Abstract
Diabetes mellitus is a complex disorder of the energy metabolism of the human body. In the last WHO/ADA classification, the main forms of this disease are Type 1 (T1DM) and Type 2 (T2DM) diabetes mellitus. According to the same classification, the pathogenesis of T2DM is considered to include a progressive insulin secretory defect on the background of insulin resistance. Recently, controversies surround the concept of peripheral insulin resistance emerging in the 70's as an attempt to explain the differences from the T1DM phenotype whose autoimmune nature was as that time revealed. The insulin resistance hypothesis was based on the supposition that high plasma insulin levels are the result of a primary molecular defect against which the normal beta cells will react with an increased insulin secretion. This hypothesis has been used to explain both the pathogenesis of T2DM and the metabolic syndrome, named also for a short period of time "the insulin resistance syndrome". We will try to argue that what it is attributed to peripheral insulin resistance belongs in fact to obesity. Special emphasis is put on the role of the adipocytes, including the secretion of different adipokines with the secondary lipotoxicity, oxidative stress and pro-inflammatory reaction, explaining the complex relationship between obesity and diabetes.

Keywords: Insulin resistance / sensitivity, insulin receptor, type 2 diabetes, obesity.

INTRODUCTION
The statement that type 2 diabetes results from the pathogenic association of peripheral insulin resistance with a β-cell defect was so often reported that it seems to be a classic acquisition that does not require any further proof. Nonetheless, while β-cell defect secretory defects can be quantified and identified at the level of molecules encoded in the genes associated with this phenotype (1), peripheral insulin resistance remains a vaguely defined disorder as a state in which the effects of insulin are lower than those recorded normally. A similar definition applied to that of the β-cell defect could sound thus: a state in which insulin secretion is diminished compared with that recorded

*Correspondence to: C. Ionescu-Tîrgoviste*, MD “N.C. Paulescu” National Institute of Diabetes, Nutrition and Metabolic Diseases, I. Movila str., 5-7 Bucharest 020475, Romania, E-mail: cit@paulescu.ro

normally. However, the β-cell defect could be characterized quantitatively as a decrease in the oscillatory pattern of insulin; a decrease or disappearance of first-phase insulin secretion; after an i.v. glucose load, a delay of insulin response versus blood glucose increase, or a decrease in the area under the curve of insulin in parallel with the increase in area under curve of blood glucose; increased proinsulin/insulin ratio, and others (1, 2, 3). In addition, the relationship β-cell genomic-proteomic is better known and there is a justified hope that future studies will clarify the relationship between β-cell function and β-cell mass (1, 2).

The notion of peripheral insulin resistance presented as a primary and obligatory pathogenetic mechanism (in its absence, β-cell derangements remain latent) contains a series of uncertainties, which clarification cannot be made unless answers are given to several questions: where is the seat of this insulin resistance? What is its molecular basis? Can peripheral glucose uptake correctly represent the status of other metabolic functions?

A "compensatory" hyperinsulinemia does exist in the natural history of diabetes? Can dissociation between peripheral insulin resistance and obesity be done? Finally does the hypothesis of peripheral insulin resistance overlap or even substitute for obesity? Can low-grade inflammation, oxidative stress, lipotoxicity (invoked as explications for the presence of insulin resistance) be dissociated from obesity? If the answer is that they cannot, then is it not right to "give unto Caeser what is Caeser’s" and to "give unto obesity what is obesity’s"? In this context, is it not right that, instead of seeking miraculous solutions to an elusive insulin resistance, appropriate to seek a solution to obesity, even if it would be a difficult on task?

The sinuous journey to the knowledge of the pathogenesis of diabetes

After the discovery of insulin in 1921 (4) and its clinical application in 1922 (5), interest in the study of "juvenile diabetes" increased considerably, gaining speed after 1958 (6), so that already in 1974 the immunogenetic theory of type 1 diabetes (T1DM) was established in a form similar to that known also today (7,8). The history of this great diabetologic success was inspired presented several years ago in Diabetologia (9). The success in "discovery in type 1 diabetes" was based on the fact that the "red line" of immunogenetics remained a valuable guideline (10) in order to understand why the destruction of beta cells in this phenotype is so rapid.

For type 2 diabetes, soon after insulin assay became available, a beta cell secretory defect (a reduced first phase insulin release) has been found by Luft and Cerasi even in early phase of type 2 diabetes (see for review 11). Later, this defect has been well documented (1, 2, 10, 12), so that such defect could had been the "red line" for pathogenesis of this phenotype. Because this mechanism has been challenged by the hypothesis that the primary cause could be a putative primary peripheral insulin resistance (13, 14) the pathogenesis of type 2 diabetes (T2DM) remained "suspended" somewhere between the pancreatic β-cell defect and the insulin resistance. The main problem is not related with their existence, but with...
their signification and succession in time: which is the primary and which is the secondary defect? This distinction is extremely important for the therapeutic approach which must result from a correct interpretation of the pathogenesis of diabetes. If the beta cell defect is the primary mechanism, the target of our therapy must have in view the preservation of this function. On contrary, if insulin resistance is the primary defect, this must be the first therapeutic target to have in view. The dramatic history of glitazone class is the result of switching a secondary diabetogenic mechanism (the decrease in insulin sensitivity) to a primary one. This is why the term and mechanism "insulin resistance" need to be reanalyzed in order to avoid the confusion existing today, when it is used for explaining a large array of unhomogenous metabolic conditions, becoming like an over-inflated balloon on the verge of bursting.

