

Bed-Side Quantum-Biophysical-Semeiotic Evaluation of PPARs Activity.

(up-dated paper, 2011)

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Introduction.

Peroxisome proliferator-activated receptors (PPARs) are notoriously ligand-activated transcription factors belonging to the nuclear factor receptor super-family which influence both the size and number of peroxisomes, performing various metabolic functions (peroxide derived respiration, beta oxidation of fatty acids, cholesterol metabolism, etc.) within the cell.

Nuclear receptors are transcription factors activated by specific ligands (fatty acids, LDL, a.s.o.) which play an important role during cell signalling. They belong to the steroid-thyroid-retinoid receptor super-family; these include receptors for steroids, thyroid hormone, vitamin A and D derived hormones and some fatty acids.

Three different PPARs have been identified to date (PPAR-alfa, PPAR-beta or -delta, and PPAR-gamma, that plays a pivotal role in insulin-sensitivity and glucose metabolism). PPARs are endogenously activated by ligands such as fatty acids, thyroid hormone, and eicosanoids. PPARs are known to modulate gene expression for pathways involved in fat, lipid and glucose metabolism, inflammation, cell cycle, and immune responses (1). The experimental use of PPAR ligands has also demonstrated their capacity to ameliorate glyco-lipidic metabolism (2) as well as myocardial fibrosis (3, 4). Like other nuclear receptors, after activation by ligand, PPARs bind a specific element in the promoter region of target genes. The hetero-dimerization of **PPAR** with **RXR** and the presence of coactivators are necessary for the transcriptional activity of PPAR responsive element (**PPRE**) in DNA (1, 9).

PPAR can be activated by small molecules such as glitazones and lead to decreases in glucose and lipid serum levels. These properties of glitazones have been used for the treatment of type 2 diabetes patients for which benefits are derived not only from their ability to enhance insulin sensitivity, but to ameliorate development of atherosclerosis(1).

Angiotensin II (Ang II), a known pathological modulator of cardiac remodeling, has been shown to enhance production of reactive oxygen species (ROS) via stimulation of nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase (5); therefore, the stimulation of ROS production by Ang II may constitute a means by which this humoral factor contributes to development of tissue injury in organs such as blood vessels, kidney, and the heart. In addition, enhanced production of ROS is also known to occur with hyperglycemia, and it is also thought to mediate organ damage in diabetes. Recent experimental studies have also demonstrated that high glucose blood levels stimulate the production of Ang II, hinting at the possible existence of positive feedback loops for ROS generation (6). Thus, uncontrolled diabetic patients may be at high risk of ROS-mediated

organ damage. Thus, Ang II–induced ROS-linked signaling has emerged as a possible pathway to mediate the effects of this humoral factor also on cardiac fibroblast Extra Cellular Matrix turnover (7).

Interestingly, cytochrome P450s of the 4A subfamily generally catalyze the omega-hydroxylation of fatty acids. The induction of P450 4A enzymes by peroxisome proliferators or fatty acids is mediated by peroxisome proliferator-activated receptors (PPARs), which, as referred above, are members of the nuclear receptor superfamily that regulates the expression of genes that control fatty acid synthesis, storage, cell cycle, and catabolism (8).

As regards our argument, it is really important that PPARs bind as heterodimers with another member of the nuclear receptor family, e.g., the retinoid X/O receptor (RXR/ROR), stimulated also by melatonin, as we demonstrated clinically in previous papers (2, 3), to peroxisome proliferator response elements (PPREs) in the P450 4A1 and 4A6 genes (8).

Early, promptly, and clinical recognizing, i.e., on very large scale, such as dysfunction of PPARs, sometimes due to inherited mutations of codifying genes (13), in individuals apparently healthy, but involved by dyslipidemic and/or diabetic constitutions evolving to Pre-Metabolic syndrome (See www.semeioticabiofisica.it/microangiologia.it) allows to prevent the occurrence of serious diseases and their complications which are developing years or decades before diseases are recognized (14-19).

