5; 1% Triton X-100, 150 mM NaCl, 1 mM EDTA, and 1% sodium deoxycholate) supplemented with a cocktail of protease and phosphatase inhibitors (Sigma, USA). Total protein concentration was determined using the Lowry method. Electrophoretic separation (50 µg of protein per lane) was performed in 10% SDS-PAGE according to the Laemmli system, followed by the electrotransfer of proteins onto a nitrocellulose membrane (conducted at 350 mA for 1 hour). Membranes were then incubated with the following primary antibodies: VDR (1:500, Cat# NBP1-51322) from Novus Biologicals; CYP2R1 (1:500, Cat# PA5-22166), CYP27B1 (1:500, Cat# PA5-79133), CYP24A1 (1:500, Cat# PA5-79130), and CYP27A1 (1:500, Cat# PA5-103310) from Thermo Fisher Scientific. Monoclonal antibodies against β-actin (1:2000, Sigma, Cat# A2228) were utilized as a loading control. Following incubation with appropriate seconda-

ry antibodies (Santa Cruz, Sigma), immunoreactive signals were detected using the enhanced chemiluminescence (ECL) method and quantified using GelPro Analyzer 3.2 software.

Statistical analysis. The data were expressed as the mean ± standard deviation (SD). Experimental groups were compared using Student's *t*-test (for two groups) or one-way ANOVA (for more than two groups) followed by Tukey post hoc test and $P \leq 0.05$ was considered significant.

Results and Discussion

The conducted study was aimed at a comprehensive investigation of the mechanisms of liver adaptation to conditions of prolonged hypovitaminosis and the evaluation of the effectiveness of subsequent vitamin replacement therapy (nutritional correction). The utilized model of alimentary vitamin D_3 deficiency allowed us to trace the dynamics of changes at the systemic, enzymatic, and molecular levels, enabling the formation of a holistic picture of the metabolic response of the organism.

The initial stage of the work was the assessment of the D-vitamin status of rats, which confirmed the development of a profound D-deficiency. It was established that keeping animals on a diet devoid of cholecalciferol for 60 days led to a critical and statistically significant reduction in the content of the main marker of the body's vitamin D status – 25(OH)D in the blood serum (Fig. 1, *A*). In the VDD group, the concentration of this metabolite was only 19.45 ± 2.52 nmol/l, which corresponds to an 80% drop compared to the control group (97.27 ± 10.05 nmol/l). Based on recent clinical guidelines, this condition is classified as severe vitamin D deficiency, a state that triggers the metabolic decompensation of vitamin D-dependent pathways, including those regulated by the CYP27B1. To investigate the potential for recovery, a therapeutic stage was initiated using a 30-day oral administration of cholecalciferol (oily vitamin D_3) at a dose of 1000 IU/kg of body weight. This intervention period effectively restored homeostasis; serum 25(OH)D concentrations in the experimental group increased nearly fourfold relative to untreated controls, reaching 78.24 ± 12.57 nmol/l ($P < 0.05$). This result fully aligns with the established physiological sufficiency range, demonstrating the successful restoration of the systemic 25(OH)D pool and ensuring adequate substrate availability for active metabolite synthesis [20].

Since the concentration of the circulating metabolite is a direct result of its synthesis in the liver tissue, the next step was to study the functional capacity of the first stage activation enzymes. In the liver, this process is provided by two main isoforms of cytochrome P450: CYP2R1 and CYP27A1. CYP2R1 is considered the major microsomal 25-hydroxylase with high affinity for vitamin D, responsible for maintaining the basal level of the metabolite in the blood. In contrast, CYP27A1 is a mitochondrial enzyme with broad substrate specificity (also involved in bile acid synthesis) that engages in vitamin metabolism mainly under conditions of its high concentrations [21].