In fact, the aim of this for debate material is not to reject the term of insulin resistance from the medical dictionaries. On the contrary, the aim is to stimulate an open discussion regarding the significance of the terms insulin resistance and insulin sensitivity in order to use them in a clinical appropriate manner.

We think we should avoid to involve an insulin resistance as a primary mechanism leading to type 2 diabetes, metabolic syndrome, obesity and other acquired metabolic disorders. For these conditions, a more appropriate term could be the "decrease in insulin sensitivity" which, according to Himsworth, can be higher in some diabetic patients and lower in other diabetic patients. Insulin sensitivity can increase (after physical exercise for instance) and decrease (after an overfeeding period with increase in body weight). It is obvious though that insulin sensitivity can be modulated by environmental factors. These are tipically secondary pathogenic mechanisms. This is important for the correct understanding of the various diabetogenic mechanisms, but also for an appropriate therapeutic approach.

**The concept of insulin-resistance: a short historical view**

Insulin resistance is a term which has been used in three different circumstances: (a) in 1931, Wilhelm Falta (1875-1950) was the first to use this term in order to describe the increased requirements of insulin in some patients treated with pork or beef semi-purified insulin available at that time (15). This type of insulin resistance has been mainly associated to the production of insulin antibodies. Their incidence decreased after the replacement of old insulin with the "monocomponent" ones (16) and became very rare after the introduction of human insulin. Harold Himsworth (1905-1993) describes the same phenomenon, but he interprets it more subtly as a variable of insulin sensitivity: some patients are "insulin sensitive", while others are "insulin insensitive" to exogenous insulin (17). Later, he suggested that "a state of diabetes might result from inefficient action of insulin as well as from a lack of insulin." Since studies of insulin secretion were not available at the time, he was unable to asses what belongs to the β-cell defect and what belongs to the peripheral action of insulin.
insulin; (b) the second circumstance was that related to the true insulin resistance, which results from the absence of insulin receptors, from the molecular defects in the insulin receptor, or by their blockage by specific antibodies (18, 19). Here are also included the inherited or acquired lipodystrophies (20). All these disturbances have been included in the extreme insulin resistance and appear in the classification of diabetes under the heading "Genetic defects of insulin action". The various models of knockout mice for some molecules might very well refer to this type of insulin resistance; (c) finally, the third circumstance in which the insulin resistance term was used is that referring to the molecular mechanisms involved in the pathogenesis of type 2 diabetes, and then, by extension to other previously unclear clinical circumstances like the metabolic syndrome (14), polycystic ovary syndrome (PCOS) or NAFLD (non-alcoholic fatty liver disease) (21) and CVD (cardio-vascular disease) (14).

The classic definition of insulin resistance is that of a state in which a given concentration of insulin produces a less than normal biological response. While the metabolic effects of insulin are numerous and differ in various tissues, the insulin resistance concept has been based almost exclusively on the study of glucose uptake or by the interaction between plasma glucose and insulin levels. This relationship is so complex that this approach leaves room for interpretation. According to Reaven (22), insulin mediated glucose uptake varies by 600% to 800% in apparently healthy persons. In addition, the intra-individual reproducibility of the tests used for this purpose is not quite good (23). This variability has been explained by both genetic and environmental factors, such as diet, physical activity or degree of adiposity. An increase in body weight is associated with a decrease in insulin sensitivity, whereas an increase in physical activity is associated with an increase in insulin sensitivity (24). Who has a curiosity to look into the results of various studies in whom the sensitivity to insulin is divided into quartiles will observe that, as a rule, BMI progressively increases from q1 to q4. To conclude, among the factors determining insulin sensitivity, body fat is of a particular importance. This is also reflected in the high percentage (>90%) of obesity among T2DM patients (25). So, if we will substract overweight / obesity from any cohort, type 2 diabetes decreases drastically and metabolic syndrome disappears almost completely.

Insulin resistance concept in type 2 diabetes

In Europe, more than in North America, the primary cause involved in the pathogenesis of Type 2 diabetes has been considered the β-cell secretory defect (11, 12). In North America, and less in Europe, the primary diabetogenic mechanism has been considered to be a presumed peripheral insulin resistance, conceived as expressing a primary and genetically determined molecular defect (26). In other words, insulin resistance precedes and conditions the β-cell defect. This view was recently expressed as such: "Individuals destined to develop type 2 diabetes inherit a set of genes from their parents that make their tissue resistant to
Insulin resistance

Insulin" (26) or "Insulin resistance is not only the hallmark, but also a determinant of type 2 diabetes" (27).
The tissues considered as insulin resistant have been in 1998 (28) three: muscle, liver, and β-cells ("triumvirate"), but they reached eight in 2009, forming "the ominous octet" by adding fat cells, gastrointestinal tract, β-cells, kidney and brain (26).

