Effect of Coenzyme Q_{10} on the Proteomic Profile of the Cytosolic and Microsomal Fractions from Rat Hepatocytes upon Dietary Consumption of Various Lipid Components during Ontogeny

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We studied proteomic features of subcellular fractions from rat hepatocytes and intensity of enzymatic and non-enzymatic free radical oxidation depending on the type of dietary fat during adaptation of animals to modified nutrition. Our results illustrate the formation of specific nutriproteomes in the microsomal and cytosolic fractions of rat hepatocytes (measurement of the macro-component and micro-component composition of diet).

Key Words: *proteomics; coenzyme Q_{10}; polyunsaturated fatty acids; F2-isoprostanes*

Coenzyme Q_{10} (CoQ_{10}) is one of the most potent natural antioxidants that plays a role in metabolic processes in all tissues of animals and humans (primarily in tissues with high metabolic activity, including the heart, kidneys, liver, and muscles) [15]. As differentiated from antioxidants that lose activity in functioning (*e.g.*, vitamin A and β-carotene), CoQ_{10} can be regenerated in the body [14]. Incorporation of CoQ_{10} into the basal diet of experimental animals (in addition to other antioxidants) has a geroprotective effect [11] and improves the resistance to metabolic stress [1,8,9]. The consumption of dietary polyunsaturated fatty acids (PUFA) that enter the composition of tissue lipids and membranes in various stages of ontogeny is followed by an increase in the content of potential substrates for LPO [5,6]. Age-related activation of free radical oxidation can induce multiple protein modification, preterm aging, and change in apoptosis. The study of complex metabolic changes will elucidate the basic signs that reflect the nature of exogenous and endogenous influences.

This work was designed to study proteomic features and processes of enzymatic and non-enzymatic free radical oxidation in dependence on the type of dietary fat and age-related characteristics of rats during adaptation to modified nutrition. The diet included a bioactive food component, CoQ_{10}, which serves as a natural endogenous mediator of antioxidant and energy homeostasis.

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats (nursery of the Institute of Nutrition) weighing 90-110 g. The animals fed a standard semisynthetic diet. CoQ_{10} (100 mg per 1 kg body weight) was added to the diet. Depending on the type of dietary fat, the rats were divided into 5 groups of 32 specimens each. The diet included the following sources of fat: lard and sunflower oil (1:1; group 1, control); lard and sunflower oil+CoQ_{10} (group 2); fish oil+CoQ_{10} (group 3); linseed oil+CoQ_{10} (group 4); and palm oil+CoQ_{10} (group 5). According to the physiological standards,

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all animals received water-soluble vitamins (dried brewer's yeast) and fat-soluble vitamins A, D, and E (sunflower oil). The duration of the experiment was 12 months. Eight animals of each group were examined after 1, 3, 6, and 12 months, respectively, to evaluate the effect of the length of dietary regimen on the test parameters.

The fatty acid composition of the liver was estimated by means of gas-liquid chromatography on a Carloerba strumentazione HRGC 5300 chromatograph with a DB-23 phase (Agilent Technologies) and Mul'tiKhrom for Windows hardware-software system [7]. The coefficient of metabolic conversion efficiency (CMCE) for fatty acids was evaluated as described elsewhere [2]. The intensity of enzymatic LPO was determined from the contents of conjugated dienes (CD) and MDA in the plasma and liver of animals [10]. Non-enzymatic LPO (plasma concentration of F2-isoprostanes, F2isoPr) and antiapoptotic factor IGF-I were assayed with EIA kits for laboratory rats (ImmunoDiagnosticSystems Ltd.).

A chromatographic study of CoQ_{10} in blood plasma and homogenates of the liver and heart was conducted as described previously [3].

The microsomal and cytosolic fractions of rat hepatocytes were obtained by the standard method of differential centrifugation. Protein separation by means of two-dimensional electrophoresis and staining with silver nitrate were performed as described previously [3]. Protein hydrolysis with trypsin and recording of the mass spectra were conducted as described elsewhere [4].

The statistical significance of differences between continuous values was evaluated by nonparametric Mann–Whitney *U* test for independent variables. The significance of differences between the parts was estimated by Student's *t* test. The differences were significant at $p<0.05$.