This article aims to demonstrate that biophysical-semeiotic PPARs activity evaluation, realized for the first time at the bed-side, represents a paramount event in clinical Medicine and research, indicating metabolic abnormality starting before the occurrence of clinical and serological phenomenology.

Biophysical-Semeiotic Methods in assessing PPARs Activity.

There are two methods, really easy to apply, similarly reliable, although different in technical difficulty and elegance, based on stimulating the secretion of thyroid hormone and respectively melatonin:

A) Firstly, doctor must evaluate the basal microcirculatory activity of liver and/or abdominal adipose tissue, and/or any other tissue, of course, either assessing the duration of vasomotility and vasomotion (NN = 7,5 sec. as “Plateau Line”, in both *absorptive* and *post-absorptive* state: microcirculatory activation type I, associated) (10) (See also website HONCode 233736 www.semeioticabiofisica.it as well as www.semeioticabiofisica.it/microangiologia.it) or easier the latency time of hepatic (or adipose tissue)-aspecific gastric reflex, caused by mean intense digital pressure upon cutaneous projection area of liver and, obviously, by pinching with same intensity lateral abdominal adipose tissue (NN = 12 sec. and respectively 8 sec.)

Subsequently, after stimulation of TSH-RH secretion – lasting 15-20 sec. – by “mean-intense” digital pressure on cutaneous projection area of TSH-RH neuronal center (i.e., 1 cm. above and 3 cm in front of external acoustic meatus), doctor evaluates a second time above-referred parameter values (Fig. 1).

Analogously, the second evaluation may occur after stimulation (duration of 15-20 sec.) of thyroid hormone secretion with the aid of cutaneous pinching of thyroid trigger-points, i.e., the cervical basal skin laterally the sternocleidomastoideous muscle of both side.

In healthy, Plateau Line duration increases significantly, raising from 7,5 sec. to about 9 sec., while latency time of hepatic and adipose tissue-aspecific gastric reflex increases in a significant manner (e.g., 16 sec. and respectively 12 sec.).

To corroborate this biophysical-semeiotic theory is of great importance the fact that if the same parameter values are assessed after “contemporaneous” application of both manoeuvres, we observe the highest results: 18 e 14 respectively.

Interestingly, in obese and/or dyslipidemic patients and/or prediabetics or diabetics, wherein is present impaired PPAR functions, there is not increasing of above-mentioned biophysical-semeiotic parameters values in the subsequent evaluation.

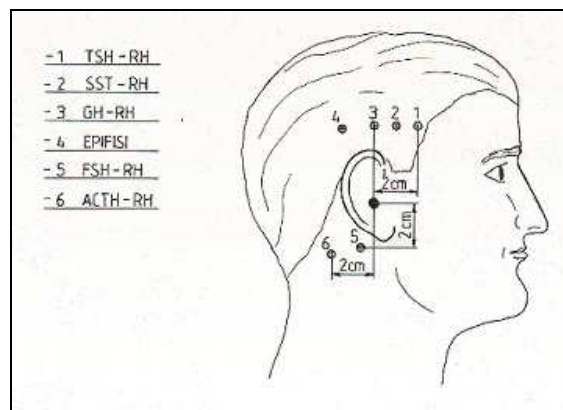
B) The second method is based on physiological nuclear receptor stimulation caused by melatonin: as illustrated above, at first, doctor must assess the same basal parameter values, i.e., duration of Plateau Line or, in a easier manner, latency time of gastric aspecific reflex, brought about by means of intense digital pressure upon cutaneous projection area of liver or with persistent pinching of lateral-abdominal adipose tissue.

A second evaluation is performed starting from 20-30 sec., the subject to be examined has closed his eyes causing melatonin secretion.

In healthy, one observes once again the same results gathered during the first procedure, outlining internal and external coherence of the method and the theory. Interestingly, the evaluation by means of both hormone stimulation contemporaneously, brings about the highest value, i.e., 18 sec. and 14 sec., corroborating the underlying physio-pathological mechanisms.