The study of hepatic vitamin D 25-hydroxylase activity (total activity of CYP2R1 and CYP27A1 isoforms) revealed a pronounced compensatory reaction in response to hypovitaminosis (Fig. 1, *B*). In the VDD group, the total hydroxylation rate almost doubled compared to the control, reaching 28.93 ± 1.50 pmol of 25(OH)D_3 formed per 10^6 cells/h (vs 15.04 ± 1.09 pmol/h in the control). This indicates the mobilization of enzymatic reserves of the liver for the maximally rapid conversion of minimal residual substrate, likely realized through intensified activity of the high-affinity CYP2R1 isoform. However, after a 4-week course of vitamin D_3 correction, 25-hydroxylase activity significantly decreased to 13.22 ± 0.95 pmol/h. Such dynamics indicate the existence of a rigid negative feedback mechanism that inhibits enzyme activity upon reaching a physiologically sufficient concentration of the product in the body, preventing an uncontrolled increase in the level of active metabolites.

To clarify the molecular basis of the recorded changes in enzymatic activity, a detailed analysis of the expression of key liver proteins was performed using the Western blotting method. The study of the master 25-hydroxylase isoform – CYP2R1 – showed that its level in the deficiency group was significantly increased 1.66-fold relative to the control ($P < 0.05$), Fig. 2, *B*. This directly confirms that the aforementioned increase in enzyme activity is ensured precisely by the enhancement of *de novo* CYP2R1 protein synthesis. After normalization of vitamin status, the level of this protein returned to values comparable to the control, which indicates the restoration of regulatory balance. In contrast, the analysis of the alternative isoform – CYP27A1 – revealed a different adaptation strategy: its expression in D-deficiency was moderately reduced (by 29% relative to control)

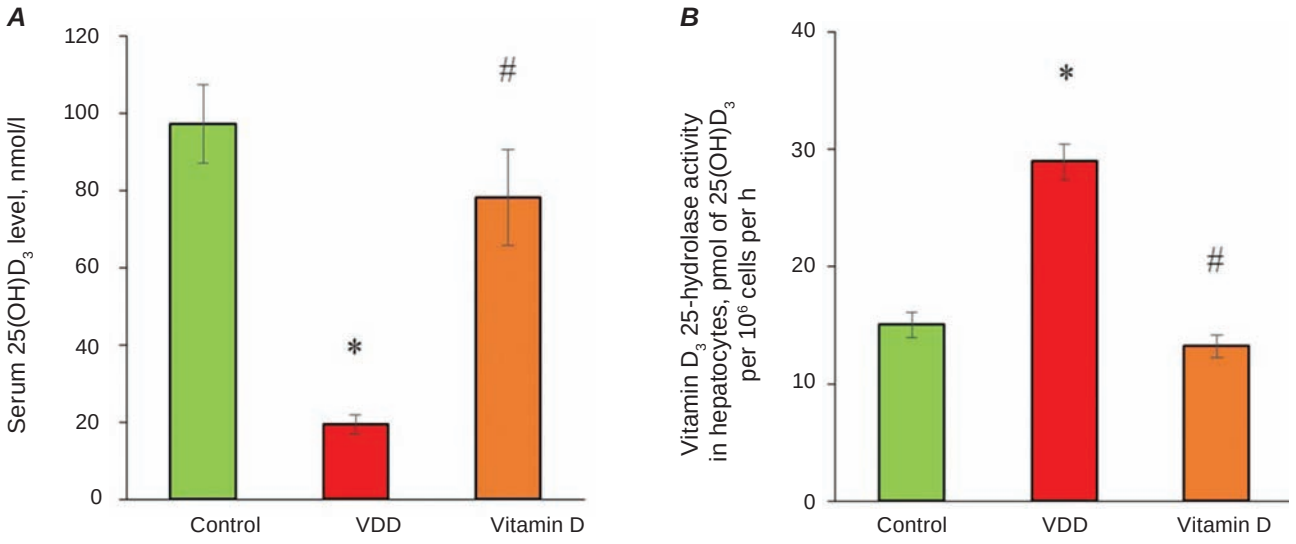

*Fig. 1. Serum 25-hydroxyvitamin D_3 level (**A**) and vitamin D_3 25-hydroxylase activity in isolated hepatocytes (**B**) of vitamin D-deficient (VDD) rats and after supplementation of vitamin D_3 (1,000 IU/kg of b.w, 30 days). All data are presented as mean ± SD, n = 6; *P < 0.05 vs. the control group, $^{\#}P < 0.05$ vs. the vitamin D deficiency group*

and only recovered after correction (Fig. 2, *A*). This suggests that CYP2R1 is the most plastic and significant link in the processes of urgent adaptation of the liver to hypovitaminosis, while CYP27A1 demonstrates less sensitivity to substrate starvation.

