Oxidative Stress in Diabetes
A model of complex thinking applied in medicine

ANCA PANTEA STOIAN[1], GRIGORINA MITROFAN[2]*, FLORIAN COLCEAG[3], ANDRA IULIA SUCEVEANU[4], RAZVAN HAINAROSIE[5,6], SILVIU PITURU[5], CAMELIA CRISTINA DIACONU[5,7], DANIEL TIMOFTE[9], CORNELIA NITIPIR[5], CATALINA POIANA[5,8], CRISTIAN SERAFINCEANU[1,2]

[1] Carol Davila University of Medicine and Pharmacy, Department of Diabetes and Nutrition, 5-7 Ion Movila Str., 051577, Bucharest, Romania

[2] Prof. Dr Nicolae Paulescu National Institute of Diabetes, Nutrition and Metabolic Diseases, 5-7 Ion Movila Str., 051577, Bucharest, Romania

[3] Human Knowledge Research and Development Institute, Bucharest, Romania

[4] Ovidius University, Faculty of Medicine, 1 Universitatii Str., 900470, Constanta, Romania

[5] Carol Davila University of Medicine and Pharmacy, 37 Dionisie Lupu Str., 020021, Bucharest, Romania

[6] Prof. Dr D. Hociota Institute of Phonoaudiology and Functional ENT Surgery, 21st Mihail Cioranu Str., 050751, Bucharest, Romania

[7] Internal Medicine Clinic, Floreasca Clinical Emergency Hospital , 8 Floreasca Av., 014461, Bucharest, Romania

[8] C.I. Parhon National Institute of Endocrinology, 34 - 38 Aviatorilor Av., 011863, Bucharest, Romania

[9] Department of Surgical Sciences, Grigore T Popa University of Medicine and Pharmacy, 16 Universitatii Str.,700115, Iasi, Romania

Since their first mention, almost 60 years ago, there were a plethora of articles about oxidative stress and antioxidants, published in a wide range of journals (biochemistry, cell physiology, molecular biology or environmental biology). Also, in the last decade the definition of oxidative stress (OS) undergone different changes, and currently OS is seen as a disruption of redox signalling and control. This review aims to offer the perspective of Complexity Theory on antioxidants framework, and diabetes disease is taken as an example.

Keywords: oxidative stress, antioxidants, complexity theory, algebraic fractals

Currently, most of the scientific literature considers that OS produces adverse effects through the imbalance between the production of ROS (reactive oxygen species) and the biological neutralising capacity of the body (i.e. enzymes) [1-3,6-10]. At a macroscopic level, obesity (i.e. insulin resistance) and a sedentary lifestyle are well-known risk factors for type 2 diabetes, but at a molecular level, oxidative stress is regarded as the primary contributor to the pathogenic process [7,22-23]. As a consequence, in the progression of cardiovascular or renal disease (diabetes complications), OS is also taken as the primary pathogenic mechanism [1]. Although there are studies that show the benefit of antioxidant treatment in chronic pathology, particularly in diabetes and its complications, there is not a general consensus among medical practitioners [2]. This review brings the attention on how our thinking models influence the way we judge pathogenic mechanisms and considers the importance of looking at a phenomenon from multiple points of view.

Experimental part

Materials and methods

We made a literature review by searching in the MEDLINE database for the most representative articles published since 1980. The searched terms used were *oxidative stress* and *diabetes antioxidants*. We selected 29 articles published in specialised journals excluding those that were not directly related to the topic. Also, Complexity Theory in the understanding of the latest mathematical signs of progress made by Colceag was considered.

Upgrading our thinking models

Currently, one of the latest perspectives in biological research is based on the system's dynamics and Complex Systems Theory. The network concept is increasingly used for analysing complex systems, such as living organisms [3]. Fractal varieties and algebraic fractals theory represent one of the main alternatives to understanding how to characterise a phenomenon, without losing its complexity [4]. Informational feedback is an object with several properties: 1) every two nodes generate the third one, 2) functionality is associated to each node, 3) each node bears a semantic content. Extended research in this field was done by Colceag [5]. Based on informational feedback, the process of oxidative stress can be characterised if several questions are answered:

1. What is it understood through oxidative stress?
2. Which are the properties of reactive oxygen species?
3. Do these properties maintain if the complexity of the system is changed? (i.e. *in vivo* versus *in vitro*)
4. How do the effects of oxidative stress express in the body?
5. How can oxidative stress be quantified and characterised?
6. Which are the strategies of the organisation when dealing with reactive oxygen species and oxidative stress?