TABLE 1. Composition of Major Fatty Acids in Liver Lipids and CoQ_{10} Concentration in Blood Plasma, Liver, and Heart of Rats after Modification of Dietary PUFA and Administration of CoQ_{10} in Various Periods of Ontogeny

Age	Parameter	Group				
		1 (control)	2 (control+CoQ_{10})	3 (FO+CoQ_{10})	4 (LO+CoQ_{10})	5 (PO+CoQ_{10})
1 month	PUFA ω6/ω3	12.46±2.06	11.54±1.76	2.17±0.78*	2.89±0.38*	9.15±1.02
	CMCE	5.20	4.54	1.15	1.36	5.75
	Plasma CoQ_{10}, μg/ml	0.58±0.29	1.23±0.25*	0.65±0.31	0.71±0.19	1.25±0.87
	Liver CoQ_{10}, μg/ml	27.5±7.5	56.2±9.4*	50.7±9.9*	40.3±12.8	62.6±16.7*
	Heart CoQ_{10}, μg/ml	5.63±3.56	4.92±3.27	1.94±0.33	2.20±0.71	2.79±1.12
3 months	PUFA ω6/ω3	14.51±1.82	11.88±2.41	1.21±0.27*	1.59±0.23*	11.06±1.94
	CMCE	5.68	4.81	0.56	1.25	6.08
	Plasma CoQ_{10}, μg/ml	1.76±0.76	4.34±1.17*	0.25±0.09*	0.31±0.15*	3.83±1.75
	Liver CoQ_{10}, μg/ml	21.7±7.4	42.4±3.3	36.6±9.3	24.7±5.2	51.8±12.7*
	Heart CoQ_{10}, μg/ml	9.06±2.49	4.96±1.58	7.70±3.10	4.03±1.46*	4.55±1.71*
6 months	PUFA ω6/ω3	6.58±0.92	7.35±0.64	1.83±0.25*	1.91±0.30*	7.78±0.23
	CMCE	2.63	2.97	0.53	0.69	4.64
	Plasma CoQ_{10}, μg/ml	3.23±1.25	5.65±3.38	10.92±1.06*	6.21±0.73*	22.62±5.10*
	Liver CoQ_{10}, μg/ml	19.9±8.9	38.2±12.7	87.1±26.1*	82.4±30.6*	23.2±7.6
	Heart CoQ_{10}, μg/ml	5.11±1.70	3.83±1.05	5.48±2.22	7.70±3.03	7.51±3.59
12 months	PUFA ω6/ω3	15.8±1.0	19.4±2.62	3.42±0.58*	1.82±0.29*	12.82±2.02
	CMCE	6.52	4.17	0.99	0.98	4.67
	Plasma CoQ_{10}, μg/ml	3.17±1.17	1.68±0.76	1.34±0.73	2.30±1.08	1.51±0.68
	Liver CoQ_{10}, μg/ml	17.5±3.5	26.9±2.0	81.7±24.3*	113.9±36.3*	45.5±13.7*
	Heart CoQ_{10}, μg/ml	5.06±1.93	6.60±1.75	7.29±3.32	5.70±1.49	7.11±4.44

Note. Here and in Table 2: *$p<0.05$ compared to the control. FO, fish oil; LO, linseed oil; PO, palm oil.

RESULTS

Plasma CoQ_{10} concentration in animals feed a modified lipid diet until the 3rd month of life did not differ from the control, but was below the level in group 3 and 4 rats (Table 1). The concentration of CoQ_{10} in blood plasma was shown to increase significantly after 6 months. This parameter did not differ in animals of the treatment groups after 12 months.

In all periods of the study, CoQ_{10} concentration in the liver from animals of the four treatment groups was higher than in the control. The elevated level of CoQ_{10} in liver samples from animals of groups 3, 4, and 5 by the 12th month of life probably serves as an adequate response to a deficiency of PUFA (palm oil) or low content of PUFA ω6 in the diet (fish oil and linseed oil). CoQ_{10} concentration in the heart tissue from animals of the four treatment groups remained unchanged in all periods of the study. CoQ_{10} concentration in the heart tissue was lowest in 3-month-old rats of groups 4 and 5. It is probably associated with the pseudo-deficiency of CoQ_{10} due to an increased demand for this substance upon activation of free radical oxidation in the period of active growth. The PUFA ω6/ω3 ratio in group 3 and 4 animals was lower than in rats of other groups, which can be considered as a risk factor for the increase in peroxide concentration.

