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## Effects of COVID-19 and diabetes mellitus on AMPK $\alpha$ 1 and IRS-1 amount in the blood plasma of patients

*Presented by Corresponding Member of the NAS of Ukraine M.D. Tronko*

*The aim of the study was to determine the levels of AMPK $\alpha$ 1 and IRS-1 in the blood of patients with diabetes mellitus and COVID-19. AMPK $\alpha$ 1 and IRS-1 were determined using enzyme-linked immunosorbent assay (ELISA) (Elabscience, USA). AMPK controls the energy balance of the cell, stimulates catabolic processes – absorption of glucose, fatty acids, and their conversion by mitochondrial oxidation and glycolysis. With type 2 diabetes and obesity, its activity decreases, and the activity of protein kinases mTORC1/p70S6K increases, leading to phosphorylation of insulin receptor substrate-1 (IRS) and insulin resistance. The level of AMPK $\alpha$ 1 in the blood of diabetic patients was significantly higher than in the blood of healthy people. The amount of AMPK $\alpha$ 1 in the blood of people recovered from COVID-19 demonstrated the further growth of AMPK $\alpha$ 1. The level of AMPK $\alpha$ 1 was much higher in the blood of patients with DM during a COVID-19 disease. IRS-1 amounts in the blood plasma of patients with diabetes was higher than normal values. The level of IRS-1 in the blood plasma of patients with COVID-19 was much higher than in the blood of healthy people and patients with diabetes. The level of IRS-1 in the blood plasma may be one of the promising markers of COVID-19.*

**Keywords:** 5'AMP-activated protein kinase  $\alpha$ 1, insulin receptor substrate-1, COVID-19, diabetes mellitus.

AMPK (5'AMP-activated protein kinase) is a heterotrimer consisting of a catalytic subunit ( $\alpha$ ) and two regulatory subunits ( $\beta$  and  $\gamma$ ). The  $\gamma$ -subunit contains four potential sites that bind adenine nucleotides [1, 2]. The  $\alpha$  subunit has 2 isoforms,  $\alpha$ 1 and  $\alpha$ 2 [3]. AMPK $\alpha$ 1 and  $\alpha$ 2 are encoded by different genes and co-expressed in most tissues to regulate energy metabolism.

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With energetic stress in the cell and an increase in the AMP concentration, ATP is replaced in the exchange centers with AMP, resulting in the allosteric activation of AMPK via phosphorylation of the 172 threonine of the  $\alpha$  subunit by the LKB1 (liver kinase B1) complex in response to changes in cell energy, or CAMKK $\beta$  (calcium-/calmodulin-dependent kinase  $\beta$ ), which is activated by intracellular  $\text{Ca}^{2+}$  [4].

AMPK controls the energy balance of the cell. By direct phosphorylation of metabolic enzymes and transcription factors, AMPK stimulates catabolic processes – absorption of glucose, fatty acids, and their conversion via mitochondrial oxidation and glycolysis. In addition, AMPK suppresses anabolic processes – the synthesis of glucose, glycogen, and lipids in liver [5]. With type 2 diabetes and obesity, its activity decreases, and the activity of protein kinases mTORC1/p70S6K (mammalian target of rapamycin complex 1/ribosomal protein S6 kinase beta-1) increases, leading to phosphorylation of insulin receptor substrate-1 (IRS-1) and insulin resistance [6]. The effect of hypoglycemic drugs, such as metformin, is associated with the activation of AMPK [7].

Insulin receptor substrate is a key adapter protein mediating effects of insulin and insulin-like growth factors (IGF) in cells [8]. The PTB (phosphotyrosine binding) and PH (pleckstrin homology) domains are involved in the interaction of the receptor with the IRS. Phosphorylated IRS are a platform for the propagation of insulin signals in the cell, which it shares with other receptor tyrosine kinases, such as IGF-1R. IRS-1 is a member of the insulin receptor substrate family, which is also associated with tumor initiation and progression [9]. Overexpression of IRS-1 promotes the growth of cells, reduces oxidative stress-induced autophagy, and diminishes oxidative stress-mediated autophagy-dependent cell death [10, 11].

