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EFFECT OF THE INSULIN ON THE APOLIPOPROTEIN A-I GENE EXPRESSION IN HUMAN MACROPHAGES

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The aim of the article — to study the effect of insulin on apolipoprotein A-I gene expression level in human macrophages and to reveal the main signal cascades which take part in the insulin-mediated regulation of apolipoprotein A-I gene.

Materials and methods. The experiments were carried out on the macrophages differentiated from acute monocytic leukemia cell line THP-1 and on the macrophages differentiated from the monocytes isolated from peripheral human blood. The analysis of *apoA-I gene* expression was performed by RealTime RT-PCR (on the mRNA level) and by flow cytofluorometry. To study the signalling cascades which take part in the insulin-mediated regulation of *apoA-I* gene the inhibitory analysis was used.

Results. Insulin induces the human *apoA-I* gene transcription in macrophages, but decreases the level of the ApoA-I protein which binds to outer cytoplasmic membrane of macrophages. The insulin-mediated transcription of *apoA-I* gene depends on PI3K-AKT signal cascade and transcription factors NF-κB and LXRs.

Conclusions. Taking into account our previous data it is plausible to conclude that the elevation of ApoA-I mRNA in human macrophages after insulin treatment leads to an increase of the amplitude of macrophages anti-inflammatory response, which consists in a sharp rise in the level of surface ApoA-I in macrophages under the some proinflammatory stimuli (TNFα, LPS).

Keywords: apolipoprotein A-I; insulin; human macrophages; NF-κB; LXR; AKT; PI3K.

ДЕЙСТВИЕ ИНСУЛИНА НА ЭКСПРЕССИЮ ГЕНА АПОЛИПОПРОТЕИНА A-I В МАКРОФАГАХ ЧЕЛОВЕКА

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Цель исследования — изучить влияние инсулина на уровень экспрессии гена аполипопротеина A-I (*apoA-I*) в макрофагах человека и выявить основные сигнальные каскады, ответственные за инсулин-опосредованную регуляцию.

Материалы и методы. Работа выполнена на макрофагах, дифференцированных из линии острой моноцитарной лейкемии THP-1, и на макрофагах, дифференцированных из моноцитов периферической крови человека. Анализ экспрессии гена *apoA-I* на уровне РНК проведен методом полимеразной цепной реакции в реальном времени, на уровне белка — методом проточной цитофлуорометрии. Для выявления сигнальных каскадов, ответственных за инсулин-опосредованную регуляцию гена *apoA-I* в макрофагах, использован ингибиторный анализ.

Результаты. Инсулин индуцирует транскрипцию гена *apoA-I* в макрофагах человека, но приводит к снижению уровня аполипопротеина A-I, связанного с наружной поверхностью мембраны. За индукцию транскрипции *apoA-I* в ответ на стимуляцию макрофагов инсулином отвечает сигнальный каскад PI3K – AKT и факторы транскрипции NF-κB и LXRs.

List of abbreviations

HDL — high-density lipoproteins; LDL — low-density lipoproteins; LPS — lipopolysaccharides; PCR — polymerase chain reaction; real-time PCR — real-time polymerase chain reaction; ABCA1 — ATP-binding cassette transporter of the subfamily A1; apoA-I — apolipoprotein AI; BSA — bovine serum albumin; FCS — fetal calf serum; PBS — phosphate-buffered saline; PI3K — phosphatidylinositol-3 kinase; TNFα — tumor necrosis factor-alpha.

Заключение. С учетом полученных ранее данных можно предположить, что стимуляция макрофагов инсулином повышает уровень мРНК аполипопротеина A-I и таким образом увеличивает амплитуду антивоспалительного ответа, заключающегося в резком возрастании уровня поверхностного аполипопротеина A-I в макрофагах при действии на них провоспалительных стимулов (фактора некроза опухоли альфа, липополисахаридов).

Ключевые слова: аполипопротеин A-I; инсулин; макрофаги человека; NF-κB; LXR; AKT; PI3K.