However, the adaptation of the hepatic metabolic system was not limited only to the first stage of activation; significant changes were also found in the synthesis link of the hormonally active form of vitamin D. The traditional paradigm considers the kidneys to be the main source of calcitriol. However, our results support the current concept of extrarenal vitamin D metabolism [22]. The synthesis of CYP27B1 in the liver tissue of deficient rats increased almost threefold (2.94-fold) compared to control ($P < 0.05$), Fig. 3, *A*. The recorded increase indicates the activation of a powerful mechanism of extrarenal calcitriol synthesis directly in hepatocytes. This is a critically important systemic response aimed at supporting intracellular functions under conditions of systemic scarcity of the active hormone and a reduction in its supply from the kidneys. Consistent with this sustained metabolic shift, post-correction VDR levels significantly declined but remained 1.8-fold higher than in control rats, indicating the preservation of a long-term adaptive state (Fig. 3, *B*).

Simultaneously with the induction of activation pathways, cellular homeostatic mechanisms recalibrated degradation processes to minimize turnover and stabilize the available vitamin pool. The key role in this process belongs to the enzyme 1,25-dihydroxy-vitamin D 24-hydroxylase (CYP24A1) – the major catabolic enzyme of vitamin D metabolism. Its primary function is the multi-stage hydroxylation of both 25(OH)D and active $1\alpha,25(OH)_2D$, converting them into water-soluble, biologically inert calcitroic acid for subsequent excretion. In a healthy organism, CYP24A1 acts as a critical regulator, preventing the excessive accumulation of calcitriol and the development of hypercalcemia [23].

Analysis of the CYP24A1 revealed substantial modulation in its expression, which appears to be dictated by the prevailing vitamin D status and the intricate feedback mechanisms involving PTH, FGF23, and $1\alpha,25(OH)_2D$. During the deficiency phase (VDD group), expression was minimal (1.64-fold decrease relative to normal physiological levels), Fig. 3, *B*. This marked suppression is an adaptive physiological mechanism to block vitamin D catabolism and maximize the preservation of the limited available active metabolites under conditions of substrate starvation. The deficiency state is typically associated with high PTH levels, which act to suppress *CYP24A1* gene expression. Following the therapeutic correction course, the CYP24A1 protein level increased significantly compared to the deficiency group (reaching 1.05, $P < 0.05$). This robust upregulation is a direct consequence of the increased sys-

