Riboflavin Content in Male Spermoplasm

V. M. Kodentsova, O. A. Vrzhesinskaya, and V. V. Evdokimov

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Riboflavin concentration in male spermoplasm depends on consumption of vitamin B_2 and is 3-50 times higher than in the blood plasma, depending on the riboflavin status. Normal levels of riboflavin content in the spermoplasm were evaluated by investigating the metabolic relationship between riboflavin concentration in the spermoplasm and blood plasma and the relationship between riboflavin saturation of the spermoplasm and the duration of treatment with vitamin B_2. Both methods yielded close values: 60-130 and 85-96 ng/ml; hence, 80 ng/ml can be taken for the lower normal threshold level of riboflavin in the spermoplasm.

Key Words: *riboflavin; spermoplasm; blood plasma*

The reproductive function depends on hormonal regulation and vitamin supply [1,7]. The levels of vitamins E [10] and A [11] in the ejaculate of infertile men are decreased. The mobility of spermatozoa is maximum at the optimal levels of ascorbic acid (43-80 μg/ml) in the spermoplasm and decreases at higher or lower concentrations of this vitamin [6,9]. The concentration of riboflavin (RF) in the spermoplasm, corresponding to the optimal concentration of vitamin B_2 in the body, is unknown.

We developed a method for measurement of RF in SP and determined the concentrations characteristic of normal vitamin B_2 supply in men.

MATERIALS AND METHODS

Blood and seminal specimens were collected from 21 men (mean age 36 years) with idiopathic infertility (infertile marriage over 2-5 years), consulted at Institute of Urology, Ministry of Health of Russian Federation. Spermograms showed decreased fertility parameters (mobility and normal forms of spermatozoa).

After the first examination 6 men were prescribed Essentiale forte (France). Each capsule contains vitamins B_1, B_2, B_6, E (6 μg each), nicotinamide (30 mg), vitamin B_{12} (6 μg), and essential phospholipids (300 mg). Each patient received 2 capsules three times daily for 6 months.

The content of vitamin B_2 was evaluated before and 1, 3, and 6 months after vitamin therapy by measuring RF concentration in venous blood collected after overnight fast [3,8].

The concentration of RF in SP was measured by fluorescent method of titration with RF-binding protein, based on the capacity of this protein to bind RF selectively with high affinity in equimolar ratio and completely quench its fluorescence. This method is used for measurement of RF in the urine, plasma, breast milk, and other biological fluids [2-4,8].

The concentration of RF in SP was measured in the supernatant obtained after addition of 0.05 ml 100% trichloroacetic acid to 0.5 ml SP and 5-min centrifugation at 3000*g*. Then 0.2 ml 4 M K_2HPO_4 and 2.2 ml distilled water were added to 0.2 cm^3 supernatant. The fluorescence intensity was measured on an F-2000 spectrofluorimeter (Hitachi) at excitation and emission wavelengths of 465 and 525 nm, respectively. After that 0.03 ml standard RF solution (2 μg/ml) was added and the measurement was repeated. Then 0.03 ml solution of RF-binding apoprotein isolated from hen egg white [3] with binding capacity of 5-15 μg RF/mg protein was repeatedly added and mixed, and the fluorescence intensity was measured after each addition of the protein. The protein was added until stabilization of fluorescence intensity (decreased no more after addition of two aliquots). The difference in

Laboratory of Vitamins and Mineral Substances, Institute of Nutrition, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** spirichev@ion.ru. Kodentsova V. M.

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fluorescence intensity before and after addition of RF-binding protein was proportionate to the content of RF in SP.

RESULTS

Two approaches were used to elucidate the range of RF concentrations in SP characteristic of adequate supply with vitamin B_2 in men. The first approach consisted in plotting a curve reflecting the relationship between two parameters for individuals: RF content in the plasma and SP (Fig. 1). If vitamin B_2 supply was insufficient (plasma concentration below 5 ng/ml [4,8]) or below optimal (plasma concentrations 5-10 ng/ml), the content of RF in SP changed negligibly. In men with adequate vitamin B_2 supply its concentrations in SP increase with increasing its plasma concentrations from 10 to 30 ng/ml, and at blood plasma concentration of 30 ng/ml SP concentrations reaches a plateau.

High linear (R=0.83, n=21, $p\leq 0.01$) and ranked (ρ=0.64, n=21, $p<0.01$) correlations between the two parameters were observed for the entire range of RF concentrations.

Table 1 shows that the mean RF content in SP of men with sufficient vitamin B_2 supply is 1.5 times higher than in men with insufficient vitamin supply.

The concentration of RF in SP was higher than in the blood plasma in all the cases (Table 1, Fig. 1). This excess, more expressed in men with vitamin B_2 deficiency, reached 10-50 times. In men with adequate vitamin supply the difference was less pronounced (3-10-fold).