Conceptual reversal of insulin resistance from secondary to primary diabetogenic mechanism is not just merely academic. From this the philosophy behind, therapy results as expressed by DeFronzo in his unexpected, somewhat imperative advice contained in the phrase: "Collectively this eight players comprises the ominous octet and dictate (our emphasis) that: 1) multiple drugs used in combination are required to correct the multiple pathophysiological defects" (26). As long as such genetic determined molecular mechanism of insulin resistance is not known, how can we solve this problem? This invitation addressed to pharmaceutical companies for an assault upon an invisible enemy is illogical, but not without major risk. The history of glitazone class known as "insulin sensitizers" reflects a wrong solution born from a wrong pathogenic understanding of type 2 diabetes (29, 30, 31).

The emergence of the peripheral insulin resistance concept arises from an error

In the Yalow & Berson paper (32) describing the plasma insulin levels (determined by the new method developed by them) in newly diagnosed patients with "maturity onset" diabetes (the term of T2DM at that time), basal insulin levels were rarely decreased. Ignoring the concomitant blood glucose level, more often the insulin level was normal or even increased. This is why they hypothesized that in this diabetes phenotype insulin secretion is normal or even increased, but its effects on the peripheral tissues are decreased (32). Because at the time the method of assessing β-cell function was not yet available, the idea of a contrasting pathogenetic mechanism of the two main phenotypes of diabetes seemed very attractive and, in fact, for their time quite logical (1, 11, 12).

Rabinowitz and Zierler (33) provided the first evidence of insulin resistance in men when they demonstrated that intra-arterial administration of insulin induces a less glucose uptake by forearm muscle in obese individuals than in normal subjects. Because T2DM is often associated with obesity, the cause of the decrease in glucose uptake in this phenotype of diabetes was explained as an expression of insulin resistance in the adipose tissue and the high levels of plasma insulin in these patients as the result of it.

These two observations was the first erroneous piece to be placed at the base of this hypothesis. A simple correction of plasma insulin level in accordance with blood glucose values, would have led to the quasi-constant identification of reduced levels of insulin in T2DM patients (34). In this context, the hypothesis of peripheral insulin resistance as the primary cause of T2DM may never be proposed.
The first error led to the second one: hyperinsulinaemia

The idea of a compensatory "hyperinsulinaemia" (also called "hyperinsulinism") appeared in the seventh decade (13, 32, 35), and was described as a characteristic of the "maturity onset diabetes", in contrast to the "juvenile diabetes" which was already described to be insulinopenic and insulin dependent (11). At that time, the pathogenesis of the "maturity onset diabetes" looked to be straightforward: due to its association with obesity, these patients are insulin resistant, so that a "robust" pancreatic β-cell will secrete an excess of insulin in order to maintain normal blood glucose. The frequent association of T2DM with obesity (25) suggested adipose tissue as the most probable site of insulin resistance, while the insidious onset of the disease could be explained by the prolonged struggle of the pancreatic β-cell with the putative insulin resistance (35, 36).

An important argument for sustaining insulin resistance as the main pathogenic mechanism in T2DM hypothesis has been the identification of increased levels of plasma insulin in certain population groups. For instance, the studies performed on an USA population during the 1999-2002 period showed an increase of plasma insulin with 5% compared with the previous period 1994-1998. However, this increase could be full explained by the increase with 30.5% of weight excess in the second period (1997-2002) versus first period (1994-1998) (37). It is possible also that some of the increased insulin plasma immune-reactivity in this USA population may be explained in part increased plasma proinsulin levels (38). We make this mention since interpreting the increase in insulin as expression of a primary peripheral insulin-resistance in patients with T2DM or metabolic syndrome is hard to sustain. It starts from a putative asymmetry in the response of peripheral tissues to insulin, in muscles (26), adipose tissue (39), liver (21) or in other tissues (40). This interpretation, has been used later in order to explain the pathogenesis of the metabolic syndrome and cardiovascular disease (22, 41, 42). Such interpretation has the major disadvantage of placing the cart (insulin resistance) before the horse (obesity) (35). In our view the real cause of the increase in plasma insulin levels is the weight gain, induced by increased caloric intake and sedentary lifestyle (43). It is obvious that the metabolic syndrome cannot be conceived in the absence of obesity, so that the pathogenic cycle attributed to insulin resistance seems in fact to be triggered by weight gain. More recently, Shanik et al. (35) tried to reconcile the two hypotheses suggesting that hyperinsulinemia could be both, the cart and the horse, a position difficult to explain physiologically.