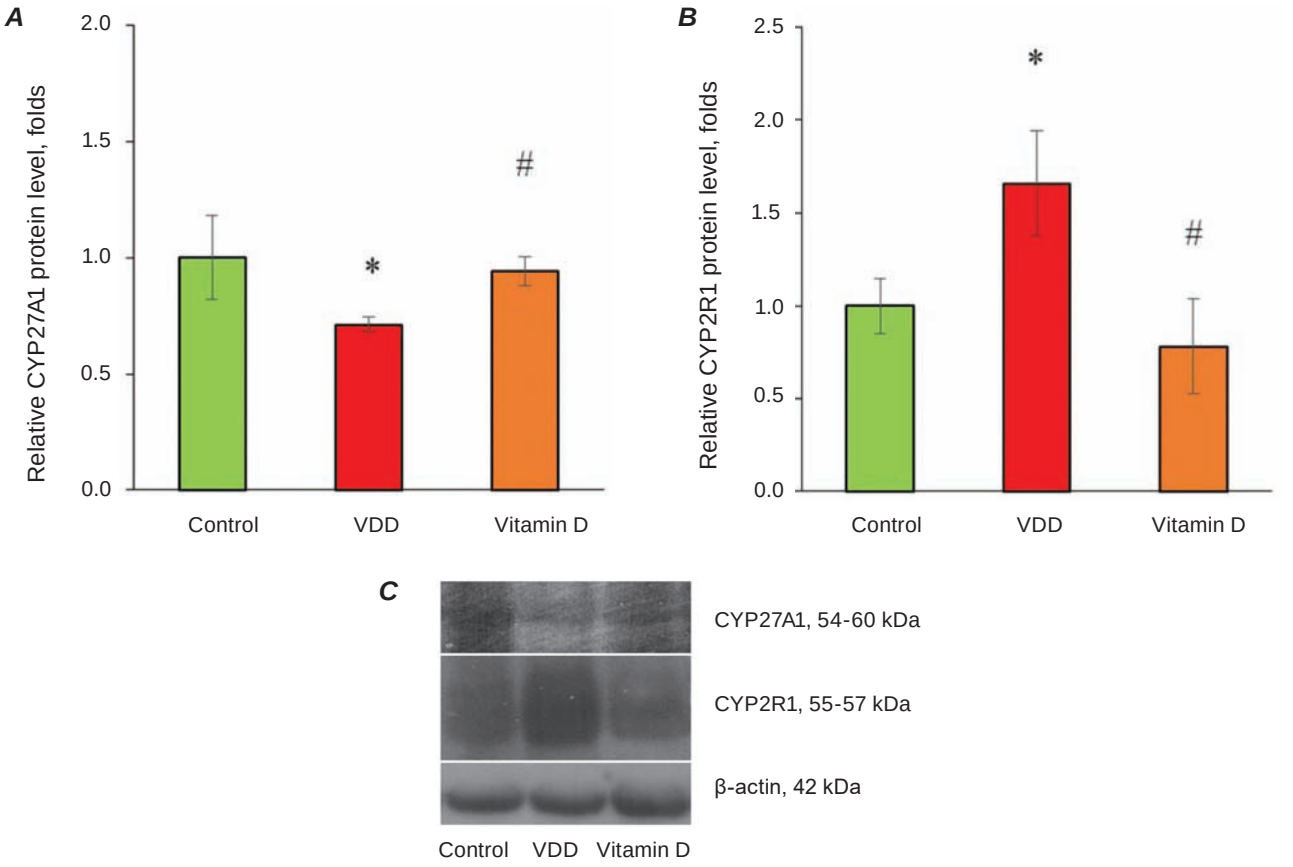

*Fig. 2. Relative protein levels of liver cytochromes CYP27A1 (**A**) and CYP2R1 (**B**) in vitamin D-deficient (VDD) rats and after supplementation of vitamin D_3 (1,000 IU/kg of b.w, 30 days). The representative immunoblots are shown (**C**). All data are presented as mean ± SD, n = 8; *P < 0.05 vs. the control group, #P < 0.05 vs. the vitamin D deficiency group*

temic 1α,25$(OH)_2$D and is largely mediated through calcitriol binding to the VDR, which then activates VDREs in the *CYP24A1* gene promoter region.

The observed induction of the CYP24A1 enzyme signifies the restoration of a functional metabolic degradation system and the activation of key protective inactivation pathways. These mechanisms are critical for mitigating the risk of toxic effects, such as hypercalcemia, following significant exogenous cholecalciferol intake. Ultimately, maintaining a dynamic, reciprocal balance between the synthesizing enzyme (CYP27B1) and the degrading enzyme (CYP24A1) acts as a key homeostatic regulator in tissues, ensuring equilibrium between functional need and metabolic safety.

The effectiveness of the entire multi-component enzymatic system of the liver and the full realization of vitamin D's biological effects directly depend on the functional state of the vitamin D receptor. As a highly specific ligand-activated nuclear transcription factor, VDR performs the role of a fundamental metabolic "sensor" [3, 8]. In hepatocytes, it coordinates the expression of a wide pool of genes responsible not only for mineral metabolism but also for lipid homeostasis, xenobiotics detoxification process, and the regenerative potential of the parenchyma [24]. In addition, VDR is a central link in the self-regulation system: its activation by calcitriol initiates the transcription of catabolic enzymes, thereby closing the negative feedback loop to prevent hypercalcemia.