Most of the papers published so far focus solely on one of these questions, but an integrative perspective is needed to avoid one of the most common thinking biases, such as forced generalisation.

What is it understood through oxidative stress?

The reactive oxygen species, as well as reactive nitrogen species (RNS), have multiple sources of generation: as byproducts of normal cellular respiration given by the new cooperation between mitochondria and cellular membrane networks, deliberate production by immune cells killing pathogens or random products of non-

* email: mitrofan.grigorina@gmail.com; Phone:+40766646399 *All the authors have contributed equally to this paper.*

enzymatic and enzymatic processes. To compensate for this potentially harmful species, several mechanisms that neutralise these effects have been developed, such as scavenging enzymes, non-enzymatic antioxidants (free radical scavengers), compartmentalisation, repair of damaged components and metal sequestration [6].

A generic definition of an anti-oxidant is given as a structure that can neutralise/inhibits oxidation of other molecules. The following equation summarises this dynamics:

$$X\bullet + Y \rightarrow X + \bullet Y, \quad (1)$$

where X• is the harmful free radical, which becomes X (non-radical) and Y is the scavenger, which becomes a more stable radical. Also, the by-products lose their adverse effect because X is metabolised, while •Y is excreted. It is worth mentioning that this explanation is valid only when speaking about antioxidants from a chemical point of view.

In the beginning, oxidative stress was defined solely as a disturbance of the equilibrium between increased free radicals production and reduced antioxidant capacity of the organism [7]. This was a quantitative approach, implying that different biological systems respond in a similar way to the lack of equilibrium. In the last decade, it was proposed an alternative definition for OS, as a *disruption of redox signalling and control* [8], shifting to a qualitative model. Thus, OS can cause pathway-specific toxicity related to processes such as uncontrolled fibrotic process, inappropriate apoptosis, immune dysfunction, altered membrane permeability and barrier functions [9]. In other words, this new concept opens the possibility that OS could emerge without an overall imbalance of pro-oxidative and anti-oxidative factors [9]. This property suggests that there may be semantic properties associated with chemical structures because some of the ROS effects are linked more with signalling, while the others are connected more with negative given impacts by oxidative stress.

It should also be noted that the effects are dose mediated, known as hormetic effect [10]. Hormetic behaviour plays a significant role in the dynamics of redox biology because it encompasses both physiological and pathological roles ROS/NOS. Overproduction of ROS leads to oxidative stress, a process that profoundly impacts cell structures including proteins, lipids or DNA. On the other hand, beneficial effects occur at low/moderate concentrations and play a role in physiological pathways in cellular responses to infectious agents or induction of mitogenic response [11]. The most intriguing behaviour is that various ROS-mediated processes have a protective effect against ROS-induced oxidative stress and re-establish or maintain *redox balance* [12]. This fact opens up the idea that there may be a chemical, behavioural vocabulary of ROS/NOS family that generates different effects depending on the various contexts.

Shifting the definition of oxidative stress from the view of a global imbalance of pro-oxidants and antioxidants to one that addresses disruption of specific redox signalling and control pathways would stimulate the development of therapeutic strategies, targeting a particular circuit of redox and eventually would contribute to disease prevention.

In the same time, different definitions of OS were adapted concerning a specific scientific field such as dietary oxidative stress, physiological oxidative stress, photo-oxidative stress, radiation-induced oxidative stress, oxidative stress status, reductive stress, nitrosative stress [8].

Although there are many types of reactive species, in this paper will be considered mostly reactive oxygen species.

Which are the molecules that can be regarded as antioxidants?

Keeping in mind that oxidative stress represents a disruption in a specific oxidative pathway, at a cellular level, one can speak of either direct and indirect oxidative stress or antioxidants.