Introduction

The circulation of lipids in the blood plasma of mammals is the most critical process that ensures the vital activity of the body. Disorders of lipid metabolism in humans (dyslipoproteinemia) lead to the development of several serious diseases, including atherosclerosis. A decrease in the level of high-density lipoprotein (HDL) that are involved in the reverse transport of cholesterol from peripheral tissues to the liver in the blood plasma is one of the risk factors for atherosclerosis [1]. In addition to its involvement in the reverse transport of cholesterol, apolipoprotein A-I (ApoA-I) functions as a cofactor of lecithin cholesterol acyltransferase [2]. It also exhibits antioxidant properties [3] and can reduce inflammatory reactions. In particular, the ability of ApoA-I to block the activation of macrophages by T-lymphocytes and limit the production of tumor necrosis factor-alpha (TNFα) and interleukin 1 beta has been demonstrated [4, 5]. ApoA-I also inhibits another pro-inflammatory factor, C-reactive protein [6]. On the other hand, ApoA-I serves as a negative indicator of the acute phase of the inflammatory response, along with the development of inflammation, and a sharp decrease in the expression of the *apoA-I* gene in the liver and small intestine [7–9]. In addition, the ApoA-I protein circulating in the blood plasma is displaced from HDL by serum amyloid and destroyed by serum proteases [4]. HDL is the primary transporter of cholesterol to the sites of steroid hormone synthesis in steroidogenic tissues and organs of mammals (including humans), and ApoA-I is the protein that controls this process [10]. The main protein component of HDL in mammals is ApoA-I (more than 70% of the total HDL protein in humans) [11]. In humans, ApoA-I is primarily synthesized in the liver and small intestine [12]. In previous studies, we showed that the expression of the human *apoA-I* gene occurs at the level of mRNA and protein in monocytic macrophage cells [13–15]. The differentiation of monocytes into macrophages leads to two separate populations of monocytes with different ApoA-I levels: ApoA-I-poor and ApoA-I-rich macrophages [13, 14]. The level of endogenous ApoA-I correlates with the level of the ATP-binding cassette transporter of the subfamily A1 (ABCA1). Moreover, endogenous ApoA-I can stabilize ABCA1 [13]. Unlike hepatocytes, in which ApoA-I secretion induces the formation of HDL in blood plasma, ApoA-I secreted by macrophages is associated with the outer surface of the cytoplasmic membrane (surface ApoA-I of macrophages). This is mainly due to the interaction of ApoA-I with the ABCA1 cassette transporter [13]. The suppression of ApoA-I synthesis in human macrophages enhances their pro-inflammatory activity, in particular, increases the primary production of the pro-inflammatory cytokine TNFα, the toll-like receptor 4 (TLR4) (lipopolysaccharide (LPS) receptor, can also interact with modified low-density lipoprotein (LDL)) and also increases the level of the inflammatory response to LPS [13]. In turn, the level of *apoA-I* expression in monocytes and macrophages increases (both at the mRNA and protein levels) under the stimulation of macrophages by TNFα [14] or under hypoxia [15]. The data obtained suggest that endogenous ApoA-I is an essential modulator of the functional state of macrophages.

The regulation of *apoA-I* gene expression in human macrophages is mirror-like regading the regulation of apoA-I gene in the main sites of its synthesis — hepatocytes and enterocytes of the small intestine. Stimulation of cells with the pro-inflammatory cytokine TNFα suppresses the activity of the *apoA-I* gene in HepG2 cells (hepatocyte model) and Caco-2 cells (enterocyte model) [7–9]. However, it activates the expression of the *apoA-I* gene in human monocytes and macrophages [13, 14]. Moreover, the transcription factors LXRα and LXRβ, which are ligand-dependent repressors of the *apoA-I* gene in hepatocytes [8, 16], activate the *apoA-I* gene in monocytes and macrophages [14]. Such mirror-like regulation may be caused by

protein-protein interactions between the nuclear receptors LXRs and transcription factors FOXO1 and FOXA2 (specific for hepatocytes and enterocytes, absent in monocytes and macrophages) [17, 18].

Insulin is the most significant component involved in maintaining the homeostasis of carbohydrates and lipids in the body [19]. Disorders of insulin signaling lead to the development of type 2 diabetes mellitus and make a significant contribution to the development of metabolic syndrome [20–22]. The role of insulin in the inflammatory process is quite controversial. On the one hand, insulin is known to block several pro-inflammatory signaling pathways and inhibits the expression of pro-inflammatory genes [23–25]. On the other hand, insulin can exhibit pro-inflammatory properties. For example, insulin enhances the secretion of TNFα and IL-6 in macrophages that differentiated from THP-1 cells (acute human monocytic leukemia) induced by LPS [26]. In addition, insulin enhances macrophage uptake of modified LDL by inducing the CD36 expression (one of scavenger receptors) and limits the efflux of cholesterol from macrophages to HDL, by decreasing the level of ABCA1 in macrophages [27]. In hepatocytes, insulin inhibits the synthesis of ApoA-I through transcription factors FOXO1 and LXRs [17]. Nothing is known about the possible regulation of the expression of the *apoA-I* gene by insulin in human macrophages.

This work aimed to study the effect of insulin on the expression of the *apoA-I* gene in human macrophages and identify the main signaling cascades involved in this regulation.

Materials and methods

Laboratory reagents were obtained from international (Sigma-Aldrich, ThermoFisher, R&D Systems) and Russian manufacturers. LXRs agonist (TO901317), signaling pathway inhibitors (phosphatidylinositol-3 kinase inhibitor (PI3K) LY2940023, NF-κB inhibitor (QNZ) and insulin were received from Sigma-Aldrich. Monoclonal murine antibodies to human ApoA-I were purchased from Bio-Rad (cat. No. 0650-0050). Donkey antibodies against murine immunoglobulins labeled with DyLight-649 (Abcam, cat. No. Ab6669-1) were used as secondary antibodies.

Cell cultures and macrophages differentiated from human peripheral blood monocytes

In this work, we used the THP-1 cell line obtained from the cell culture bank of the Institute of Cytology Rus. Acad. Sci. (St. Petersburg, Russia). THP-1 cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS) (HyClone) in an atmosphere of 5% CO_2 at 37 °C. Differentiation of THP-1 monocytes into macrophages was induced by incubating monocytes with phorbol 12-myristate 13-acetate (PMA) (50 ng/ml) according to the protocol described previously [28].

