

THE IMPACT OF HYDROXYCITRIC ACID ON THE LIPID METABOLISM PROFILE UNDER EXPERIMENTAL INSULIN RESISTANCE SYNDROME OF SYRIAN HAMSTERS

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The syndrome of insulin resistance (IR) is one of the leading reasons for the increased risk of cardiovascular diseases and their complications. Among the key components of IR are obesity and dyslipidemia. Hydroxycitric acid (HCA), an inhibitor of a key enzyme of lipogenesis ATP citrate lyase (ACLY) is a promising obesity treatment agent. The aim of this work was to investigate the effect of HCA on lipid and lipoproteins content in the blood serum, as well as lipid content and activity of some lipid metabolism enzymes in the liver of hamsters with IR. IR was modeled by keeping animals on high-fat diet with addition of fructose. Lipid content was determined by using standard reagent kits, the level of lipoproteins, the activity of glucose 6-phosphate dehydrogenase and ACLY – spectrophotometrically, lysosomal lipase activity – fluorimetrically. Development of hyperlipidemia and atherogenic dyslipidemia, lipid accumulation in the liver, activation of lysosomal lipase and ACLY and reduction of glucose 6-phosphate dehydrogenase activity were shown under IR. The treatment by HCA reduces the manifestations of hyperlipidemia, but enhances the lipid accumulation in the liver.

Key words: ATP-citrate lyase, hydroxycitric acid, insulin resistance, dyslipidemia, hepatic lipidosis.

Insulin resistance syndrome (one of the key metabolic syndrome components) is the most common systemic disease characterized by disturbance of all types of metabolism, and high risk of complications associated with cardiovascular system impairment. Great social significance of this disease is that it leads to early disability and high mortality. Some of the key components of insulin resistance are obesity and dyslipidemia [1].

ATP-citrate lyase (ACLY) is a key lipogenesis enzyme that converts citrate into acetyl-CoA in the cytoplasm; acetyl-CoA is an initial precursor that after connection of carboxyl group gives a malonyl-CoA for the fatty acids biosynthesis. Cytosolic citrate is a derivative of the mitochondrial tricarboxylic cycle, that is why ACLY catalyzes a critical reaction which connects glucose catabolism and lipid synthesis [2].

Lipogenesis is a metabolic pathway that is coordinately regulated in the response to nutritional and hormonal stimuli. It has been shown that dysregulation of lipogenesis significantly contributes to the occurrence of dyslipidemia and metabolic disorders [3]. At the same time, the metabolic contribution of each separate lipogenic enzyme in lipid metabolism

and also in whole body energy homeostasis is poorly understood [4-6].

According to the literature, the amount of both acetyl-CoA and malonyl-CoA is markedly decreased in liver, irrespective of dietary fat intake, and also the level of triacylglycerol and free fatty acids drop in the blood with ACLY knockout. On this background the liver lipid range also changes [2].

Among the drugs and functional foodstuffs that are used to the obesity treatment, the popularity of products from *Garcinia Cambogia* (*Garcinia gummi-gutta* L.) is coming up now; hydroxycitric acid (HCA) is an active substance of mentioned *Garcinia* products. The proposed mechanism of HCA action lies in a competitive ACLY inhibition, but the experimental studies of *Garcinia* drugs are isolated cases [7, 8].

Based on the above, the exploration of ACLY activity and lipid metabolism profile using the hydroxycitric acid appears to be topical.

Materials and Methods

24 male Syrian hamsters (*Mesocricetus auratus* L.) at the age of 20 weeks at the beginning of experiment were used. Animals have been divided

into 3 groups by 8 in each one: 1) intact animals that were kept on a standard diet 2) group of animals with insulin resistance syndrome (IR) – group which diet contained 29% of animal fat [9] with addition of fructose (2.5 g/100 g of body weight per day) [10], 5 weeks (IR group) 3) animals which have been administered intragastrically the HCA (STIFIMOL preparation manufactured by Kyiv Vitamin Factory) at the daily dose of 5 mg/kg body weight starting from 3rd week of insulin resistance syndrome modeling (IR+ST group). After 5 weeks the animals were decapitated under chloralose-urethane anesthesia. The studies were conducted in accordance with national General ethical principles of animal experiments (Ukraine, 2001), which are consistent with provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

The studied objects were the blood serum and liver of laboratory animals. A 25% homogenate and postmitochondrial and mitochondrial fractions were prepared from perfused liver by differential centrifugation.