Our results revealed a pronounced dynamic in the content of the VDR protein. Under conditions of deficiency, the level of VDR protein in the liver tissue significantly increased by 2.19-fold (a 119% increase) relative to control values ($P < 0.05$), Fig. 4. Given the VDR's role as the primary mediator of vitamin D-dependent pathways, this hyperexpression indicates the activation of a compensatory positive regulatory mechanism. In a situation of criti-

cal substrate depletion, the hepatocyte activates *de novo* synthesis of receptor molecules, effectively increasing the density of nuclear receptor sites. This mechanism is physiologically aimed at enhancing the system's affinity for the limited ligand: by increasing the number of available binding "traps", the cell attempts to utilize minimally available fractions of the active metabolite to sustain the transcription of vital genes.

Such an adaptive restructuring of the receptor apparatus allows the liver to maintain effective metabolic control even when the systemic level of 25(OH)D falls to threshold values. This phenomenon illustrates the exceptional plasticity of the liver tissue, capable of dynamically correcting its sensitivity to humoral signals depending on the body's nutritional status. The molecular "tension" of the reception system serves as a temporary buffer that prevents systemic decompensation of vitamin D-dependent processes during prolonged hypovitaminosis.

Subsequent corrective administration of cholecalciferol over four weeks resulted in a significant reduction in VDR expression, effectively returning the levels to their physiological baseline. This normalization likely reflects the stabilization of biochemical processes in hepatocytes and suggests the restoration of ligand-receptor signaling. The return of receptor expression to physiological levels confirms the adequacy of the administered therapy. By saturating the system with the ligand, the cell presumably bypasses reactive "emergency" pathways, thereby restoring stable metabolic equilibrium and transitioning back to normal physiological function.

In total, these results comprehensively prove that the liver acts as a central regulatory node. Through the finely tuned coordination of 25(OH)D levels, metabolic enzymes (CYP2R1,