The content of primary products of lipid peroxidation (CD) was highest in 1-month-old rats of groups 3 and 4 (Table 2). Dietary fat composition in these animals was mainly presented by unsaturated fatty acids. By the 12th month of life, plasma CD concentration in these rats was lowest (particularly in group 4 animals). It was probably related to the highest concentration of CoQ_{10} in blood plasma of these animals.

The contents of CD and MDA in blood plasma of 1-month-old animals practically did not depend on dietary fat composition. F2isoPr concentration was highest in group 5 rats, but lowest in group 3 animals. The contents of CD and MDA in blood plasma were shown to decrease in 3-month-old animals of various groups. These changes accompanied an increase in the

TABLE 2. Contents of IGF-I and F2-isoprostanes in Blood Plasma and Concentration of LPO Products in Blood Plasma and Liver of Rats after Modification of Dietary PUFA and Administration of CoQ_{10} in Various Periods of Ontogeny

Age	Diet	Plasma CD, arb. units/ml	Plasma MDA, nmol/ml	Liver MDA, nmol/g	F2isoPr, ng/ml	IGF-I, ng/ml
1 month	Control (group 1)	2.39±0.10	10.74±0.07	221.10±4.32	0.57±0.03	1309.0±72.3
	Control+CoQ_{10} (group 2)	2.22±0.09	11.16±0.45	228.62±5.02	0.45±0.14	627.9±173.3
	FO+CoQ_{10} (group 3)	3.19±0.13*	12.41±0.25*	265.08±2.88*	0.32±0.20	702.5±301.1
	LO+CoQ_{10} (group 4)	3.12±0.11*	8.86±0.17*	249.05±3.14	0.47±0.10	518.3±139.0*
	PO+CoQ_{10} (group 5)	2.41±0.11	9.56±0.11	215.04±2.25	1.19±0.57	659.8±173.3*
3 months	Control (group 1)	1.56±0.08	7.35±0.21	245.38±2.25	-	-
	Control+CoQ_{10} (group 2)	1.02±0.04*	6.78±0.29*	225.76±5.99*	-	-
	FO+CoQ_{10} (group 3)	1.18±0.05*	6.81±0.09	314.74±1.22*	-	-
	LO+CoQ_{10} (group 4)	1.01±0.04*	6.52±0.16	246.79±2.76	-	-
	PO+CoQ_{10} (group 5)	1.14±0.03*	5.47±0.30*	237.43±5.96	-	-
6 months	Control (group 1)	2.22±0.04	10.26±0.18	193.03±2.51	2.08±0.95	523.7±99.7
	Control+CoQ_{10} (group 2)	2.43±0.03*	10.70±0.12*	192.43±3.76	2.05±1.29	689.0±135.2
	FO+CoQ_{10} (group 3)	1.95±0.01	9.11±0.14	176.47±4.12	0.63±0.12*	645.7±77.4
	LO+CoQ_{10} (group 4)	1.58±0.04*	9.16±0.10	163.46±2.63*	0.54±0.17*	552.4±105.4
	PO+CoQ_{10} (group 5)	2.23±0.09	9.41±0.11	187.90±2.89	2.13±0.78	496.2±120.3
12 months	Control (group 1)	1.97±0.06	10.24±0.10	294.61±1.95	2.17±0.86	500.8±80.8
	Control+CoQ_{10} (group 2)	2.18±0.05*	11.66±0.14*	291.61±2.91	2.00±1.29	489.8±80.2
	FO+CoQ_{10} (group 3)	1.94±0.06	9.96±0.12	306.28±3.21	0.90±0.09*	556.9±202.2
	LO+CoQ_{10} (group 4)	1.49±0.01*	9.84±0.09	270.97±2.97	1.41±0.97	581.3±207.9
	PO+CoQ_{10} (group 5)	2.18±0.02	10.19±0.29	272.73±2.09	1.48±0.69	489.8±104.9

Note. –: no data.

TABLE 3. Proteomic Features of the Microsomal and Cytosolic Fractions of Rat Hepatocytes after Dietary Consumption of CoQ_{10} upon a Modified Lipid Diet

Protein	Age	Group				
		1 (control)	2	3	4	5
Microsomal fraction						
Transmembrane emp24 protein	1 month					
	3 months	*				
	6 months	*				
	12 months	*				
Catalase	1 month					
	3 months		*			
	6 months		*			
	12 months		*			
Cytochrome b5	1 month		*			*
	3 months		*			*
	6 months	*	*	*		*
	12 months	*		*		
Homogentisate 1,2-dioxygenase	1 month		*			*
	3 months		*			*
	6 months		*			
	12 months					
Transthyretin	1 month	*		*	*	*
	3 months	*		*	*	*
	6 months	*				*
	12 months					
Leucine rich protein (FLII)	1 month		*	*		*
	3 months					
	6 months			*		*
	12 months			*		
rCG26823	1 month		*			
	3 months		*			
	6 months	*	*			
	12 months	*				
Cytosolic fraction						
C-type lectin	1 month	*		*		
	3 months	*	*			
	6 months	*	*	*		
	12 months		*	*		*
60S ribosomal protein L29	1 month		*	*		
	3 months		*	*		
	6 months			*		
	12 months					

Continuation of Table 3.