When analyzing separately the hyperinsulinism and insulin-resistance (35, 41), between these two parameters exist an important rate of discordance (of ~30-40%). There is high uncertainty regarding the real significance of these two parameters, of which the first (plasma insulin), being directly measurable, is a more reliable parameter in comparison with that emerged from
The fasting plasma insulin/glucose ratio (HOMA - IR) or by the clamp studies, irrespective of which from the many existing variants is used (45, 46, 47). However, if we shall analyze the subjects not according to the calculated indicator of insulin resistance but in accordance with their BMI, we shall observe that insulin resistance (HOMA - IR) increases progressively with the BMI quartiles from 23±3 in q1, to 24±3 in q2, 29.5 ±5 in q3 and 30±4 in q4. So, BMI could be the simplest and easiest to measure parameter (23, 48).

Compensatory hyperinsulinaemia, as pathogenetic mechanism in type 2 diabetes, was the second great error consequent upon the first. Now it is well known that insulin secretory dysfunction of the β-cell is an early defect in T2DM (1, 11, 12, 49). In the UKPDS cohort including newly T2DM patients, plasma insulin levels were quite constantly under normal. At the onset of T2DM no indicator of the presence of insulin resistance (such as HOMA-IR) has been found (50). Moreover, neither hyperinsulinemia nor insulin resistance has been found clearly associated with cardiovascular risk (51). The hyperinsulinemia proposed by Modan et al. (41) a quarter century ago, as reflecting insulin resistance making a link with hypertension, obesity and glucose intolerance in human has not been proved. This, despite the effort made to convince medical community that insulin resistance might be the core pathogenetic mechanism operating in the metabolic syndrome (14). While hyperinsulinemia can occur in obese patients, an insulin resistance as a primary diabetogenic mechanism is not operating in the pathogenesis of T2DM as a change independent of obesity (36).

The second error led to the third error

The third error comes as a consequence of the first two. By the mid-20th century, researchers already anticipated that in "maturity onset diabetes", the pancreatic islets of these patients should be either normal or even enlarged. Since the histological examination of the pancreas at that time was far from being standardized and the clear phenotypical data regarding the distinction between T1DM and T2DM were difficult to assess, it was not difficult to obtain "histological pictures" to confirm the tempting hypothesis of peripheral insulin resistance and the effort of a β-cell to overcome it.

In fact, Ogilvie (52) already reported islet hypertrophy in obese subjects, so its presence in obese diabetic subjects (53) seemed to be natural. Indeed, the pathogenetic triad of peripheral insulin resistance/ hyperinsulinemia/β-cell and islet hypertrophy seemed a solid construction that explained its spread among the practitioners eager to have a logical explanation of various complex disorders such as T2DM, obesity, metabolic syndrome and cardiovascular disease.

In contrast with this view, many researches (12, 54, 55) demonstrated that at onset of clinical diabetes (the decompensation of blood glucose regulation, including also IGT and IFG) the β-cell mass is already severely decreased. Such a decrease in β-cell mass/function is considered as sine qua non condition for the decompensation
of blood glucose regulation and a preliminary condition for it (56, 57, 58).

The primary or real insulin resistance does exist, but is very rare

A key point in underpinning the insulin resistance hypothesis as the primary cause of T2DM was the discovery of the insulin receptor (IR) (59, 60). As insulin itself, IR is a vital molecule. Thus, homozygote IR knockout mice express precocious neonatal death (61, 62). Interestingly, mice with deletion of the IR gene only in one insulin-dependent tissue survive a long period of time (63).

In the years following the discovery and characterization of the IR, coinciding with the spectacular progresses of genetics, many researchers focused on the study of putative mutations/genetic defects of the two molecules (insulin and its receptor) in order to explain insulin resistance. This led to the description of several mutation of the insulin gene (64). However, their frequency proved to be very low (less then 1% in diabetic patients), with variable clinical expression and practically no relevance for the common T2DM phenotype.

The same was true for the IR gene. Several mutations have been identified on both ? and ?-chains of the molecule (60, 65). These mutations can lead to the decreased binding of insulin up to complete blockage by generating truncated or uncleaved IR (67). Other mutations can decrease or abolish the tyrosin kinasic action of the ?-chains. Again these gene defects proved to be very rare (18, 19, 60, 68). The main characteristic of subjects carrying these mutations was severe resistance to exogenous insulin (19, 61), accompanied by markedly increased plasma insulin levels, sometimes higher than 1000 µU/mL in the absence of hypoglycemia. They are mediated either by defects in the IR - type A syndromes (60) or by anti-IR antibodies - type B syndromes. Indeed, this is a true hyperinsulinemnic and insulin resistant state. They are known as "syndromes of severe/extreme insulin resistance" and included as such in the classification of diabetes (69). In this group is included also the insulin resistance associated with various types of lipodistrophy (20).

Severe insulin resistances are perhaps the only true hyperinsulinemic and true insulin-resistant states, having known molecular defects. They were "strategically" named extreme insulin resistances in order to make room for the "common" or supposed "mild" insulin resistance, proposed as we have showed, as the main pathogenetic mechanism in T2DM (26). What we contest is the involvement of such a physiological relationship as primary mechanism in pathogenesis of this diabetes phenotype.