The development of IR was confirmed by the blood serum level of glucose and insulin in fasting state, calculating insulin resistance index using the algorithm Homeostasis Model Assessment (the data are not included in the article).

Total lipids concentration was determined using a standard kit (Eagle Diagnostics, USA) in a reaction with vanillic reagent. The content of triacylglycerols was determined using a standard enzymatic glyceroloxidase assay kit (KONE Oy, Finland). Total cholesterol content was determined by standard enzymatic cholesteroloxidase kit (Boehringer Mannheim GmbH diagnostica, Germany). The content of free fatty acids (FFA) was determined using colorimetric method [11].

Lipoproteins of blood serum were fractionated by disc electrophoresis in vertical polyacrylamide gel plates (160×140×2 mm) [12]. α -lipoprotein, pre- β - and β -lipoproteins, the amount of which is called apo-B-containing lipoproteins were identified at the electropherograms. Stained by black sudan 10 lipoproteins were eluted, the light absorption of eluate was measured spectrophotometrically at 595 nm [13].

The activity of glucose 6-phosphate dehydrogenase (G-6-PDG, EC 1.1.1.49) was determined spectrophotometrically by NADP⁺ reduction [14]. The activity of lysosomal acid lipase (LL, EC 3.1.1.3) was determined in lysosomal-mitochondrial liver

fraction by the hydrolysis of substrate – 4-methylumbelliferyl oleate; hydrolysis product content was determined fluorimetrically (E = 449 nm, 410 nm) [15]. The activity of ATP-citrate lyase (ACLY, EC 4.1.3.8) was determined using malate dehydrogenase associated method by the reduced NADH that is oxidized by oxaloacetate formed in cytrate lyase reaction [16]. Protein content was determined by the Lowry method in the Miller modification.

Statistical analysis of the data was performed using Statistica software package (StatSoft Inc., USA, version 6.0). The significance of differences between groups was assessed by non-parametric Mann-Whitney test.

Results and Discussion

Keeping animals on the described diet for 5 weeks led to insulin resistance development, which was confirmed by insulin resistance index increasing 2.6 times in the parallel experiment (3.02 vs 1.78 in the intact animals).

The obtained data are the evidence that high energy diet lead to the hyper- and dyslipidemia that are the typical signs of IR (Table 1): the content of total lipids (TL) (mainly due to neutral lipids), lipoproteins (due to apo-B-containing ones) and non-esterified fatty acids are increased in the blood of experimental animals. Level of high-density lipoproteins (HDL) is somewhat reduced in this case.

All these changes have the pronounced proatherogenic character and may point the lipid metabolism shift in animal's organism towards the lipids accumulation and adipose tissue redistribution in favor of the visceral one. The latter fact is confirmed by free fatty acids (FFA) accumulation in blood, at that the main FFA source in metabolic syndrome is lipolysis activation in adipose tissue. The use of HCA significantly reduces all the studied parameters, except FFA and total cholesterol (TC), their content remains increased. Such changes are the indicators of: 1st, the fatty acids source in the serum is not their synthesis *de novo* that should be slightly suppressed by HCA action, but the active lipolysis, and 2nd, more complex cholesterol metabolism regulation exists along with its synthesis from components of non-carbohydrate nature.