**Materials and Methods.** The study was conducted in the Diabetology department of the Institute. The study protocol was approved by the Institute's ethics committee. All patients signed informed consent to conduct further diagnostic and research studies.

Blood was obtained via standard venipuncture and stored in EDTA-coated vacutainer tubes. Plasma was separated via centrifugation within 10 min after blood sampling. The samples were

### Plasma AMPK $\alpha$ and IRS-1 levels of patients with diabetes and COVID-19

Groups	AMPK $\alpha$ , pg/ml	$m \pm$
Control ( $n = 7$ )	688.06	98.09
DM ( $n = 60$ )	944.42	61.97*
DM after COVID-19 ( $n = 8$ )	1310.00	118.69** <sup>+</sup>
DM + COVID-19 ( $n = 21$ )	1824.8	190.5** <sup>+</sup>
Groups	IRS-1, ng/ml	$m \pm$
Control ( $n = 7$ )	0.092	0.01
DM ( $n = 60$ )	0.121	0.007*
DM after COVID-19 ( $n = 8$ )	0.16	0.0076** <sup>+</sup>
DM + COVID-19 ( $n = 21$ )	0.414	0.029** <sup>+</sup>

Note.  $M \pm m$ . Differences from control are significant: \* $P < 0.05$ ; \*\* $P < 0.01$ . <sup>+</sup>Differences from previous group are significant,  $P < 0.05 - 0.01$ .

stored at  $-80^{\circ}\text{C}$  until use. AMPK $\alpha$  and IRS-1 were determined ( $n = 81$ ) using enzyme-linked immunosorbent assay (ELISA) kit (Elabscience, USA). The measurement was carried out at an optical wavelength of 450 nm on the immunoenzymatic plate analyzer Stat Fax 3200 (Awareness Technology, USA).

Glycated hemoglobin was determined using one HbA1c FS kit (DiaSys Diagnostic Systems GmbH, Germany). The measurement was carried out at an optical wavelength of 660 nm.

Statistical analysis and data presentation were performed using Origin 7.0 software. The results of the study are presented as  $M \pm m$ . To compare the data groups, Student's  $t$ -test was used. Values of  $P \leq 0.05$  were considered as significant.

**Results and discussion.** The blood plasma of 60 type 2 diabetes patients and 21 patients with diabetes and COVID-19 was used. As a control, we used the blood of healthy people ( $n = 7$ ) without concomitant diseases, representative of age. The level of Hb1Ac in diabetic patients was  $9.62 \pm 0.27$ ; BMI –  $30.69 \pm 1.06 \text{ kg/m}^2$ . The fasting glucose content in the blood of patients with COVID-19 and diabetes was  $9.6 \pm 0.92 \text{ mmol/l}$ , at the time of discharge –  $6.72 \pm 0.62 \text{ mmol/l}$ . Average  $\text{O}_2$  saturation was  $87.3 \pm 0.7 \%$  that indicates a severe course of the disease.

The average level of AMPK $\alpha$ 1 in the blood of healthy people is 688.06 pg/ml (Table). In diabetic patients, this indicator is significantly higher. The amount of AMPK $\alpha$ 1 in the blood of people recovered from COVID-19 demonstrated the further growth of AMPK $\alpha$ 1. In patients with diabetes and COVID-19, the content of AMPK $\alpha$ 1 in the blood is almost 3 times higher than normal values. Thus, the level of AMPK $\alpha$ 1 is much higher in the blood of patients with DM during COVID-19 disease.

The data on the reason and significance of AMPK $\alpha$  appearance in the blood are limited. Plasma levels of AMPK $\alpha$ 1 were decreased in patients with Alzheimer disease [12]. Recent evidence also indicates novel roles for AMPK in the pathogenesis of cardiovascular diseases. AMPK $\alpha$ 2 deletion increases atherosclerosis in ApoE $^{-/-}$  mice, probably via enhanced oxidative stress and endoplasmic reticulum stress. AMPK $\alpha$ 1 deficiency impairs autophagy-mediated monocyte differentiation and decreases monocyte/macrophage survival, which attenuates atherosclerosis in ApoE $^{-/-}$  mice *in vivo* [12, 13].