The crossing year of 1979

An important chance for confirming or infirming the insulin resistance hypothesis as a molecular defect appeared at the end of the 7th decade of the last century. Thus, in 1979 Cox et al. (70) reported for the first time the results of a detailed study of the insulin receptor gene, cloned a few years before (71). Analyzing both the exonic and
Intrinsic regions of this gene on affected sib-pair families with T2DM, the authors could find no significant genetic defect that could sustain the involvement of IR in the putative insulin resistance proposed as the pathogenic mechanism for T2DM. This report was overlooked at that time, considering that surely, further genetic studies could soon bring the genetic proof of this concept. As we shall see, such genetic proof has yet to be found (48).

In the same 1979 year, a completely different impact of insulin resistance hypothesis had the publishing by Ralph DeFronzo et al. (13) of the euglycemic-hyperinsulinemic clamp method (13). Using this important method, an increased uptake of glucose by peripheral tissues implied good insulin sensitivity, while a decreased glucose uptake implied the presence of peripheral insulin resistance. The sustainers of the insulin resistance concept considered that, finally, a measurable method for insulin resistance was provided. Using two separate infusion lines (one for glucose and the other for insulin), the insulin level has been raised and then maintained at ~100µU/ml whereas the rate of glucose infusion has been increased or decreased in order to obtain a steady-state momentum. The rate of glucose infused (mg / kg b.w. or so called Ed or M value) in such a steady-state period was considered the main indicator for the sensitivity/resistance to insulin.

Since at that time diabetes was considered to be a disorder specific to glucose metabolism and not a disorder of the whole energy metabolism (including lipid and protein), as clearly showed by Paulescu in 1921 (4) and fully accepted today (34, 72), no one questioned why only glucose was chosen in order to explore the complex function of insulin receptor. What happens with the free fatty acids and amino-acids during a clamp test?

The laborious and time consuming clamp technique, accessible only for a few elite laboratories, becomes soon the "golden standard" for the investigation of insulin resistance (16, 47, 73, 74). In fact, this valuable method furnished more useful information on insulin secretion than on insulin resistance. However, some researchers signaled the large variations in the uptake of glucose induced by slight variations in the manipulation of the two venous infusion lines (insulin and glucose) in order to obtain the "steady state" level (75, 76) so that a result obtained in one laboratory could not be identical with that obtained in another one (77). A meta-analysis of the papers using the clamp technique on bigger or smaller animals showed that only half of them were performed according to the classic protocol (76). In other words, at least half the information provided by this kind of studies, supporting insulin resistance hypothesis could be erroneous (75). Unfortunately, a similar critical analysis for the various researches carried out with clamp methods in humans has not yet been made (76).

To this observation was added another one, even more important, specifically the non-physiologic nature of the increased levels of insulin during euglycemic-hyperinsulinemic clamp. Indeed, maintaining during 2-4 hours a supra-physiologic increased insulin level (~100 µU/mL) is never encountered in real life and current clinical practice, raising questions about the interpretation
of the final results. Finally, the euglycemic-hyperinsulinemic clamp provides no information on the molecular basis of the putative peripheral insulin-resistance.

The year 1979 marked also the onset of the "mathematical euphoria" in generating complicated formulas and abstract indexes for the assessment of the elusive insulin resistance. Apart the euglycemic/hyperinsulinemic clamp (13), in the same year (1979), Bergman et al (78) has published also "the minimal model approach" (MINIMOD), named also FSIVGTT (Frequently Sampled Intravenous Glucose Tolerance Test). The last was generated over a decade (1978-1989) by assembling 24 concepts constructed by 18 working groups of 1-4 researchers and theoretically could provide many information regarding insulin secretion, insulin action, and glucose effectiveness or disposition index (79). In fact, the parameters included in this model are several of the 24 glucose and insulin values (meaning 24 blood sampling), obtained along 3 hours of investigations. The MINIMOD program is used from time to time in some research laboratories.

The uncertainties or difficulties related to the exact significance of the clamp methods led to the generation of a large number of "insulin resistance indexes" as the result of some complex mathematical formulas based on the study between the basal or stimulated glucose and insulin level (47).

In 1985, Mattews et al. (80) proposed as index of insulin resistance a static and simple test, using the measurement of fasting glucose and insulin levels. Based on some mathematical formulas one can obtain the HOMA (homeostasis model assessment) index, with its two variants: HOMA IR (HOMA-R) and HOMA-S, indicators of insulin resistance, respectively of insulin secretion. By its simplicity, this technique is most often used in current practice despite the fact that is considered inferior to the gold standard for insulin resistance (26, 73, 74). It has been considered that the information provided by this index is inferior to the clamp method (26).

Both methods have been criticized (80, 81). For instance, the euglycemic clamp method gives an inter-subject coefficient of variation is 21% in normal volunteers (82) rising to 40% in subjects with T2DM. The relativity of the results provided by the clamp studies was recently evidenced by a study in which the results obtained in one elite laboratory could not be reproduced (even when using the same protocol) by other elite laboratories (77).