Preserved donor blood, unsuitable for transfusion, was purchased at a blood transfusion station (Moskovsky avenue, 104), transported to the building of the biochemistry department of the Institute of Experimental Medicine in a cooled state. Donors signed informed voluntary consent. Primary macrophages were obtained from mononuclear cells that were isolated from human blood by centrifugation in a density gradient of ficoll, as described in [29]. For this purpose, 15 ml of ficoll was poured into 50 ml tubes, 35 ml of blood was layered on top, and centrifuged for 30 minutes (2,000 rpm (1500 g), 18 °C). Then, a layer containing mononuclear cells was selected and washed twice with Hank's solution. The residual matter was resuspended in RPMI-1640 culture medium containing 10% FCS, poured into the wells of the plates, and incubated at 37 °C in an atmosphere of 5% CO_2 for two hours to adhere the monocytes. After this, the monocytes were washed from cells (lymphocytes) not attached to the substrate in Hank's solution, fresh RPMI-1640 culture medium containing 10% FCS was added to the washed monocytes, and the monocytes were differentiated into macrophages at 37 °C in an atmosphere of 5% CO_2 for five days.

RNA isolation, reverse transcription reactions, and real-time polymerase chain reaction

Isolation of RNA, conducting reactions of reverse transcription, and real-time polymerase chain reaction (PCR) (real-time PCR) have been described previously [8]. The total RNA was isolated from cultured cells using RNA STAT-60 reagent (Tel-Test) following the manufacturer's instructions. Genomic DNA residues were removed by treatment with RNAse-free DNAse I (Roche Applied Science) for 30 min at 37 °C.

The reaction was stopped by adding sodium salt of ethylenediaminetetraacetic acid (EDTA) to a final concentration of 2 mM, followed by inactivation of DNAse I by heating at 70 °C for 15 min. RNA concentration and purity were determined using a Synergy 2 plate spectrophotometer (BioTek). The ratio of optical densities at wavelengths of 260 and 280 nm was more than 2, and the ratio of optical densities at 260 and 230 nm was more than 1.7. The absence of RNA degradation was checked by 1% agarose gel electrophoresis to preserve the integrity of ribosomal RNA. The reverse transcription reaction was conducted by taking an equal amount of RNA at all points. The reverse transcription reaction (with 1 μg of total RNA) was performed using oligo-dT primers and specific 3′-primers for the *apoA-I* gene and Promega reagents.

The real-time polymerase chain reaction was performed using Taqman technology or SYBRGreen intercalation using a Bio-Rad CFX-96 amplifier. The components of the reaction mixture were purchased from Synthol (Moscow, Russia). Primers and fluorescent samples for *apoA-I* and reference genes encoding 60S acidic ribosomal protein P0 (RPLP0), cyclophilin A, and β-actin were described previously [8, 13, 17, 30]. The relative level of mRNA of the *apoA-I* gene was evaluated together with the detection of reference genes in the same reaction (multiplex PCR). The results were normalized by the geometric mean of the three reference genes, as described previously [31]. The number of PCR cycles for each gene at which the fluorescence level exceeded 10 times the standard deviation of fluctuations in the background fluorescence was determined using the CFX-96 RealTime PCR System and automated software (Bio-Rad). The relative values of the *apoA-I* mRNA level (in percent relative to the control sample) were calculated by the formula

$$2^{(\mathrm{Ct\,(control)} - \mathrm{Ct\,(experience)})} \cdot 100.$$

Flow cytofluorometry

Macrophages were fixed in 4% formaldehyde for 10 min at 22 °C, washed three times in sodium phosphate buffer (PBS) with 0.1 M glycine, and incubated for 40 min at 22 °C with blocking buffer (PBS, 1% bovine serum albumin (BSA), 3% FCS, non-specific human immunoglobulins G (1 μg/ml), and 0.02% Tween-20). THP-1 cells were treated with murine monoclonal antibodies against human ApoA-I (Bio-Rad, Cat. No. 0650-0050), 1/250 dilution (PBS, 1% BSA and 0.02% Tween-20) for 2 hours at 22 °C. They were washed three times in PBS and incubated with secondary donkey polyclonal antibodies against murine immunoglobulin G proteins conjugated with DyLight-649 stain (Abcam, cat. number ab6669-1), 1/1000 dilution (PBS, 1% BSA, and 0.02% Tween-20), for an hour at 22 °C. Then, the cells were washed three times in PBS and fixed in PBS with 1% formaldehyde for flow cytofluorometry. THP-1 cells were treated with secondary antibodies but were not incubated with anti-ApoA-I antibodies. They were used as a control for the specificity of immunostaining (isotype control). Flow cytofluorometry and cell sorting were performed using the Epics Altra flow cytofluorimeter (Beckman Coulter, USA) and the FCSalyzer program (https:// sourceforge.net/projects/fcsalyzer/).

Statistical analysis

The results were presented as the arithmetic mean ± standard error of the mean. Statistical analysis of the differences between the compared groups was performed with the Student's test (non-paired *t*-test) and Dunnett's test. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using Statistica 5.0 software (StatSoft, Inc., USA).

Results and discussion

In previous studies, we demonstrated a dose-dependent effect of insulin on the activity of the *apoA-I* gene in hepatocytes [17]. At a concentration of 0.7 nM, *apoA-I* expression was enhanced after 24 hours, and the effect disappeared after 48 hours. The addition of insulin to hepatocytes at a concentration of 100 nM did not affect the level of *apoA-I* mRNA after 24 hours of incubation. However, it led to the inhibition of gene expression after 48 hours of incubation. The potential effect of insulin on the synthesis of *apoA-I* in human macrophages was evaluated by real-time PCR using two insulin concentrations, 0.7 and 100 nM. The first concentration corresponds to the physiological level of insulin in the blood after a meal. Insulin at a concentration of 100 nM is used in experiments *in vitro* as standard. TNFα (20 ng/ml), which stimulates the