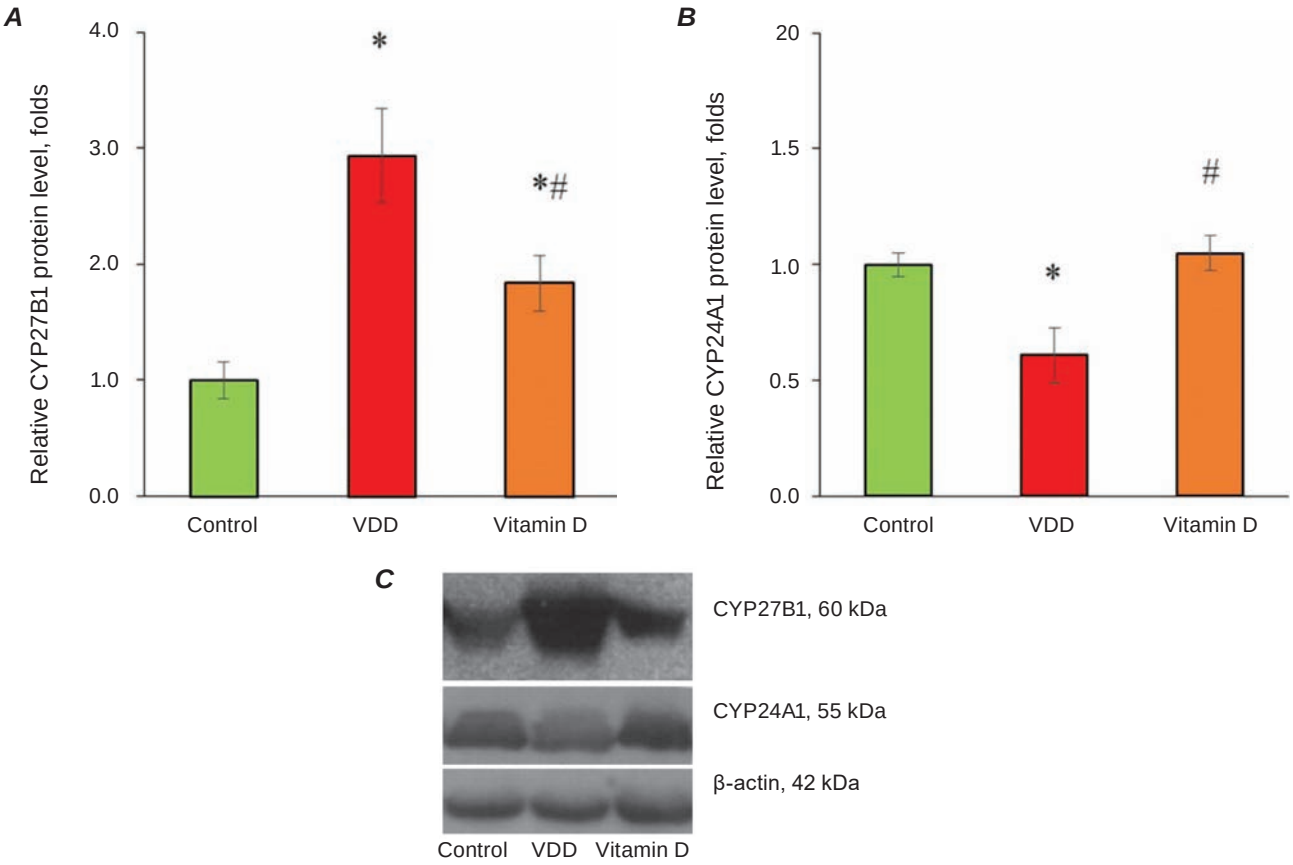

*Fig. 3. Relative protein levels of the key vitamin D metabolism enzymes: CYP27B1 (25-hydroxyvitamin D 1α-hydroxylase, **A**) and CYP24A1 (25-hydroxyvitamin D-24-hydroxylase, **B**) in the liver of vitamin D-deficient (VDD) rats and after supplementation of vitamin D_3 (1,000 IU/kg of b.w, 30 days). The representative immunoblots are shown (**C**). All data are presented as mean ± SD, n = 8; *P < 0.05 vs. the control group, #P < 0.05 vs. the vitamin D_3 deficiency group*

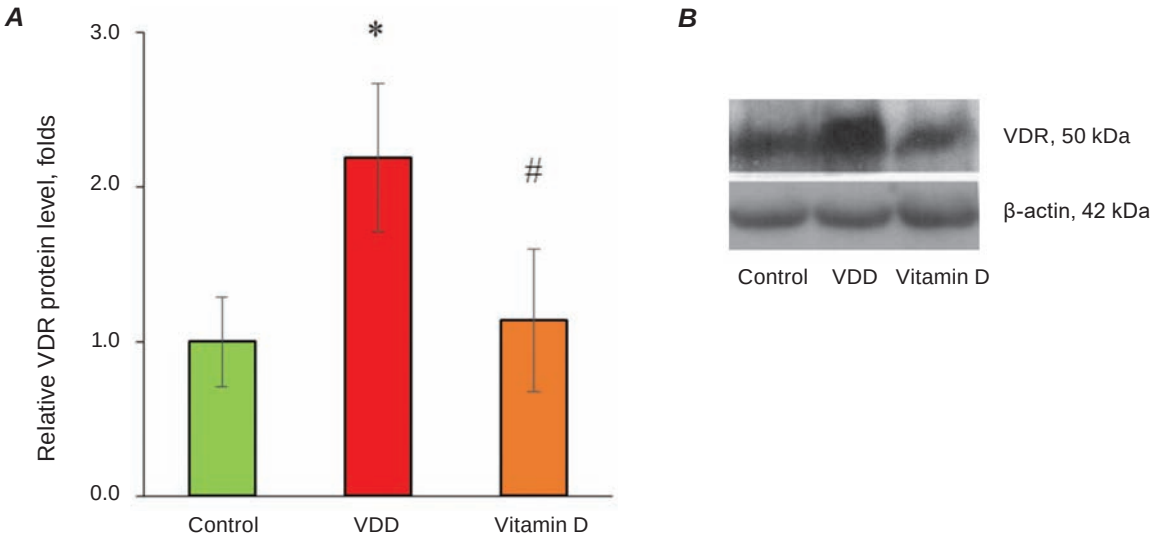

*Fig. 4. Relative protein level of VDR (**A**) and the representative immunoblot (**B**) in the liver of vitamin D-deficient (VDD) rats and after supplementation of vitamin D_3 (1,000 IU/kg of b.w, 30 days). All data are presented as mean ± SD, n = 8; *P < 0.05 vs. the control group, $^{\#}P < 0.05$ vs. the vitamin D_3 deficiency group*

CYP27B1, CYP24A1), and the receptor apparatus (VDR), it ensures multi-stage adaptation of the organism to changes in vitamin status, restoring metabolic homeostasis after correction.