From a chemical point of view, the underlying properties of partially reduced species are given by molecular orbital oxygen structures [6]. Highly reactive molecular species with unpaired electron persist only for very short duration 10^{-9}-10^{-12} s, and then they collide with another molecule, donate the electron and achieve stability. Molecular oxygen is itself a radical, and by adding one electron forms superoxide anion radical ($O_2^{\bullet -}$), is considered the *primary* ROS. The *secondary* ROS results from reactivity of superoxide with other molecules either directly or through enzyme or metal catalysed processes [6]. The chemical reactions of generating free radicals are presented in the formulas below (2):

(2) Free radicals generations

$$O_2 + e^- \Rightarrow O_2^{\bullet -}$$
$$O_2^{\bullet -} + 2H^+ + e^- \Rightarrow H_2O_2$$
$$H_2O_2 + H^+ + e^- \Rightarrow HO^\bullet + OH^-$$
$$HO^\bullet + e^- \Rightarrow H_2O$$

Hydrogen peroxide has a limited ability to cause tissue damage, depending on the interaction with transition metal ions, when it forms hydroxyl radical, which is the most potent of all oxygen radicals. When speaking about free radicals, Fenton reaction (3) and Haber-Weiss reaction (4) are the always mentioned as the main sources of hydroxyl radical. Although Fenton reactions are specific to *in vitro* reactions, *in vivo* their significance is not clear [13].

(3) Fenton reaction

$$Fe^{+2} + H_2O_2 \Rightarrow Fe^{+3} + HO^\bullet + OH^-$$
$$Fe^{+3} + O_2^{\bullet -} \Leftrightarrow Fe^{+2} + O_2$$

(4) Haber-Weiss reaction

$$O_2^{\bullet -} + H_2O_2 \Leftrightarrow HO^\bullet + OH^- + O_2$$

The main properties of reactive oxygen species are summarized in table1.

From a cellular signalling point of view, a closer look is needed when speaking about anti-oxidants and pro-oxidants molecules. There are molecules able to induce gene expression of components known to play a role in scavenging free radicals or interfere with signalling pathways. For example, the synthesis of a group of antioxidant enzymes is regulated via antioxidant response element (ARE) and transcription factor NfR2 [14]. Another example is represented by α-tocopherol, which prevents phosphorylation of P47 and assembly of the active oxidase, and subsequently inhibits NADPH oxidase in macrophages and the oxygen burst [15]. On the other hand, in the mitochondrial complex III, the electron flow is blocked by antimycin A, which doesn't have any reactive oxygen groups, but induces superoxide and hydrogen peroxide release [16], therefore can antimycin A be regarded as pro-oxidant?

By giving these examples, we support the idea of some authors [9, 17] that the definition and properties of antioxidants or pro-oxidants should be regarded more than

Reactive Species	Properties [11]
Superoxide anion ($O_2^{\bullet-}$)	Produced mainly by the electron transport chain. Cannot diffuse so far from the site of origin. Generates other ROS
Hydrogen peroxide (H_2O_2)	Not a free radical, but can produce free radicals by reaction with transition metal (i.e. Fe+2). Can diffuse into and through cell membranes
Hydroxyl radical ($HO^{\bullet}$)	The highly reactive species in attacking biological molecules. Produced from H_2O_2 in the Fenton reaction in the presence of Fe2+ or Cu+
Singlet oxygen (O_2)	Oxygen with antiparallel spins. Rendered at high oxygen tensions from absorption of UV light. Decays so fast that it is not significant in vivo source of toxicity.

Table 1
OXYGEN TOXICITY AND REACTIVE SPECIES (ADAPTED AFTER DAL ET. Al., 2016)

description associated with the imbalance between reactive oxygen species production [18].

Do these properties maintain if the complexity of the system is changed? (i.e. in vivo versus in vitro)

Due to extensive research made in this field from food science, the associations between oxidative stress and diet generated the concept of *dietary oxidative stress* and it was regarded as *a substance in food that significantly decreases the adverse effects of reactive species, such as reactive oxygen species, reactive nitrogen species, on normal physiological function in humans* [8]. So far, a wide range of molecules was named as antioxidants including vitamin E, vitamin C, carotenoids, polyphenols, lycopene and other micronutrients such as selenium [11].