Stern et al. (23) analyzed the clinical relevance of different methods for measurement of insulin sensitivity / resistance such as the hyperinsulinemic / euglycemic clamp technique (13), the frequently sampled intravenous glucose tolerance test - FSIVGTT and the insulin suppression test. Analyzing 2,321 subjects for correlation between different indexes of insulin resistance and some routine clinical/biochemical parameters such as obesity, fasting glucose, insulin, lipids, and blood pressure, as well as family history, the authors reached the conclusion that it is possible to identify insulin resistant individuals by only routine clinical measures, (such as BMI and abdominal circumference,-in fact, the main indicators of obesity) possible to be
used in ordinary clinical practice (23).

How the insulin resistance concept reached its climax

In 1988, Reaven (14) proposes insulin resistance as the key pathogenic mechanism for the so called "metabolic syndrome", trying to explain the clustering of cardiovascular risk factors usually present in diabetes: obesity, dyslipidemia, and hypertension. The mechanism proposed by him had some logic: peripheral insulin resistance leads to hyperglycemia which induces high insulin levels and this in turn mediates the increase in VLDL and TG, decrease of HDL-cholesterol and even increase in blood pressure (14, 83, 84, 85).

Many authors including ourselves (34, 48) signaled the poor correlation between the various indicators of insulin resistance and the presence of the metabolic syndrome attested by fulfilling the somehow arbitrary criteria proposed by various classifications (ATP III, WHO, IDF). Thus, in our study performed on 442 randomly selected adult subjects (48) we analyzed the prevalence of the metabolic syndrome according to the level of insulin resistance defined by the HOMA-IR index. In this study, the prevalence of metabolic syndrome in the four quartiles of HOMA-IR was 20.9%, 20.72%, 21.81% and 41.4% respectively. Only the 75 percentile of HOMA-IR has been associated with a doubling of metabolic syndrome prevalence and this could be explained by higher BMI from q1 to q4. If we consider the 75% HOMA-IR as cut-point for defining insulin resistance, then the majority of metabolic syndrome subjects (60.35%) will fall below this threshold and only 39.65% of them will have insulin resistance (48). Using factor analysis in order to detect the main determinant for metabolic syndrome, did not succeeded to identify any unique factor suggesting that insulin resistance is not the real pathophysiological factor involved (fig 1).

The inconsistencies of the association of metabolic syndrome with insulin resistance assessed by various methods lead to the doubt regarding the mechanistic validity of this syndrome. So along the time the concept of metabolic syndrome was a subject for large controversies (51, 86, 87, 88, 89). A recent WHO Expert Consultation Report (90) stated that the metabolic syndrome could have a practical utility in informing about the cardiovascular risk, but the mechanistic explanation based on the presence of the insulin resistance as a common denominator has not been proved. Following that correct decision, Borch-Johanson and Wareham (91) announced the "death" of the metabolic syndrome as expressing insulin resistance. However, it has been accepted as an useful concept for clinical practice, that for two decades generated thousands of articles, a lot of monographs and many congresses (38). In our view, the statement that insulin resistance is the main primary defect in T2DM is equally unacceptable and need an appropriate correction. I think that this time is already here.

The flaws of insulin resistance concept

Insulin resistance is a theoretical...
concept that, obviously, cannot be measured directly but only deduced by analyzing the changes in peripheral utilization of proteins, lipids and carbohydrates, normally controlled by insulin. Since this concept was born by the erroneous interpretation of some data (subsequently infirmed), from time to time the concept required numerous changes. As this concept has not a precise content and boundaries, this explains its association with lipotoxicity, low grade inflammation status, oxidative stress and endoplasmic reticulum stress in various cells, among others. This is how, in the pathogenesis of insulin resistance, were included in time not only the adipocytes (39), hepatocytes (92) and miocytes (26), but also the pancreatic beta cells (93), the intestine epithelium (secretion of incretin hormones), the endothelium, the brain (43, 94, 95), and even platelets devoid of insulin receptors! (96). This is an exaggerate attempt to link insulin resistance and the prothrombotic risk induced by hyperactivity of platelets.

It is difficult to understand why despite its evident and numerous inconsistencies this concept never underwent a careful critical analysis. Two important problems would have required clarification: (1) first, is that of defining the molecular basis of insulin resistance, able to explain the pathogenesis of T2DM (26), of the metabolic syndrome (14), of NAFLD (21), of PCOS or of peripheral vascular disease (85). Fatty liver, for example, was sometimes considered the cause of insulin resistance and at other times, a consequence of this (21); (2) the second is that of defining precisely which of the clinical entities mentioned above results from a primary molecular defect related to its receptor and which are secondary to other biochemical alterations. Our point of view is that in the pathogenesis of T2DM, what is attributed to peripheral insulin resistance is closely related to obesity.