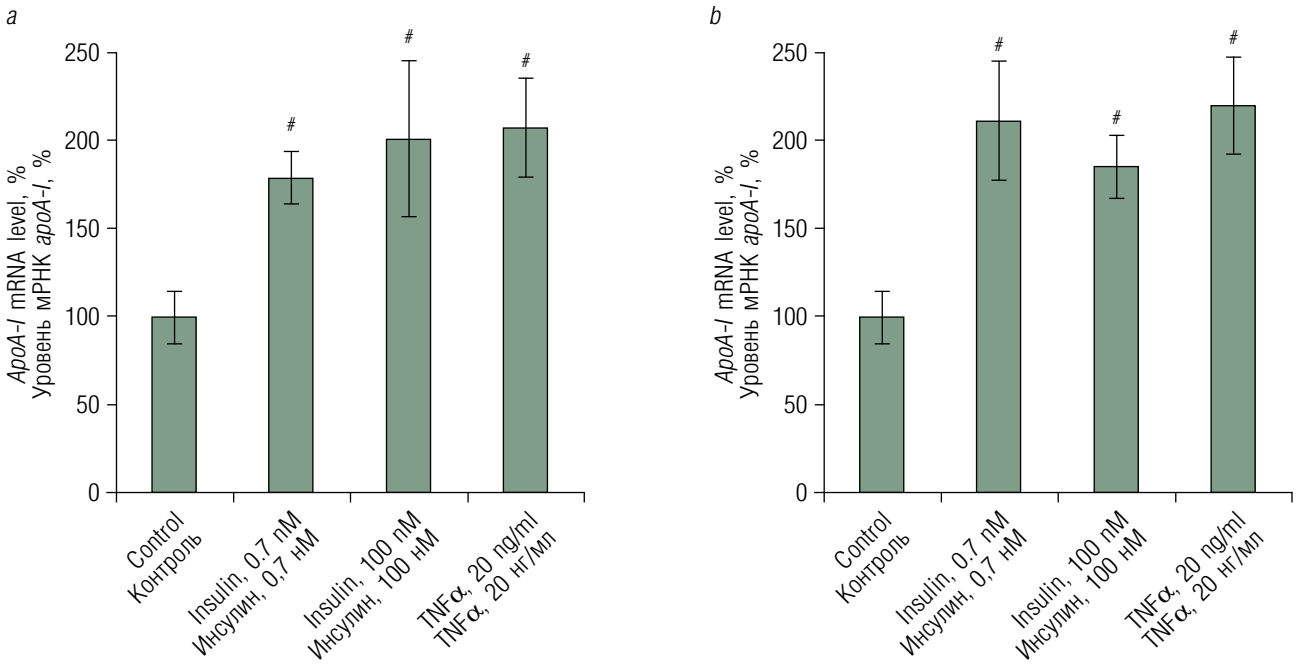

Fig. 1. The elevation of *ApoA-I* mRNA level in human macrophages after insulin treatment. The macrophages differentiated for 5 days from human monocytes isolated from peripheral blood (*a*), THP-1 macrophages (*b*). The diagrams show the relative *apoA-I* gene expression level (100% in the unstimulated macrophages). The diagrams show the mean values ± the standard error of mean. $^{\#}p < 0.01$ (*t*-test)

Рис. 1. Повышение уровня матричной РНК *apoA-I* в макрофагах под действием инсулина. Макрофаги, дифференцированные из моноцитов периферической крови человека в течение 5 сут (*a*), макрофаги THP-1 (*b*). Указан относительный уровень экспрессии мРНК *apoA-I,* где за 100 % принят уровень мРНК в нестимулированных макрофагах. На диаграммах представлены средние значения ± ошибка среднего. $^{\#}p < 0{,}01$ (*t*-критерий)

expression of the *apoA-I* gene in human macrophages, was used as a positive control [14]. At all points, the incubation time of cells (macrophages differentiated from human peripheral blood monocytes for five days, or macrophages THP-1) with insulin was 24 hours. The average results of the four experiments are presented in Fig. 1.

Both insulin concentrations stimulated *apoA-I* gene expression significantly in macrophages differentiated from both THP-1 cells and human peripheral blood monocytes. The level of stimulation was comparable to the level of stimulation with TNFα. ApoA-I protein synthesis is controlled not only through transcription but also at the post-transcriptional level. In addition, this gene is characterized by multidirectional regulation at the level of mRNA and protein. For example, stimulation of human HepG2 hepatoma cells with gramoxone (an oxidative stress inducer) led to the simultaneous induction of transcription of the *apoA-I* gene. It accelerated the degradation of *apoA-I* mRNA [32]. In our studies, the incubation of HepG2 cells with insulin (100 nM) for 24 hours did not cause noticeable changes in the level of *apoA-I* mRNA but increased the amount of intracellular ApoA-1 protein significantly [17]. Unlike hepatocytes and enterocytes, where the vast majority of ApoA-I is secreted with nascent HDL, normal full-fledged secretion does not occur in macrophages. Instead, ApoA-I remains in a membrane-bound state and forms a complex with ABCA1 and/or with cytoplasmic membrane rafts [13]. Moreover, preliminary results suggest that the membrane-bound form of ApoA-I is of functional importance in macrophages. This is because strong correlations exist between the amount of membrane-bound ApoA-I and the functional activity of macrophages (inflammatory activity, the capture of modified LDL, and migratory activity) (Nekrasova et al., unpublished data). The effect of insulin on the ApoA-I protein surface level was checked by flow cytofluorometry. Macrophages differentiated from human peripheral blood monocytes for five days were incubated with insulin at a concentration of 100 nM for 24 hours and then stained with antibodies to ApoA-I (see Materials and methods). The results are presented in Fig. 2. The treatment of primary