When taken as a supplement, usually an antioxidant follows the route of intestine, plasma and either liver or directly to the target tissues, but it has been noticed that through methylation or glucuronidation the antioxidant properties are lost, and the molecules may still have effects at a cellular level, in a similar way as the initial antioxidant [17]. This observation may lead to the previous hypothesis that some antioxidant's effects are exerted considering semantic functionalities of chemical structures.

In the same time, molecules proved to have antioxidant properties *in vivo* might have additional features *in vivo* and their first functionality changes. For example, retinol, which is an antioxidant *in vitro* initiates the process of vision together with rhodopsin, due to isomerisation of the pigment and not due to its antioxidant properties, but in some studies, it was classified as an antioxidant [19]. Melatonin also has antioxidant properties *in vitro*, but through G protein-coupled receptors takes place its primary function of generating circadian rhythm [20, 21].

Their bioavailability represents another issue regarding oral supplements, and it is well known that dietary antioxidants are bioavailable but in a decreased quantity per portion.

How do the effects of oxidative stress express in the body?

An increased quantity of free radicals or modified signal transductions has been associated with almost all of the chronic diseases [6]. In clinical research two approaches are considered: either if there are specific nutritional interventions able to prevent a particular disease or, nutritional interventions able to alleviate progression, symptoms or complications of the diseases. In the same time, the diseases break into two groups: (i) the first involves the so-called *mitochondrial oxidative stress* conditions (cancer and diabetes) and (ii) the second group involves the so-called *inflammatory, oxidative conditions* (atherosclerosis, chronic inflammation, and ischemia and reperfusion injury [6, 22].

In the case of diabetes, there are several structural transformations given by hyperglycemia: protein oxidation and lipid oxidation are the most recognised. Free radicals induce damage to sulfhydryl groups and proteins are no longer recognised as self, leading to cross-reactions and autoimmune diseases.

Also, peroxidation of plasmatic lipids produces abnormal LDL which are not identified by liver's LDL receptors and subsequently, macrophage scavenger receptors take

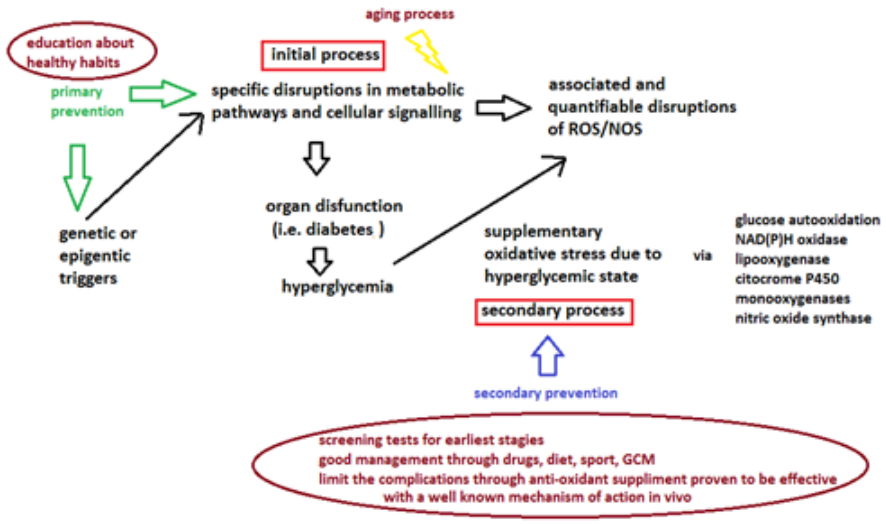

Fig. 1. Primary prevention of diabetes can be made through education about healthy lifestyle, while secondary prevention can be realised through approaches that limit the impact of hyperglycemia

modified LDLs, forming engorged lipid macrophages (LEM), and infiltrate under blood vessel endothelium.

Another mechanism of lipid peroxidation involves loss of membrane functions and integrity. A chain reaction between polyunsaturated fatty acids and ROS affects membrane lipids, which leads to increased permeability, increased calcium influx and subsequent mitochondrial damage. The dynamics can be described as follows:

A). Initiation by OH• attack of polyunsaturated lipids, extracting one proton, B) free radical chain reaction with O2, C) Lipid peroxyl radical propagates and degrades lipidic structures

D) Lipid soluble antioxidants such as vitamin Estop the chain reaction.