The distinction between which
derangement is primary and which is secondary is not only an academic debate, since the therapeutic approach is dependent on this. For a primary derangement, the target molecule (for example anti-receptor antibodies) could be borne in mind as a therapeutic solution. On the other hand, if it is secondary (to obesity, for example), the therapeutic target must be to prevent or to remove this derangement.

The need for this clarification is stringent, since thousands of researchers in thousands of laboratories seek to identify a "magic bullet" (38) for the putative molecules responsible for the insulin resistance hoping that their work for years was not a lost of time and resources. Unfortunately, the notion of insulin resistance was so abstract and permissive that the temptation to include in it any orphan pathology, lacking a mechanistic explanation was so great that the danger of a diagnostic "derailment" was great.

A cunning method for rescuing the insulin-resistance concept: intra-cell signalomics
An ingenious solution to rescue the concept of peripheral insulin resistance as pathogenic mechanism in T2DM, was to place the putative molecular defect in the hidden and complex intracellular molecular machinery, generating a new chapter known as post-receptor signalomics (62, 97). This refers to some of more than 100 intra-cellular proteins activated by trans-phosphorylation.

In a first stage, it was found out that signaling on the IRS-1 pathway could be decreased because instead of the phosphorylation of thyrosine, this occurs on serine/threonine, leading to the decrease in post-receptor insulin signal (62, 97, 98). A wave of genetic and metabolic studies followed in order to identify the putative genetic defect associated with this molecule. Numerous studies using different KO mice showed that IRS-1 molecule is essential for insulin intra-cellular signaling (98). A genetic defect consisting in the substitution of Glycine with Arginine in position 972 of IRS1 (G972R or rs1801278) was studied as the possible genetic basis of insulin-resistance. In vitro studies proved that the genetic manipulation of the IRS1 972 alleles is associated with alterations of the insulin signaling in different cell types. However, we point out again that this finding doesn’t prove that human T2DM exhibit such a defect (98, 99). The same observation is valid for the studies on transgenic mice in which IRS1 G972R SNP is hyper-expressed. It is explicable why the genetic studies devoted to IRS1 are extremely numerous. Initially, studies showed a possible association of the G972 allele with T2DM in humans (100), association that was not confirmed subsequently (101). The most recent meta-analysis (102), which analyzed 32 studies (including 12,076 cases and 11,285 controls), could not find significant association of the G972R IRS-1 SNP with T2DM. The authors consider that, in order to ascertain the contribution of "low-frequency-low-risk" variants to T2DM (as is the case of G972) a number of ~200,000 study individuals would be needed to have 80% power to identify a 9% increase in diabetes risk at a genome-wide
signification level (102). The difficulty for approaching such sensitive topic related with sensitivity/resistance to insulin has been recently underlined (103).

Researchers adopted an indirect approach to explore pathogenesis of diabetes by studying animal models considered to be insulin-resistant, hoping to extrapolate that data in human diabetes. In addition the silencing some protein molecules using the small interfering RNA technique (si-RNA) and the generation of multiple types of transgenic mice (MIRKO, FIRKO, LIRKO, NIRKO, BIRKO, VENIRKO - corresponding to the deletion of IR in muscle, adipocytes, liver, neurons, β-cells or vascular endothelial cells), became a true industry in many countries (104). As expected, silencing or elimination of IRS-1 led to the generation of a true insulin-resistance, especially in conditions of high fat diet. It is obvious that these studies had no direct relevance for the human diabetes. They only proved what was anticipated, i.e. alteration of the function of some important intra-cellular kinases is followed by the alteration of various metabolic pathways inside these cells.

The new chapter of epigenetic modulation of genes opened the new field of the influence exercised by environmental factors on the determinants of the metabolic disorder (43). Such metabolic changes do not reflect a primary mechanism, but a secondary event.

**Uncertainties regarding the localization of insulin-resistance**

Initially it was suggested that adipocytes could represent the site of the putative insulin-resistance. However, insulin resistance in obesity is a nonsense, because insulin has an anabolic effect and the resistance to this effect must induce a catabolic effect and lose weight, which is contrary to the reality.

At the end of 2009, reunited in a seminar dedicated to this topic, each of the invited speakers provided successively "strong evidence" that the primary site of peripheral insulin resistance is represented by the adipocyte (39), hepatocyte (92), skeletal muscle (26), brain (94), intestinal epithelium (105), vascular endothelium (106) or pancreatic β-cell itself (107, 108). If the pathogenic basis of this syndrome can be explained in 7 different ways, than this concept has a serious problem of identity. However, if one analysis carefully all these 7 approaches, it can be easily noticed that their unique common element is overweight / obesity / lipid overflow. The inclusion of the pancreatic beta cells among the cells that could generate insulin-resistance (108) via their hyperexcitability inducing hyperinsulinism, represents an attempt to bring back the intrinsic β-cell defect at the forefront of T2DM pathogenesis (1, 29, 36, 49, 56-58).