The average level of IRS-1 in the blood of healthy people is below 0.1 ng/ml (see Table). In diabetic patients, this indicator is somewhat higher. The amount of IRS-1 in the blood of people recovered from COVID-19 demonstrated the further growth – 0.16 ng/ml. In patients with diabetes and COVID-19, the content of IRS-1 in the blood is more than 4 times higher than normal values. Thus, the level of IRS-1 substantially rises in patients with COVID-19. It should be noted that the amount of IRS-1 in the blood of people recovered from COVID-19 decreased rather quickly compared to patients with acute COVID-19 disease.

As can be seen from our data and the results obtained by other authors, the level of IRS in the blood of healthy people is quite low. However, for serious illnesses such as cancer, it more than doubles. A highly significant increase was found in serum IRS-1 of nasopharyngeal carcinoma compared with that of healthy individuals. It might be potential biomarkers in the diagnosis of cancer [11]. In the blood of patients with COVID-19 and concomitant diseases, the quantity of IRS-1 increased from 3.5 to more than 6 times. It is still difficult to assume what is the reason for the growth of this substrate and what is the mechanism of its appearance in blood plasma. Most likely, its source is blood cells or tumor cells in the case of cancer. The physiological roles of IRSs

are not limited to glucose metabolism and growth. It is known that, in addition to its participation in mediating the action of growth factors, IRS is involved in other signaling mechanisms that are still insufficiently studied. IRS-1 maintains vascular health, and IRS-1 and IRS-2 govern bone turnover and adipocyte differentiation [14, 15].

It should be noted that, in a patient with COVID-19 who died from cardiovascular complications, the level of IRS-1 was 15 times higher than the control. Perhaps, the amount of IRS-1 may be one of the markers of severity of disease.

**Conclusions.** The levels of AMPK $\alpha$ 1 and IRS-1 in the blood plasma of patients with COVID-19 were much higher than in the blood of healthy people and patients with diabetes. The levels of AMPK $\alpha$ 1 and especially IRS-1 may be promising markers of COVID-19.

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#### ВПЛИВ COVID-19 ТА ЦУКРОВОГО ДІАБЕТУ НА КІЛЬКІСТЬ АМПК $\alpha$ 1 І IRS-1 У ПЛАЗМІ КРОВІ ПАЦІЄНТІВ

Досліджено рівень АМПК $\alpha$ 1 та IRS-1 у крові хворих на цукровий діабет та COVID-19 ( $n = 81$ ). Кількість АМПК $\alpha$ 1 та IRS-1 визначали за допомогою імуноферментного аналізу (Elabscience, США). АМПК контролює енергетичний баланс клітини, стимулює катаболічні процеси — поглинання глюкози, жирних кислот і їх перетворення шляхом мітохондріального окиснення та гліколізу. У разі цукрового діабету 2-го типу та ожиріння її активність знижується, а активність протеїнкіназ mTORC1/p70S6K підвищується, що призводить до фосфорилювання субстрату інсулінового рецептора-1 (IRS) і резистентності до інсуліну. Встановлено, що рівень АМПК $\alpha$ 1 у крові хворих на цукровий діабет був значно вищим, ніж у крові здорових людей. У крові людей, які одужали від COVID-19, виявлено подальше зростання АМПК $\alpha$ 1. У крові пацієнтів із цукровим діабетом під час захворювання на COVID-19 рівень АМПК $\alpha$ 1 був значно вищим. Кількість IRS-1 у плазмі крові пацієнтів з цукровим діабетом була вищою за нормальні значення. У плазмі крові хворих на COVID-19 рівень IRS-1 був значно вищим, ніж у крові здорових людей та хворих на цукровий діабет. Рівень IRS-1 у плазмі крові може бути одним із перспективних маркерів COVID-19.

**Ключові слова:** 5'АМР-активована протеїнкіназа  $\alpha$ 1, субстрат інсулінового рецептора-1, COVID-19, цукровий діабет.