The assumption about predominant role of lipolysis activation in FFA release into blood under high-caloric diet condition is supported by the data of lysosomal acid lipase activity in the liver of experimental animals (Table 2). This activity increased

Table 1. Some parameters of lipid metabolism in Syrian hamsters males serum with insulin resistance syndrome (IR group) and under hydroxycitric acid administration (IR+ST group) ($M \pm m$, $n = 8$)

Parameters	Group of animals		
	Intact animals	IR	IR+ST
Total lipids, mg/ml	6.25 ± 0.39	7.29 ± 0.21*	5.78 ± 0.22**
Free fatty acids, mmol/l	1.64 ± 0.16	2.29 ± 0.25*	2.08 ± 0.15*
Triacylglycerols, mg/ml	1.57 ± 0.22	2.00 ± 0.13*	1.36 ± 0.11**
Total cholesterol, mmol/l	2.84 ± 0.15	3.71 ± 0.18*	3.56 ± 0.19*
Total lipoproteins, mg/ml	6.72 ± 0.33	7.77 ± 0.18*	6.89 ± 0.37**
ApoB- lipoproteins, mg/ml	5.66 ± 0.34	6.68 ± 0.21*	5.32 ± 0.28**
High-density lipoproteins, mg/ml	1.01 ± 0.02	0.85 ± 0.08*	1.15 ± 0.07**

Here and in Table 2: * the differences are substantial as related to intact animals ($P \leq 0.05$); ** the differences are substantial as related to IR group ($P \leq 0.05$).

2.35-fold compared to the intact animals and remained high when HCA was used.

As for lipogenesis inhibition that occurs because of decreased carbohydrate-derived substrates flux due to HCA usage, it correlates with a decrease of G-6-PDG activity observed under experimental IR condition and amplified under STIFIMOL action (Table 2).

We obtained data on the decrease of ACLY activity under the STIFIMOL usage (Table 2), and they confirm the assumption about HCA effect realization by virtue of this enzyme inhibition. The consequence of such action should be a switching the FFA synthesis (and, to some extent, cholesterol as well) to non-carbohydrate sources of acetyl-CoA, particularly to FFA resynthesis from food lipids.

It is needed to pay attention to the increase of total lipid level in the liver tissue, particularly tri-

acylglycerols (TAG) accumulation which is observed in animals received the energy food and this accumulation is strengthened by the HCA administration (Table 2). Such effect under IR conditions is probably a result of the FFA utilization that come to the liver from blood in the context of active lipolysis and leads to secretion of lipoproteins (LP) enriched by TAG and serves as additional pro-atherogenic factor. Since a secretion of apo-B-containing LP is apparently somewhat reduced at the HCA application (Table 1), TAG accumulation in the liver can lead to lipidosis, and later to hepatic steatosis. Perhaps, more significant LL activation at the HCA administration as compared to model pathology group (Table 2) is an attempt to compensate for TAG accumulation through their degradation and oxidation.

The dangerous consequence of HCA usage is a decrease of cholesterol content in liver that does

Table 2. Some parameters of lipid metabolism in Syrian hamsters males liver with insulin resistance syndrome (IR group) and under hydroxycitric acid administration (IR+ST group) ($M \pm m$, $n = 8$)

Parameters	Group of animals		
	Intact animals	IR	IR+ST
Total lipids, mg/g	112.62 ± 2.66	143.59 ± 2.65*	177.32 ± 1.56***
Triacylglycerols, mg/g	15.95 ± 1.23	25.01 ± 1.18*	32.12 ± 1.22***
Total cholesterol, mcmol/g	5.15 ± 0.25	7.25 ± 0.18*	4.98 ± 0.25**
Activity of glucose 6-phosphate dehydrogenase, nmol/mg·min	4.44 ± 0.28	3.13 ± 0.28*	2.29 ± 0.25***
Activity of lysosomal acid lipase, nmol/mg·min	0.54 ± 0.03	1.27 ± 0.09*	1.45 ± 0.12*
Activity of ATP-citrate lyase, mcmol NADH/mg·min	0.32 ± 0.02	0.57 ± 0.03*	0.28 ± 0.02**

not correspond to increased cholesterol level in the blood (Tables 1-2). Obviously, these changes are a manifestation of active transport of cholesterol (as esters) to the tissues, and such transport probably performs within the lipoproteins hydrophobic phase due to lack of triglycerides, and can be considered as unfavorable atherogenic factor and evidence of a disturbance of the cholesterol usage for the synthesis of biologically active steroids that may have postponed negative consequences.