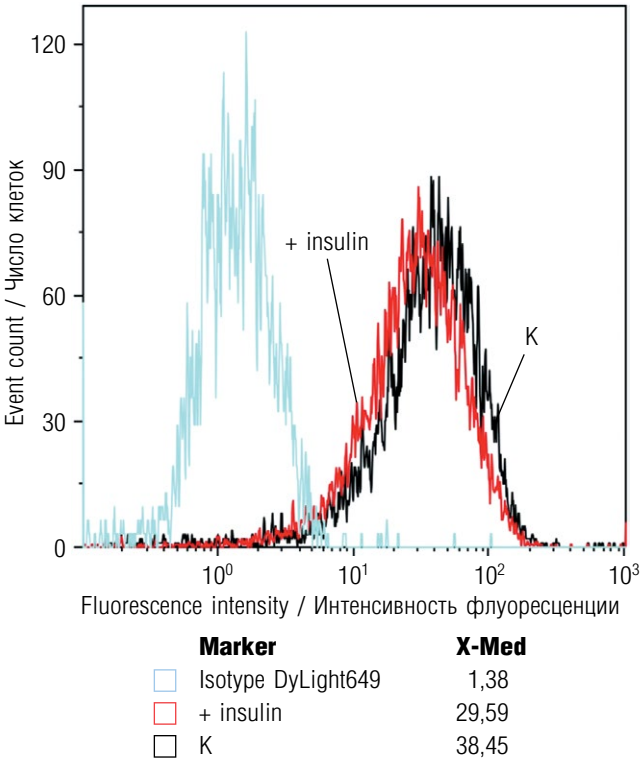

Fig. 2. The ApoA-I surface level on human macrophages decreases by insulin treatment. K — control macrophages without treatment with insulin; + insulin — macrophages, treated by insulin; X-Med — median fluorescence intensity

Рис. 2. Уменьшение уровня поверхностного белка ApoA-I в макрофагах под действием инсулина. K — контрольные, не обработанные инсулином макрофаги; + insulin — макрофаги, инкубированные с инсулином; X-Med — медиана интенсивности флуоресценции

macrophages with insulin (100 nM, 24 h) results in a decrease in the level of surface ApoA-I, even though under the same experimental conditions, an increase in the mRNA level of this gene has been noted. The results obtained once again confirm the complex and multilevel nature of the regulation of *apoA-I* gene expression, which is not limited to the regulation of transcription initiation. For a detailed analysis of these regulatory mechanisms, additional studies are required.

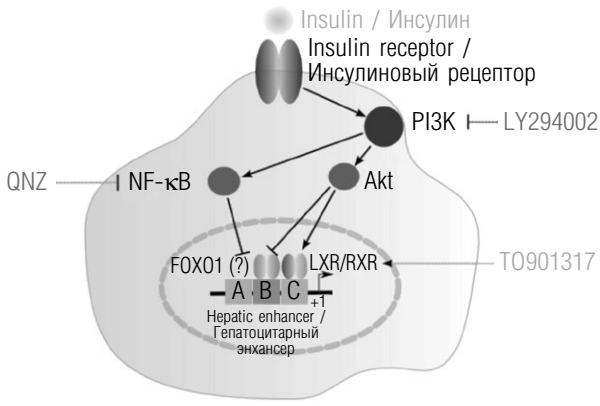

Fig. 3. The scheme of signal cascades initiated by insulin: QNZ — the inhibitor of NF-κB; LY294002 — the inhibitor of PI3K; TO901317 — the agonist of nuclear receptors LXRα and LXRβ

Рис. 3. Схема сигнальных каскадов, инициируемых инсулином: QNZ — ингибитор NF-κB; LY294002 — ингибитор PI3K; TO901317 — агонист LXRs

Insulin, while interacting with its membrane tyrosine kinase receptor, initiates a series of signaling cascades (Fig. 3). We used inhibitory analysis to determine the potential involvement of these cascades in the insulin-dependent activation of the *apoA-I* gene in human macrophages. Macrophages differentiated from human peripheral blood monocytes for five days were treated with an NF-κB QNZ inhibitor at a concentration of 10 nM, an LXR TO901317 agonist at a concentration of 5 μM, and a PI3K LY2940023 inhibitor at a concentration of 10 μM for one hour. Then insulin (100 nM) was added to the cells and incubated for 24 hours. Then, RNA was isolated from the cells, and the level of *apoA-I* expression was measured using real-time PCR. The averaged results of four experiments are presented in Fig. 4.

Blocking any of the tested signaling pathways (transcription factor NF-κB, PI3K, or activation of transcription factors LXRs) cancels the stimulating effect of insulin on the level of *apoA-I* mRNA. Therefore, the induction of *apoA-I* transcription in the presence of insulin depends on the combined action of at least two signaling cascades terminating the transcription factors NF-κB and LXRs. In addition, the potential involvement of the transcription factor FOXO1, which is capable of being complexed with LXRβ, cannot be ruled out in this process.