By contrast, in patients, with impaired PPAR activity, i.e., obese, dyslipidemic, prediabetics and diabetics, parameter basal values appear not increased, or increase but not significantly, in the second evaluation.

Analogously, in the final stages of Pre-Metabolic syndrome evolving to the metabolic one, parameter values ameliorate less than normally: e.g., lt. of hepatic- and adipose tissue-gastric aspecific reflexes increases from basal value of 12, and respectively, 8 sec. to 14 and 10 sec. (NN = 16 sec. and respectively 12 sec.).



QBS experimental evidence corroborates P. Garaiev and A Berezin's Wave Genome Theory (21).

As follows the various steps of my experiment:

A) **Firstly, at the base line**, in *post-absorptive state*, I evaluated parameter values of my Epto-Gastric Aspecific Reflex latency time: **8,5** sec. (in almost all other tissues at rest, Lt. = 8 sec.). As a matter of fact, Liver parenchyma is always functioning, thus **8,5** sec. l.t. indicates a *small* type I, microcirculatory activation, and, in turn, according to my **Angiobiopathy theory**, *slightly increased* parenchyma activity, is aiming to provide tissues with glucose, under such a condition. In fact, under such a condition, liver INTERSTITIUM appears *large*, when evaluated by means of "in toto" ureteral Reflex.

Reflex Duration = **3,5 sec.**, showing a physiologica hepato -Microcirculatory Reserve Function (**MFR**).

As regards microcirculatory data, there is a small microcirculatory activation, AL+PL+DL = **7 sec.** (NN = 6 sec. in the majority of other tissues): INTERNAL coherence of my theory.

B) Secondly, I gathered energy wave frequencies (PLURAL!) from the liver of my wife, Marina, with both crystals (diodes) of Cem Tech, Programme **2**, localized for **1 minute** on her liver cutaneous projection area.

At this point, I putted down the two crystals upon a device, able to receive the gathered energy wave frequencies, that Marina would utilize next!

C) Thirdly, when I was **about 10 cm.** near to both device "and" crystals (diodes), *simultaneously* the above-mentioned parameter values, concerning my liver, increased maximally: latency time of Epato-Gastric Aspecific Reflex raised from 8,5 sec. to **24 sec.**, which is the highest value I till now observed!;

Duration **3 sec.**, indicating an OPTIMAL MFR.

Interestingly, I observed maximal type I, associated microcirculatory activation: AL+PL+DL from **7 sec.** raised to **11 sec.**, highest value!

INTERSTITIUM was *virtual*: "in toto" ureteral Reflex resulted NULL, indicating material intense absorption by epato-cells, i.e. INTENSE metabolic activation. Contemporaneously, blood glucose appeared slightly lowered (**Stagnaro-Neri M., Stagnaro S., Il Segno di Bilancini-Lucchi nella diagnosi clinica del diabete mellito. The Pract. Ed. It. 176, 30, 1993.**), indicating intense hepatic metabolism stimulation, underlining "and" emphasizing signals brought about obviously by mit-DNA and n-DNA (See my works on the assessment of liver PPARs, Practical Applications, www.semeioticabiofisica.it).

Surprisingly, when I went away from diodes (crystals), the above-referred amelioration **lasted**....BUT for one minute only, indicating that mit-DNA and n-DNA re-**structuration** was TEMPORARY, due to the small time of the energy wave stimulatory action...

Conclusions: P. Garaiev's and A A Berezin's **Wave Genome Theory** is corroborated by Quantum Biophysical Semeiotics: both mit-DNA and n-DNA emit energy wave frequencies.

PPARs evaluation is the best clinical tool in controlling glucose and lipid metabolism.

as regards the war against ATS as well as metabolic disorders, as type 2 diabetes, a 52-year-long long well-established clinical experience allows me to state that in primary prevention of these diseases and their severe complications, as well as their monitoring, bedside evaluation of liver, artery wall, adipocyte, and skeletal muscle PPARs is far better than visceral fat assessment and chemical examination.