Thus, summarizing the factual data and proceeding to scientific analysis, our data indicate the existence of a complex, multi-level physiological system for the adaptation of vitamin D_3 metabolism to conditions of prolonged dietary deficiency. The liver acts as a key link in this adaptation, dynamically changing its enzymatic and receptor profile to maintain systemic vitamin D homeostasis.

The central marker that confirmed the development of profound hypovitaminosis D was the reduction of serum 25(OH)D levels to critical values (19.45 nmol/l). This condition initiates a cascade of compensatory reactions, the first of which is a sharp increase in hepatic 25-hydroxylase activity. Our data demonstrate an almost two-fold increase in the functional capacity of this enzyme, accompanied by a selective rise in CYP2R1 expression. According to Shinkyo et al., CYP2R1 is the primary 25-hydroxylase with high substrate affinity, and its induction under deficiency is strategically justified for the most effective capture of remaining cholecalciferol [25]. Simultaneously, a moderate decrease in CYP27A1 expression confirms the conclusions of Guo et al. that this mitochondrial enzyme plays a secondary role at low vitamin concentrations [26].

A highly significant result was the three-fold increase in CYP27B1 expression in the liver during deficiency. This finding is consistent with current scientific understanding of extrarenal vitamin D metabolism: under conditions of systemic hormone scarcity, the liver activates a paracrine mechanism of local 25(OH)D conversion into active calcitriol to support intracellular functions [22, 26-28]. This adaptation is accompanied by the positive regulation of VDR (a 2.19-fold increase), which creates a state of cellular hypersensitivity to the ligand [29, 30].

The transport link of metabolism is also involved in this process, ensuring fine regulation of vitamin D bioavailability in tissues. Although we did not perform a direct analysis of VDBP expression in our study, the established reduction of its level in the blood serum during D-deficiency is consistent with the “free hormone hypothesis” [31]. This means that the reduction in the total level of the transport protein facilitates the passive diffusion of metabolites into hepatocytes. The most recent paradigm complements this with a mechanism of active endocytosis via the megalin/cubilin system, which potentially allows cells to accumulate substrate even at its low content [29, 32].

Therapeutic correction with vitamin D_3 led to the restoration of systemic homeostasis and the reversion of most markers. The return of 25-hydroxylase activity and CYP2R1 and VDR levels to control values indicates the high sensitivity of feed-

back mechanisms. As noted by Zhu et al., CYP2R1 expression is tightly regulated by its own product level, allowing the system to promptly neutralize the state of metabolic perturbations [33]. However, the recorded inertia of CYP27B1, whose level remained somewhat elevated, points to a prolonged period of stabilization for the extrarenal activation system [34,35].

Of particular importance for therapy safety is the rapid increase in CYP24A1 expression after the administration of vitamin D_3. This reflects the launch of fundamental mechanisms of metabolic protection against hypercalcemia [36]. The strong enhancement of this protein synthesis is a direct response to the restoration of VDR-mediated signaling, as the *CYP24A1* gene promoter contains potent vitamin D response elements [37].

While the rat model is a cornerstone of preclinical vitamin D research, certain metabolic differences must be acknowledged when translating these findings to human clinical practice. Rats, as nocturnal animals, have evolved different calcium homeostasis mechanisms and exhibit a significantly higher tolerance to vitamin D than humans; for instance, the toxic threshold in rodents is markedly higher. Furthermore, the hepatic 25-hydroxylation and renal 1-alpha-hydroxylation pathways in rats can be more rapid, leading to different pharmacokinetic profiles of circulating 25(OH)D and 1α,25(OH)2D. Therefore, while the 1000 IU/kg dose demonstrates clear therapeutic potential in this model, direct extrapolation of this dosage to humans should be approached with caution, as it may exceed the safe upper intake levels and increase the risk of hypercalcemia in a clinical setting.