Thus, application of hydroxycitric acid (ACLY inhibitor) under the experimental IR is characterized by ambivalent action: on the one hand, it causes the decrease of hyperlipidemia manifestations and therefore can have somewhat adjusted the signs of lipid toxicity that are typical for IR, but on the other hand, this compound does not exhibit the inhibitory effect on lipolysis, it may further distort the distinct parts of lipid metabolism and cause the TAG accumulation in the liver, which in turn leads to hepatic lipidosis. Given the obtained experimental data, it may be concluded that further careful investigation of the HCA effect on the various organism systems and the application terms to avoid possible adverse effects is needed.

ВПЛИВ ГІДРОКСИЛИМОННОЇ КИСЛОТИ НА ПОКАЗНИКИ МЕТАБОЛІЗМУ ЛІПІДІВ У СИРІЙСЬКИХ ХОМ'ЯЧКІВ ЗА ЕКСПЕРИМЕНТАЛЬНОГО СИНДРОМУ ІНСУЛІНОРЕЗИСТЕНТНОСТІ

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Синдром інсулінорезистентності (ІР) є одним із визначальних у зростанні серцево-судинних захворювань та їхніх наслідків. Ключовими компонентами ІР є ожиріння та дисліпідемія. Гідроксилимонна кислота (ГЛК) – інгібітор ключового ензиму ліпогенезу АТР-цитратліази (АЦЛ) – перспективний коректор ожиріння. Метою роботи було дослідити вплив ГЛК на вміст ліпідів та ліпопротеїнів у сироватці крові хом'ячків, а також на вміст ліпідів й активність деяких ензимів ліпідного обміну в печінці

тварин за ІР. Синдром ІР моделювали, утримуючи тварин на високожировій дієті з додаванням фруктози. Ліпіди визначали за допомогою стандартних наборів реактивів, рівень ліпопротеїнів, активність глюкозо-6-фосфат-дегідрогенази та АЦЛ – спектрофотометрично, активність лізосомальної ліпази – флуориметрично. Виявлено розвиток гіперліпідемії та атерогенної дисліпідемії, накопичення ліпідів у печінці, активацію лізосомальної ліпази й АЦЛ і зниження активності глюкозо-6-фосфат-дегідрогенази за ІР. Застосування ГЛК зменшувало прояви гіперліпідемії, але підсилювало накопичення ліпідів у печінці.

Ключові слова: АТР-цитратліаза, гідроксилимонна кислота, інсулінорезистентність, дисліпідемія, печінковий ліпідоз.

ВЛИЯНИЕ ГИДРОКСИЛИМОННОЙ КИСЛОТЫ НА ПОКАЗАТЕЛИ МЕТАБОЛИЗМА ЛИПИДОВ У СИРИЙСКИХ ХОМЯЧКОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ СИНДРОМЕ ИНСУЛИНОРЕЗИСТЕНТНОСТИ

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Синдром инсулинорезистентности (ИР) является одним из определяющих рост сердечно-сосудистых заболеваний и их последствий. Ключевыми компонентами ИР являются ожирение и дислипидемия. Гидроксилимонная кислота (ГЛК) – ингибитор ключевого фермента липогенеза АТР-цитратлиаза (АЦЛ) – перспективный корректор ожирения. Целью работы было исследовать влияние ГЛК на содержание липидов и липопротеинов в сыворотке крови хомячков, а также на содержание липидов и активность некоторых ферментов липидного обмена в печени животных с ИР. Синдром ИР моделировали, содержа животных на высокожировой диете с добавлением фруктозы. Липиды определяли с помощью стандартных наборов реактивов, уровень липопротеинов, активность глюкозо-6-фосфат-дегидрогеназы и АЦЛ – спектрофотометрически, активность лизосомальной ли-

пазы – флуориметрически. Выявлены развитие гиперлипидемии и атерогенной дислипидемии, накопление липидов в печени, активация лизосомальной липазы и АЦЛ и снижение активности глюкозо-6-фосфат-дегидрогеназы при ИР. Применение ГЛК уменьшало проявления гиперлипидемии, но усиливало накопление липидов в печени.

Ключевые слова: АТФ-цитратлиаза, гидроксимионная кислота, инсулинорезистентность, дислипидемия, печеночный липидоз.

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Received 21.03.2016