Moreover, insulin-induced removal of such a complex from the *apoA-I* gene promoter in hepatocytes explains the repressive effect of insulin (100 nM) on the activity of this gene [17]. The role of FOXO1 in insulin-mediated stimulation of *apoA-I* gene expression in human macrophages remains to be studied. Based on the results of our previous studies and the published data, insulin in hepatocytes can regulate the expression of *apoA-I* through phosphatidylinositol-3 kinase, protein kinases B and C, and transcription factor Sp1 [17, 33]. At the same time, protein kinase B in macrophages phosphorylates IκB kinase and activates the transcription factor NF-κB. Processing the macrophages with an NF-κB inhibitor cancels not only the effect of

insulin but also those of TNFα [14]. Moreover, in hepatocytes, the transcription factor NF-κB plays a crucial role in suppressing the activity of the *apoA-I* gene under the influence of LPS [34] or TNFα [8]. Interestingly, the *apoA-I* gene promoter does not contain binding sites for the transcription factor NF-κB [34]. The primary mechanism of NF-κB action is transrepression by nuclear receptors involved in the regulation of *apoA-I* gene activity. The transcription factor NF-κB can form complexes with nuclear receptors such as HNF4α and PPARα, and the formation of such complexes results in mutual inactivation of both transcription factors [34, 35]. The mirror-like nature of the regulation of the *apoA-I* gene in hepatocytes and macrophages determines the opposite effect of NF-κB in macrophages compared with that in hepatocytes. Thus, NF-κB in hepatocytes is involved in the transmission of repressive signals to the *apoA-I* promoter (from LPS [34], TNFα [8, 9]). However, in macrophages, the same effects (LPS, TNFα) lead to the activation of *apoA-I* expression. In this case, the nuclear receptor PPARα used by NF-κB when transmitting signals from LPS and TNFα acts as an *apoA-I* activator in hepatocytes [8, 9, 34]. In contrast, in macrophages, it acts as an *apoA-I* repressor [14].

The question of the functional role of insulin in the regulation of the *apoA-I* gene remains uncertain. Can induction of *apoA-I* gene transcription in macrophages be considered insignificant if the level of surface ApoA-I does not increase in parallel but decreases? We answered this question in the negative. Previous studies analyzing the level of *apoA-I* mRNA in ApoA-I-poor and ApoA-I-rich macrophages established that the amount of *apoA-I* mRNA is greater in ApoA-I-poor macrophages [13]. Moreover, ApoA-I-poor macrophages were the cells that retained the ability to synthesize ApoA-I in response to inflammatory stimuli such as TNFα. In contrast, ApoA-I-rich macrophages lost this ability [13]. Therefore, the action of insulin enhances the future anti-inflammatory response of macrophages, which consists of a sharp increase in the level of surface ApoA-I in response to pro-inflammatory stimuli. Further research is expected to clarify this issue.

Thus, in this work, the regulation of the *apoA-I* gene by insulin in human macrophages is presented for the first time. It was found that for the induction of transcription of the *apoA-I* gene under the action of insulin, the PI3K–AKT signaling cascade, and the transcription factors NF-κB and LXRs play a crucial role. An increase in the level of *apoA-I* mRNA in the presence of insulin is accompanied by a decrease in the level of surface ApoA-I.

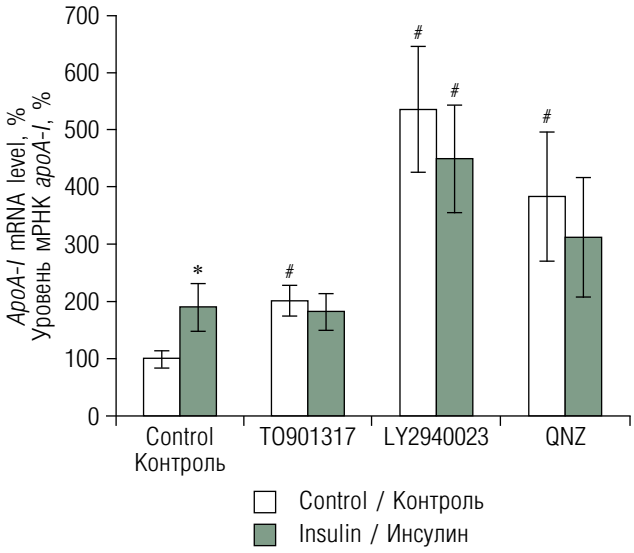

Fig. 4. The influence of insulin on *ApoA-I* mRNA level: the role of nuclear receptors LXRs, PI3K and transcription factor NF-κB. The diagram shows the relative *apoA-I* gene expression level (100% in the unstimulated macrophages). The diagram shows the mean values ± the standard error of mean. $*p < 0.05$ (*t*-test); $\#p < 0.01$ (Dunnet's test). White columns correspond to the unstimulated cells; black columns correspond to the cells treated by insulin

Рис. 4. Влияние инсулина на уровень мРНК *apoA-I*: роль ядерных рецепторов LXRs, PI3K и фактора транскрипции NF-κB. Указан относительный уровень экспрессии мРНК *apoA-I*, где за 100 % принят уровень мРНК в нестимулированных макрофагах. На диаграмме представлены средние значения уровня мРНК *apoA-I* ± ошибка среднего. Критерий Стьюдента — $*p < 0{,}05$. Критерий Даннета — $\#p < 0{,}01$. Столбцы белого цвета отображают интенсивность транскрипции в контрольных клетках, столбцы черного цвета — интенсивность транскрипции в клетках, обработанных инсулином

Conclusion

1. Insulin induces transcription of the *ApoA-I* gene in human macrophages.
2. Despite the increase in the amount of *apoA-I* mRNA in insulin-stimulated macrophages, the level of surface ApoA-I in these cells is reduced.
3. Activation of transcription of the *apoA-I* gene by insulin depends on the PI3K–AKT signaling cascade and transcription factors NF-κB and LXRs.