In summary, the obtained results expand our knowledge of the liver's role as a central dynamic regulator of vitamin D metabolism. The organ's capacity for rapid metabolic reprogramming – facilitating the precise coordination of vitamin D activation and catabolism to maintain systemic homeostasis – underscores the necessity of integrating hepatobiliary functional assessments into clinical protocols for correcting vitamin D deficiency.

Conclusions. Collectively, our findings demonstrate that under deficiency, the liver adopts an adaptive metabolic profile by upregulating activation enzymes (CYP2R1, CYP27B1) and receptor sensitivity (VDR), while downregulating catabolic pathways (CYP24A1). This strategic reprogramming serves to maximize the utilization of limited vitamin D resources. Successful correction saturates the system and restores the equilibrium between synthesis and degradation, facilitating a return to systemic homeostasis. These findings underscore the liver's role as a central hub of metabolic plasticity, dynamically adjusting its enzymatic and receptor landscape to safeguard vitamin D status.

Conflict of interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ФУНКЦІОНУВАННЯ АВТО-/ ПАРАКРИННОЇ СИСТЕМИ ВІТАМІНУ D У ПЕЧІНЦІ ЩУРІВ ЗА УМОВ РІЗНОГО СТАТУСУ ВІТАМІНУ D

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Печінка відіграє центральну роль у метаболізмі вітаміну D, забезпечуючи його первинну активацію та транспорт. Проте молекулярні механізми адаптації печінкової системи гідроксилювання та рецепції до тривалого дефіциту субстрату залишаються недостатньо з'ясованими. Метою роботи було оцінити стан системи метаболізму та рецепції вітаміну D у печінці щурів за умов аліментарного гіповітамінозу та після введення холекальциферолу. Дослідження проведено на щурах-самицях лінії Wistar. Аліментарний дефіцит вітаміну D моделювали шляхом утримання тварин на відповідному раціоні протягом 60 днів. Корекцію здійснювали пероральним введен-

ням холекальциферолу (1000 МО/кг маси тіла) протягом 30 днів. Рівень 25(OH)D у сироватці крові визначали методом ELISA. Активність печінкової 25-гідроксилази оцінювали радіоізотопним методом. Експресію протеїнів CYP2R1, CYP27A1, CYP27B1, CYP24A1 та VDR у тканині печінки досліджували методом вестерн-блоттингу. Встановлено, що 60-денний D-дефіцит призводив до критичного зниження рівня 25(OH)D у сироватці крові до 19,45 ± 2,52 нмоль/л (20% від контролю). За цих умов зафіксовано дворазове зростання загальної активності печінкової 25-гідроксилази та компенсаторне підвищення експресії CYP2R1 (у 1,66 раза) і VDR (у 2,19 раза). Найбільш виражене зростання (у 2,94 раза) виявлено для експресії екстраренальної 1-α-гідроксилази (CYP27B1), що свідчить про інтенсифікацію локального синтезу кальцитріолу. Натомість рівень інактивуючого ензиму CYP24A1 вірогідно знижувався. Введення вітаміну D_3 забезпечувало відновлення його, а також рівня молекулярних маркерів трансформації та рецепції у печінці, за винятком CYP27B1, що зберігала помірно підвищену експресію. Таким чином, метаболічна адаптація за умов вітамінної недостатності проявлялася через компенсаторне посилення синтезу вітаміну D та підвищення чутливості рецепторів на тлі пригнічених метаболічних шляхів. Нутрієнтна корекція відновлювала фізіологічну рівновагу між процесами активування та інактивування метаболітів вітаміну D, забезпечуючи стабільний гомеостаз.

Ключові слова: вітамін D_3 (холекальциферол), печінка, активність 25-гідроксилази, CYP2R1, CYP27A1, CYP27B1, CYP24A1, VDR, D-гіповітаміноз.

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