The dynamics of free radical interaction in lipid peroxidation follows the logic of laticeal automata described by Colceag [5]. A so-called defect appears in an initial structure, which is transmitted to the surrounded structures to get to an equilibrium state, inducing conformational changes of the initial structure.

Figure 1 presents the dynamic of the pathogenic process in diabetic disease and how to tackle the illness through primary and secondary prevention.

How could oxidative stress be quantified and characterised?

There are many lines of evidence that are suggesting that oxidative reactions play a role in the progression of age-related pathologies (in the field of healthy ageing) and significant disease processes including cardiovascular disease, diabetes, cancer and neurodegenerative diseases [6].

Although extensive research on oxidative damage was achieved, there is no consensus for routine clinical assessment of oxidative stress. The main burden is that most of the assays do not assess the balance of pro-oxidants and antioxidants, but instead focus on either oxidants and oxidation products or antioxidants and antioxidant systems. For example, disease-oriented research goes for the pro-oxidant side, while nutrition research in more often focused on the antioxidants side.

Several methods have been described previously in order to assess oxidative stress: HPLC and CG-MS for antioxidants (ascorbate, α-tocopherol, glutathione, carotenoids, polyphenols), d-ROMs and ESR for oxidants (superoxide anion, H2O2), immunoassays, HPLC, LC-MS/MS GC-MS for oxidation products (isoprostanes, protein carbonyls), mRNA expression, immunoassay, enzyme activity (catalase, SODI-2, GPx1,2, Trx 1,2, Prx1,2)[9]. However, the large number of antioxidant systems limits the utility of antioxidant assays for assessment of oxidative stress under clinical conditions.

Some authors describe the balance of GSH/GSSH antioxidant system as an essential alternative for assessment [9]. GSH has a central role as an antioxidant, maintaining thiol/disulfide redox state proteins, and it also supports the redox state of ascorbate and indirectly vitamin E. On the other hand, GSSG is reduced back by GSSG reductase, which is ubiquitously distributed in tissues. Therefore, there is a direct link between their respective tissular concentration and plasma concentration, providing useful indications about oxidative stress. More than this, analysing the interaction between GSH/GSSH with Cys/CySS in healthy individuals, the investigators obtained that there is a lack of equilibrium between the two systems, suggesting that the concept of a single balance between pro-oxidant and antioxidant system is a simple approach of oxidative stress [9]. Recognition of the existence of multiple, discrete redox signalling pathways suggests that a more suitable definition for oxidative stress is a condition that disrupts redox signalling control.

Due to the difficulty in assessing cellular redox processes (reactive species are present at very low concentrations in cells, and it is difficult to measure them directly), there is limited evidence of specificity in redox signal transduction mechanisms, although most studies have an underlying assumption of uniqueness. The main burden is represented by the difference of experimental conditions *in vivo* compared with *in vitro*. To study a redox pathway, three critical components are needed: a redox-signal generator, a redox signal sensor and a redox signal. If the sensors could have an increased sensitivity in discriminating between hydrogen peroxide and superoxide these circuits could exist within the same physical space and transmit biological information independently [9]. Therefore, the description of *oxidative stress* should be considered if the molecular details of the imbalance are known.

Which are the strategies of the body when dealing with reactive oxygen species and oxidative stress?

As mentioned above, there are many strategies by which the body tries to cope with an increased flow of free radicals; some of them are short or medium term reactions, while others can be considered as long-term defence mechanisms.

In the physiopathological chain of T2D, insulin resistance (IR) is seen as the primary factor involved in hyperglycemic state, and the central dogma considers IR a high risk to the body and should be counteracted at any cost, but recently insulin resistance is seen as a *physiological defence against metabolic stress* [23][24]. Although most of the therapeutic strategies are focused on lowering glucose concentration and HbA1c, it is questionable whether if this approach is the most beneficial. For example, the ACCORD trial showed that aggressive glycemic control compared with standard treatment increased all cause of mortality including death from cardiovascular causes [25]. Also, a meta-analysis of UKPDS, ADVANCE, ACCORD and VADT showed that a strategy based on intensive versus standard glycemic approaches were associated with only a 15% reduction in myocardial infarction [26].