Additional information

Ethical considerations. All international, national, and/or institutional principles for work with donated blood have been observed.

Conflict of interests. The authors declare no conflict of interest.

References

1. Hopkins PN. Molecular biology of atherosclerosis. *Physiol Rev*. 2013;93(3):1317-1542. https://doi.org/10.1152/physrev.00004.2012.
2. Никифорова А.А., Хейфиц Г.М., Алкснис Е.Г., и др. Акцепция холестерина из мембран эритроцитов подфракцией ЛПВП2b и роль лецитин-холестерин-ацилтрансферазы в этом процессе // Биохимия. – 1988. – Т. 53. – № 7-12. – С. 1334–1338. [Nikiforova AA, Kheifits GM, Alksnis EG, Parfenova NS, Klimov AN. Aktseptsiya kholesterina iz membran eritrotsitov podfraktsiey LPVP2b i rol' letsitin-kholesterin-atsiltransferazy v etom protsesse. *Biokhimiia*. 1988,53(7-12):1334-1338. (In Russ.)]
3. Shah PK, Kaul S, Nilsson J, Cercek B. Exploiting the vascular protective effects of high-density lipoprotein and its apolipoproteins: an idea whose time for testing is coming, part II. *Circulation*. 2001;104(20):2498-2502. https://doi.org/10.1161/hc4501.098468.
4. Hyka N, Dayer JM, Modoux C, et al. Apolipoprotein A-I inhibits the production of interleukin-1beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood*. 2001;97(8):2381-2389. https://doi.org/10.1182/blood.v97.8.2381.
5. Burger D, Dayer J-M. High-density lipoprotein-associated apolipoprotein A-I: the missing link between infection and chronic inflammation? *Autoimmun Rev*. 2002;1(1-2):111-117. https://doi.org/10.1016/s1568-9972(01)00018-0.
6. Wadham C, Albanese N, Roberts J, et al. High-density lipoproteins neutralize C-reactive protein proinflammatory activity. *Circulation*. 2004;109(17):2116-2122. https://doi.org/10.1161/01.CIR.0000127419.45975.26.
7. Haas MJ, Horani M, Mreyoud A, et al. Suppression of apolipoprotein AI gene expression in HepG2 cells by TNF alpha and IL-1beta. *Biochim Biophys Acta*. 2003;1623(2-3):120-128. https://doi.org/10.1016/j.bbagen.2003.08.004.
8. Mogilenko DA, Dizhe EB, Shavva VS, et al. Role of the nuclear receptors HNF4 alpha, PPAR alpha, and LXRs in the TNF alpha-mediated inhibition of human apolipoprotein A-I gene expression in HepG2 cells. *Biochemistry*. 2009;48(50):11950-11960. https://doi.org/10.1021/bi9015742.
9. Orlov SV, Mogilenko DA, Shavva VS, et al. Effect of TNFalpha on activities of different promoters of human apolipoprotein A-I gene. *Biochem Biophys Res Commun*. 2010;398(2):224-230. https://doi.org/10.1016/j.bbrc.2010.06.064.
10. Connelly MA, Williams DL. SR-BI and HDL cholesteryl ester metabolism. *Endocr Res*. 2004;30(4):697-703. https://doi.org/10.1081/erc-200043979.
11. Lewis GF, Rader DJ. New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circ Res*. 2005;96(12):1221-1232. https://doi.org/10.1161/01.RES.0000170946.56981.5c.
12. Higuchi K, Law SW, Hoeg JM, et al. Tissue-specific expression of apolipoprotein A-I (apoA-I) is regulated by the 5'-flanking region of the human apoA-I gene. *J Biol Chem*. 1988;263(34):18530-18536.
13. Mogilenko DA, Orlov SV, Trulioff AS, et al. Endogenous apolipoprotein A-I stabilizes ATP-binding cassette transporter A1 and modulates Toll-like receptor 4 signaling in human macrophages. *FASEB J*. 2012;26(5):2019-2030. https://doi.org/10.1096/fj.11-193946.
14. Shavva VS, Mogilenko DA, Nekrasova EV, et al. Tumor necrosis factor alpha stimulates endogenous apolipoprotein A-I expression and secretion by human monocytes and macrophages: role of MAP-kinases, NF-kappaB, and nuclear receptors PPA-Ralpha and LXRs. *Mol Cell Biochem*. 2018;448(1-2):211-223. https://doi.org/10.1007/s11010-018-3327-7.
15. Богомолова А.М., Шавва В.С., Никитин А.А., и др. Гипоксия как фактор регуляции экспрессии генов *apoA-1*, *ABCA1* и компонента комплемента *C3* в макрофагах человека // Биохимия. – 2019. – Т. 84. – № 5. – С. 692–703. [Bogomolova AM, Shavva VS, Nikitin AA, et al. Hypoxia as a factor involved in the regulation of the *apoA-1*, *ABCA1*, and complement *C3* gene expression in human macrophages. *Biokhimiia*. 2019;84(5):692-703. (In Russ.)]. https://doi.org/10.1134/S0320972519050075.
16. Huuskonen J, Vishnu M, Chau P, et al. Liver X receptor inhibits the synthesis and secretion of apolipoprotein A1 by human liver-derived cells. *Biochemistry*. 2006;45(50):15068-15074. https://doi.org/10.1021/bi061378y.
17. Shavva VS, Bogomolova AM, Nikitin AA, et al. Insulin-mediated downregulation of apolipoprotein A-I gene in human hepatoma cell line HepG2: the role of interaction between FOXO1 and LXRbeta transcription factors. *J Cell Biochem*. 2017;118(2):382-396. https://doi.org/10.1002/jcb.25651.
18. Shavva VS, Bogomolova AM, Nikitin AA, et al. FOXO1 and LXRalpha downregulate the apolipoprotein A-I gene expression during hydrogen peroxide-induced oxidative stress in HepG2 cells. *Cell Stress Chaperones*. 2017;22(1):123-134. https://doi.org/10.1007/s12192-016-0749-6.
19. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol*. 2011;11(2):98-107. https://doi.org/10.1038/nri2925.
20. Bansilal S, Farkouh ME, Fuster V. Role of insulin resistance and hyperglycemia in the development of athero-