For many researchers, the main "competitor" for the primary site of peripheral insulin resistance remained the skeletal muscle which, in normal conditions, "burns" ~75% of the total daily metabolized glucose (109, 110, 111, 112). A mitochondrial dysfunction has been involved via increased IRS1-serine phosphorylation and a decrease in glucose uptake and utilization (110, 113). However it is worthy of note that the direction of this disorder is not the
classical downstream from the IR to mitochondria, but upstream, passing along the multiple steps from mitochondria, back to the IRS1 and then to IR. This is a typical secondary defect, strongly related to what I called, the high biochemical pressure induced inside the energy system, which in turn is secondary to low muscular activity.

**The relationship obesity-diabetes**

The "couple" diabetes-obesity is known for a long time (114). In a study performed on a large cohort of diabetic patients, published by us more than 3 decades ago (25), we found that weight excess was recorded in ~90% of diabetic subjects. More recent data show that an excess of lipids in one or more compartments (subcutaneous intra-abdominal, intra-hepatic, extra- or intra-miocellular) is recorded virtually in all diabetes cases supporting the lipotoxicity hypothesis (115, 116) as one of the main diabetogenic mechanism.

Between the β-cell mass and the adipocytes-mass there are much more complex correlations than currently known. An older but very interesting study performed on human volunteers (117) demonstrated that, a hypercaloric diet is associated with an increase in plasma insulin proportionally with the magnitude of weight gain. In the same subjects, weight loss following a hypo-caloric diet led to a decrease in plasma insulin levels back to normal limits. On the other side, in a prospective study performed on Hispanic obese children with a family history of diabetes (118), it has been found that the "progressors" had a precocious β-cell dysfunction (decreased acute insulin response) even in subjects with normal insulin sensitivity and increased visceral obesity.

The pathogenic relationship diabetes-obesity can be seen as the result of the functional disequilibrium between these two cell-mass: while pancreatic β-cell seems to be a post-mitotic one (119) and in adult people any β-cell loss can not be replaced by a new one, the adipocyte cell mass can increase (by proliferation or hypertrophy) several times. The functional mutual relationship between pancreatic β-cell mass and adipocyte cell mass deteriorates when the adipose tissue has to store a surplus of energy as lipid deposits beyond its physiologic "programmed" capacity. This will transform a "quiet" adipocyte in a "restless" (dysfunctional) and then in an "aggressive" (clear pathogenic) adipocyte.

**Peripheral insulin-resistance in the light of the new evidence**

In the last years, the peripheral insulin resistance hypothesis received two serious blows that should lead to its repositioning as a secondary diabetogenic mechanism, both chronologically and as importance.

The first blow came from the recent genetic large scale GWA studies in T2DM which managed to identify ~20 T2DM associated genes. The first set of genes (~16) is associated with molecules involved in the β-cell function, even if their precise mechanism of action is not fully understood. The second set of genes is smaller and includes three genes associated both, with diabetes with BMI. The most important is the FTO gene. It
encodes some molecules (mainly expressed in hypothalamus) involved in the regulation of body weight (120). The second most important is the PPAR

gene involved in the differentiation and functioning of adipocytes (121), erroneously considered as associated with peripheral insulin resistance. Other genes associated with BMI such as MC4R or PCSK1 were recently described. However, as expressed in the title of a recent review paper ("Where are the insulin resistance genes?") none of these genes has been actually associated with insulin resistance (122).

The second blow was represented by bariatric surgery, named also "metabolic surgery" (123). The presence of peripheral insulin resistance in morbid obese diabetic patients, confirmed by using the "golden standard" technique (13) disappears rapidly in a period of days or months after surgery (123), in parallel with the weight loss (124). It is obvious that a primary, genetically encoded molecular defect, as base of insulin resistance, could not disappear in these conditions. The disappearance of "insulin resistance" in this case is secondary to the decrease of the biochemical pressure inside the energy system with the concomitant decrease of the pancreatic β-cell over-load.

Attempts to elucidate the true pathogenetic role of insulin resistance and β-cell defect in type 2 diabetes have been made in the past (11, 36) and also more recently (1). Insulin resistance was placed in doubt by these authors as a primary pathogenetic mechanism, without emphasizing the urgent need for a clarification regarding their real role in diabetogenesis. It is my hope that the more critical tone of this presentation will stimulate a useful and balanced debate regarding the real role of the β-cell dysfunction and insulin resistance, not only in pathogenesis of type 2 diabetes, but also in other metabolic derangements. Recently, in the October issue of Diabetologia Jenkins an Campbell on one side (125) and Mari and Ferrannini (58) on the other side, exchanged "letters" regarding the interrelationships between β-cell function, insulin sensitivity and glucose tolerance at a such level that, finally the readers remain to decide themselves if Ptolemy or Copernicus were right in their view on the planetary dilemma.

CONCLUSIONS

A more terrestrial approach is necessary for a practitioner in order to understand what really happen inside their patients and what therapeutically measure is more appropriate for them. Of course, avoiding mathematical formulas and refined biochemical philosophy.

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