The relationship between the two parameters (Fig. 1) indicates that RF content in SP of men with normal vitamin B_2 supply (according to its blood plasma level, ≥5 ng/ml) surpasses 60 ng/ml. The concentrations of 60-130 ng/ml seem to correspond to normal vitamin B_2 content in SP.

These conclusions are confirmed by the results obtained using the other approach: parallel evaluation of the relationship between RF levels in blood plasma and SP, on the one hand, and additional intake of vitamin B_2 (36 mg daily) as a component of the drug for 1-6 months, on the other. We considered that treatment with vitamin B_2 in high doses would lead to saturation and the vitamin concentration in the studied fluids would reach the maximum level [5]. As we see in Fig. 2, both parameters reached the plateau as soon as after 1 month and virtually did not change later, though vitamin therapy was continued. At RF plasma

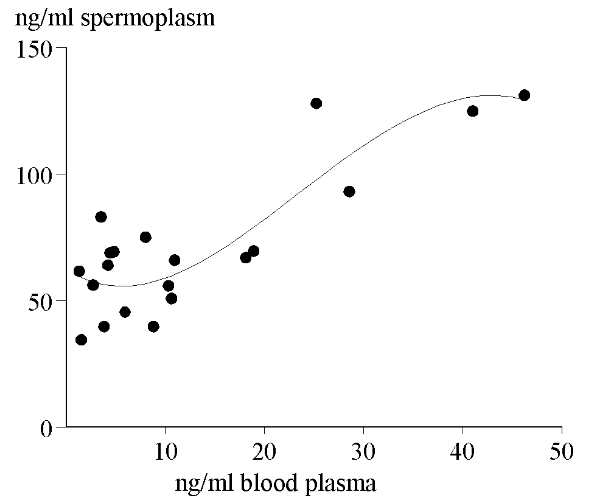

Fig. 1. Relationship between riboflavin concentrations in blood plasma and spermoplasm of men. The curve is described with an equation: $y=-0.003x^2+0.2x^2-1.91x+61$.

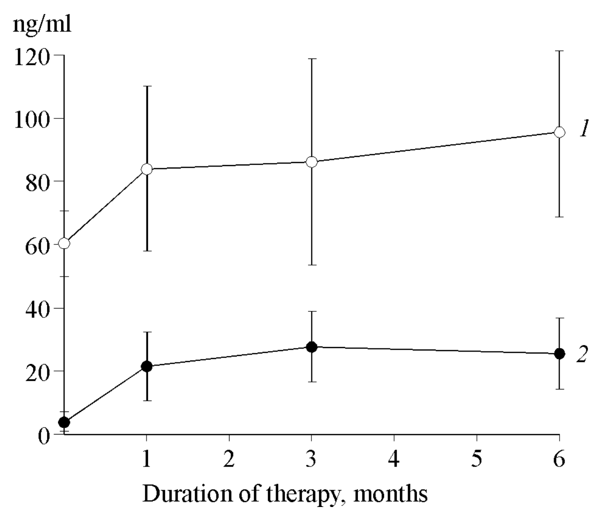

Fig. 2. Relationship between riboflavin concentrations in spermoplasm (*1*) and blood plasma (*2*) of men and the duration of vitamin therapy.

TABLE 1. Relationship between RF Concentration in Biological Fluids of Men and Their Saturation with Vitamin B_2

Object of analysis	Vitamin B_2 saturation (n=13)		Poor vitamin B_2 supply (n=8)	
	M±m	range of values	*M±m*	range of values
Blood plasma	19.6±3.7	5.9-46.2	3.3±0.5*	1.3-4.8
SP	92.5±9.1	40.0-131.2	60.0±5.6*	30.0-69.1

Note. *$p\leq 0.01$ compared to vitamin B_2 saturation.

concentrations of 21-27 ng/ml (corresponding to the upper boundary of normal) its level in SP was 85-96 ng/ml. Vitamin treatment improved fertility parameters of the ejaculate (total concentrations of spermatozoa and active mobile cell fraction; the number of live and normal spermatozoa increased in 75% men). This suggests that RF concentration in SP of men with normal vitamin B_2 supply is at least 85 ng/ml.

Both methods for evaluating the range of normal values of RF in SP gave close results: 60-130 and 85-96 ng/ml. To be on the safe side, we take the concentration of 80 ng/ml as the lower boundary of normal for RF content in SP.

This value can be used for the diagnosis of B_2 hypovitaminosis in men by noninvasive methods. The diagnosis and correction of vitamin B_2 deficiency in the ejaculate leading to improvement of spermatogenesis parameters will be useful in the treatment of infertility and in optimization of storage conditions for donor seminal fluid.

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