In fact, nowadays doctors can evaluate such as interesting nuclear receptor activity by means of Biophysical Semeiotics, i.e., on very large scale (16-19) (<http://www.semeioticabiofisica.it>, at the URLs: <http://www.semeioticabiofisica.it/semeioticabiofisica/Biography.htm> and <http://www.semeioticabiofisica.it/semeioticabiofisica/Biography.htm>). Notoriously, the developed as well as developing world is experiencing a dramatic increase in the prevalence of obesity, insulin resistance, dyslipidaemia, hypertension, impaired glucose tolerance, diabetes mellitus, endothelial dysfunction, and pro-thrombotic and pro-inflammatory states. At the cellular and molecular levels,

we have to consider with attention the roles of the adipocyte, hepatocyte, and skeletal muscle; insulin action, signaling transduction, and resistance; endothelial dysfunction; and the newly emerging area of inflammation. All these molecular-biological essential events can nowadays be assessed at the bed side by means of Biophysical Semeiotics, including PPARs biological activity, which plays a paramount role in glucose and lipids metabolism (16-19).

Discussion and Conclusion.

Nuclear receptors are transcription factors activated by specific ligands (fatty acids, LDL, a.s.o.) which play an important role during cell signalling. They belong to the steroid-thyroid-retinoid receptor superfamily; these include receptors for steroids, thyroid hormone, melatonin, vitamin A and D derived hormones and some fatty acids. Structurally, they share common features: highly conserved central DNA binding domain (binds receptor to specific DNA sequences – Hormone Response Elements, HRE), ligand binding domain in the COOH- terminal region and variable N-terminal domain (9). Recently, the three-dimensional structure of DNA binding domains of various nuclear receptors have been described (10). However, in some nuclear receptors the natural ligand (hormone) has not been identified and therefore the term “orphan” receptors (OR) was suggested a decade ago. Searching for such ligands (hormones) has introduced the concept of “reverse endocrinology” (11). A typical example of this approach is the discovery of 9-cis retinoic acid (a metabolite of vitamin A) as a high-affinity ligand for three variants of retinoid X receptors (RXR).

Currently, five families of OR are distinguished: 1) liver X receptor (LXR), 2) pregnane X receptor (PXR), 3) constitutive androstane receptor (CAR), 4) farnesoid X receptor (FXR) and 5) peroxisome proliferator activated receptors (PPARs).

Peroxisome Proliferator-Activated Receptors (PPARs) were first cloned from mouse liver in 1990 as the nuclear receptor mediating the effects of many synthetic (industrial and pharmaceutical) compounds called **peroxisome proliferators** (PPs) (12). PPs influence both the size and number of peroxisomes, which perform various metabolic functions (peroxide derived respiration, beta oxidation of fatty acids, cholesterol metabolism, etc.) within the cell.

Like other nuclear receptors, after activation by ligand, PPARs bind a specific element in the promoter region of target genes. The hetero-dimerization of **PPAR** with **RXR** and the presence of coactivators are necessary for the transcriptional activity of PPAR responsive element. PPARs activity can be compromised not only in obese, dyslipidemic, diabetic, and arteriosclerotic patients, but also in the early stages of Pre-Metabolic syndrome, evolving to the metabolic one.

In conclusion, in the paper, for the first time from the clinical view-point, an original method for assessing PPARs activation in quantitative manner is described. It is useful in both practice and research. In fact, we can utilize it in diagnosing as well as in primary prevention of the most common and serious human diseases.

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В статье приведены эксперименты и технология по дальней волновой передаче генетической информации от Донора (хирургически изолированные ткани) к Реципиенту (животный организм). Такая передача является способом управления процессами регенерации у Реципиента. Обнаружено сопутствующее явление выработки волнового иммунитета у реципиента к отравляющему веществу – аллоксану. Дано теоретическое объяснение полученных результатов.