These contradictory results could be at least partially explained if IR is accepted as a mechanism for myocardial protection against glucolipotoxicity. In healthy people there is an inverse relationship between glucose and free fatty acids level (FFA), whereas, in low controlled T2D patients, both circulating glucose and free fatty acids are elevated simultaneously, leading to an exposure of the myocardium to nutrient overload and glucolipotoxicity [27]. Therefore, treating T2D patients with exogenous insulin increased lipid content in the myocardial tissue by 80% [28-29], and more than this, even in healthy subjects, short-term hyperinsulinemia and hyperglycemia led to increase in myocardial lipid accumulation [30]. This situation was described as *insulin-mediated metabolic stress* [31], and various authors established mitochondrial oxidative stress (i.e. superoxide stress) as a predecessor of insulin resistance [32-34].

Conclusions

In this paper, we showed that the assessment of a phenomenon using a complex logic leads to a better understanding of pathogenic processes, which may avoid oversimplification or overgeneralization.

Also, the research made in the field of oxidative stress will have a meaningful impact in understanding the signalling pathways that generate chronic diseases (i.e. diabetes), with an enormous contribution in both primary and secondary prevention.

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References

1.KHAN A.N., KHAN R.A., AHMAD M., MUSHTAQ N., J.Pharmacogn. Phytochem, **3,** no. 3, 2015, p. 217-220.
2.CERIELLO A., Diabetes Care, **26**, nr. 5, 2003, p.1589**–**1596.
3.GOLDMAN A.W., BURMEISTER Y., CESNULEVICIUS K., HERBERT M., KANE M., LESCHEID D., MCCAFFREY T., SCHULTZ M., SEILHEIMER B., SMIT A., ST LAURENT III G., BERMAN B., Hypothesis and Theory,**6**, 2015, p 225.
4.*** http://austega.com/florin/CellularAutomataAlgebraicFractals.htm [accessed 25.05.2018]
5.***http://austega.com/florin/ALGEBRAIC%20FRACTALS-FRACTAL%20VARIETIES.htm [accessed 25.05.2018]
6.VALKO M., LEIBFRITZ D., MONCOL J., CRONIN MTD., MAZUR M., TELSER J., Int. J. Cell. Biol., **39**, 2007, p.44-84.
7.SOHAL R.S., ORR W.C., Free Radic. Biol. Med., 2012, **1**, nr. 52, iss. 3, p.539-555.
8. SIES H., JONED D., Oxidative Stress in Encyclopedia of Stress, (2nd ed), **3**, Elsevier, G Fink (Ed), Amsterdam, 2007, p. 45-48.
9. JONES D.P., Antioxid. Redox Signal., **8,** nr. 9, 2006, p. 1865-1879.
10.CORNELIUS C., PERROTTA R., GRAZIANO A., CALABRESE E.J., CALABRESE V., Stress responses, vitagenes and hormesis as critical determinants in aging and longevity: Mitochondria as a " chi", Immun. Ageing, **10**, nr 1, iss. 15, 2013, p. 309-321.
11.DAL S., SIGRIST S., Diseases, **4,** nr 3, iss. 24, 2016, p.1-51.
12.ESPINOSA-DIEZ C, MIGUEL V, MENNERICH D, KIERZMANN T, SANCHEZ-PEREZ P, CADENAS S, LAMAS S, , Redox Biol., **6**, 2015, p.183-197.