26S proteasome, isoform CRA_b	1 month			*		
	3 months	*		*	*	*
	6 months	*	*		*	*
	12 months	*	*		*	*
rab/GDP dissociation inhibitor-α	1 month		*			
	3 months		*			
	6 months					*
	12 months					*
ras-related protein Rab-14	1 month	*			*	
	3 months					
	6 months	*				
	12 months	*				
Proteasome complex subunit	1 month	*				
	3 months	*	*			
	6 months	*	*			
	12 months		*			
F-box protein	1 month			*		*
	3 months					*
	6 months		*			
	12 months		*			
Cyclin-dependent kinase (Cdk5)	1 month	*	*			*
	3 months					*
	6 months					
	12 months					
rCG33743	1 month		*			
	3 months				*	*
	6 months				*	*
	12 months				*	*

Note. *: protein detected.

concentration of CoQ_{10} in blood plasma. No between-group differences were found in the intensity of LPO in rats aging 6 and 12 months. By the 6th month of life, F2isoPr concentration in CoQ_{10}-receiving animals of group 5 was highest and did not differ from the control. However, F2isoPr concentration in group 3 and 4 rats was lower than in control specimens (by 70 and 74%).

The content of IGF-I in 1-month-old animals of the four treatment groups was lower than in the control. Fish oil and linseed oil are the sources of PUFA ω3, which induce *in vitro* and *ex vivo* apoptosis in various cells. It is mediated by a change in the mitochondrial transmembrane potential, reduced expression of anti-apoptotic bcl-2 proteins, and induction of Fas ligand expression [12,13].

Proteomic analysis of the cytosolic fraction revealed C-type lectin expression in group 3 animals aging 1, 6, and 12 months (Table 3). C-type lectins are involved in the immune response and apoptosis. Moreover, they can induce the synthesis of cytokines and reactive oxygen species in phagocytes. The expression of ras-related protein Rab-14 was observed in 1-month-old rats of groups 1 and 4. It was probably related to the involvement of linseed oil in cell proliferation, differentiation, and cytoskeleton formation in the early stage of ontogeny. Cyclin-dependent kinase 5 (Cdk5) was revealed in 1-month-old animals of groups 1, 2, and 5. However, the expression of Cdk5 persisted only in group 5 rats aging 3 months. Cdk5 phosphorylates some proteins, the majority of which play a role in cell morphology and mobility. F-box

protein was identified in group 3 and 5 rats receiving the diet with fish oil and palm oil over the first months of life. This protein has a regulatory role and serves as a component of the ubiquitin ligase complex SCFCOl1 (Skp1+Cullin+F-box protein), which is involved in protein degradation in the 26S proteasome with ubiquitin. Ubiquitin transfer to phosphorylated regulatory proteins is realized via F-box protein.

A proteomic study of the microsomal fraction showed that cytochrome b5 is present in group 1 and 3 animals in the late stage of ontogeny (6 and 12 months). In group 2 and 5 rats, cytochrome b5 was identified on the 1st, 3rd, and 6th months of life (Table 3). Our findings are consistent with the increase in F2-isoprostane concentration during these periods of life (by 78 and 20%, respectively; $p \leq 0.01$) and intensification of non-enzymatic free radical oxidation. Cytochrome b5 plays an important role in the metabolism of endogenous and exogenous compounds with enzymes of the cytochrome P450 system in various organs and tissues. Homogentisate 1,2-dioxygenase catalyzes the conversion of phenylalanine and tyrosine into maleylacetoacetate of homogentisic acid. Homogentisate 1,2-dioxygenase was revealed in the microsomal fraction of hepatocytes from animals receiving CoQ_{10} and palm oil (group 5) in the early stage of ontogeny.

Our results indicate that macronutrients and micronutrients induce the specific nutriproteomic changes.

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