sclerosis. *Am J Cardiol*. 2007;99(4A):6B-14B. https://doi.org/10.1016/j.amjcard.2006.11.002.
21. Fuentes L, Roszer T, Ricote M. Inflammatory mediators and insulin resistance in obesity: role of nuclear receptor signaling in macrophages. *Mediators Inflamm*. 2010;2010:219583. https://doi.org/10.1155/2010/219583.
22. Olefsky JM, Glass CK. Macrophages, inflammation, and insulin resistance. *Annu Rev Physiol*. 2010;72:219-246. https://doi.org/10.1146/annurev-physiol-021909-135846.
23. Fan W, Morinaga H, Kim JJ, et al. FoxO1 regulates Tlr4 inflammatory pathway signalling in macrophages. *EMBO J*. 2010;29(24):4223-4236. https://doi.org/10.1038/emboj.2010.268.
24. Miao H, Zhang Y, Lu Z, et al. FOXO1 involvement in insulin resistance-related pro-inflammatory cytokine production in hepatocytes. *Inflamm Res*. 2012;61(4):349-358. https://doi.org/10.1007/s00011-011-0417-3.
25. Su D, Coudriet GM, Hyun Kim D, et al. FoxO1 links insulin resistance to proinflammatory cytokine IL-1beta production in macrophages. *Diabetes*. 2009;58(11):2624-2633. https://doi.org/10.2337/db09-0232.
26. Iida KT, Shimano H, Kawakami Y, et al. Insulin up-regulates tumor necrosis factor-alpha production in macrophages through an extracellular-regulated kinase-dependent pathway. *J Biol Chem*. 2001;276(35):32531-32537. https://doi.org/10.1074/jbc.M009894200.
27. Park YM, S RK, J AM, Silverstein RL. Insulin promotes macrophage foam cell formation: potential implications in diabetes-related atherosclerosis. *Lab Invest*. 2012;92(8):1171-1180. https://doi.org/10.1038/labinvest.2012.74.
28. Tedla N, Glaros EN, Brunk UT, et al. Heterogeneous expression of apolipoprotein-E by human macrophages. *Immunology*. 2004;113(3):338-347. https://doi.org/10.1111/j.1365-2567.2004.01972.x.
29. Bennett S, Breit SN. Variables in the isolation and culture of human monocytes that are of particular relevance to studies of HIV. *J Leukoc Biol*. 1994;56(3):236-240. https://doi.org/10.1002/jlb.56.3.236.
30. Shavva VS, Mogilenko DA, Bogomolova AM, et al. PPARgamma represses apolipoprotein A-I gene but impedes TNFalpha-mediated ApoA-I downregulation in HepG2 Cells. *J Cell Biochem*. 2016;117(9):2010-2022. https://doi.org/10.1002/jcb.25498.
31. Mogilenko DA, Kudriavtsev IV, Shavva VS, et al. Peroxisome proliferator-activated receptor alpha positively regulates complement C3 expression but inhibits tumor necrosis factor alpha-mediated activation of C3 gene in mammalian hepatic-derived cells. *J Biol Chem*. 2013;288(3):1726-1738. https://doi.org/10.1074/jbc.M112.437525.
32. Cuthbert C, Wang Z, Zhang X, Tam SP. Regulation of human apolipoprotein A-I gene expression by gramoxone. *J Biol Chem*. 1997;272(23):14954-14960. https://doi.org/10.1074/jbc.272.23.14954.
33. Haas MJ, Horani MH, Wong NC, Mooradian AD. Induction of the apolipoprotein AI promoter by Sp1 is repressed by saturated fatty acids. *Metabolism*. 2004;53(10):1342-1348. https://doi.org/10.1016/j.metabol.2004.05.011.
34. Morishima A, Ohkubo N, Maeda N, et al. NFkappaB regulates plasma apolipoprotein A-I and high density lipoprotein cholesterol through inhibition of peroxisome proliferator-activated receptor alpha. *J Biol Chem*. 2003;278(40):38188-38193. https://doi.org/10.1074/jbc.M306336200.
35. Nikolaidou-Neokosmidou V, Zannis VI, Kardassis D. Inhibition of hepatocyte nuclear factor 4 transcriptional activity by the nuclear factor kappaB pathway. *Biochem J*. 2006;398(3):439-450. https://doi.org/10.1042/BJ20060169.

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