13. KAKHLON, O., CABANTCHIK, Z. I., Free Radic. Biol. Med., **33**, 2002, p. 1037–1046.
14. AOKI, Y., SATO, H., NISHIMURA, N., TAKAHASHI, S., ITOH, K. and YAMAMOTO, M, Toxicol. Appl. Pharmacol., **173**, nr. 3, 2001, p. 154-160.
15.CACHIA, O., BENNA, J.E., PEDRUZZI, E., DESCOMPS, B., GOUGEROT POCIDALO, M.A., LEGER, C.L., J. Biol. Chem. **273**, nr. 49, 1998, p. 32801-32805
16. LOSCHEN, G., AZZI, A., RICHTER, C. and FLOHE, L., FEBS Lett., **42**, nr.1, 1974, p. 68-72.
17.AZZI A, DAVIES KJA, KELLY F, FEBS Letters, **558**, nr.1-3, 2004, 3-6.
18. PASTOR, N., WEINSTEIN, H., JAMISON, E., BRENOWITZ, M. J., Mol. Biol., **304**, nr. 1, 2000, 55–68.
19. GRANADO F., OLMEDILLA B., BOTELLA F., SIMAL A., BLANCO I, Nutrition, **19**, nr. 2, 2003, p.128-132.
20. KOKKOLA T., LAITINEN J.T., Ann. Med., 30, nr. 1, 1998, p. 88-94.
21.ARSENE, A.L.; MITREA, N. , CRISTEA, A.; DRAGOI, C.M., Farmacia, **57**, nr. 2, 2009, p. 223-228.
22. MIRICESCU, D; GREABU, M; TOTAN, A, MOHORA, M; DIDILESCU, A; MITREA, N; ARSENE, AL; SPINU, T , TOTAN, C; RADULESCU, R , FARMACIA, **59,** iss. 3, 2011, p. 329-337.
23.NOLAN CJ, RUDERMAN NB, KAHN SE, PEDERSEN O, PRENTKI M, Diabetes, **64**, iss. 3, 2015, p.673-686.
24. HOEHN K.L., SALMON A.B., HOHNEN-BEHRENS C., TURNER N., HOY A.J., MAGHZAL G.J., STOCKER R., REMMEN H.V., KRAEGEN E.W., COONEY G.J., RICHARDSON A.R., JAMES D.E., Proc. Natl. Acad. Sci., 2009, **106**, nr 42, p.17787-17792.
25.GERSTEIN H.C. The Action to Control Cardiovascular Risk in Diabetes Study Group, N. Engl. Med., **358**, 2008, p.2545-2559.
26.TURNBULL F.M., ABRAIRA C., ANDERSON R.J., BYINGTON R.P., CHALMERS J.P., DUCKWORTH W.C., EVANS G.W., GERSTEIN H.C., HOLMAN R.R., MORITZ T.E., Neal B.C., NINOMIYA T., PATEL A.A., PAUL S.K., TRAVERT F., WOODWARD M., Diabetologia, **52**, nr 11, 2009, p. 2288-98.
27.STANLEY W.C., RECCHIA F.A., LOPASCHUK G.D. Physiol. Rev., **85**, no. 3, 2005, p.1093-129.
28.JANKOVIC D., WINHOFER Y., PROMINTZER-SCHIFFERL M., WOHLSCHLÄGER-KRENN E. ANDERWALD C.H., WOLF P., SCHERER T., REITER G., TRATTNIG S., LUGER A., KREBS M., KRSSAK M., PLoS One, **7**, nr. 12, 2012, p. e50077.
29.LABBÉ S.M., GRENIER-LAROUCHE T., NOLL C., PHOENIX S., GUÉRIN B., TURCOTTE E.E., CARPENTIER A.C., Diabetes, **61**, nr 11, 2012, p. 2701-10.
30.NOLAN C.J., RUDERMAN N.B., KAHN S.E., PEDERSEN O., PRENTKI M., Diabetes, 2015, **64**, nr. 3,673-686.
31.WINHOFER Y., KRSSÁK M., JANKOVIC D., ANDERWALD C.H., REITER G., HOFER A., TRATTNIG S., LUGER A., KREBS M., Diabetes, **61**, 5, 2012, p. 1210-6.
32. HOUSTIS N., ROSEN E.D., LANDER E.S., Nature, **440**, nr. 7086, 2006, p.944-8.
33.ANDERSON E.J., LUSTIG M.E., BOYLE K.E., WOODLIEF T.L., KANE D.A., LIN C.T., PRICE J.W. 3rd, KANG L., RABINOVITCH P.S., SZETO H.H., HOUMARD J.A., CORTR9

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