THE PROINSULIN-TO-ADIPONECTIN RATIO COULD BE THE BEST PRACTICAL INDICATOR OF THE EARLY TYPE 2 DIABETES

Constantin Ionescu-Tîrgovişte, Răzvan Vasilescu, Romulus Timar, Cristian Guja, Paul Gagniuc, Elena Băcanu, Ramona Gligor, and Simona Carniciu

National Institute of Diabetes, Nutrition and Metabolic Diseases, „N.C. Paulescu", Bucharest, Romania

Abstract

In 1974, the "Ariadne's thread" (from Greek Mythology, pick one's way through the Labyrinth) of autoimmunity leading to the immunogenetic theory of type 1 diabetes mellitus made it possible to conclude that in this phenotype there exists an unsolved conflict between β-cells and the body's autoreactive immune system. In type 2 diabetes mellitus (T2DM), the "Ariadne's thread" links obesity to pancreatic β-cell biology. Unfortunately, beginning from a wrong interpretation of the relationship between plasma glucose and plasma insulin, the insulin resistance hypothesis was born and claimed as the main defect of T2DM. Overlooking obesity, the understanding of T2DM pathogenesis was delayed by almost half a century. Fortunately, the adipobiology, particularly adipocytes was able to reach the "drawing board" of researchers, and has all the chances these cells to become the "cell of the century," just as the pancreatic β-cell was last the cell of century. The association of diabetes and obesity has motivated us to put pancreatic β-cells and adipocytes head-to-head, that is, we moved from the T2DM-obesity couple to the β-cell-adipocyte couple, appreciating their main secretory products, insulin/proinsulin, C-peptide, amylin (for β-cells), and adiponectin, as well as other numerous adipokines (for adipocytes). We thus propose that the proinsulin-to-adiponectin ratio could become the earliest predictor of dysfunction of these two cell types.

Knowledge means
to know the cause.
Aristotle of Stagira
(348-322 BC)

The knowledge of disease means
the knowledge of its cause.
Paulescu of Bucharest
(1869-1931)

Introduction

The association between diabetes and obesity (recently termed "diabesty") has been known for a long time. Lancereaux in 1877 made the distinction between "fat" diabetes and "lean" diabetes (1,2). Along with other researchers (2-4), we also have approached this "old couple" (3), documenting that this association is related to the degree of obesity and also with its duration. About 90% of 10 000 cases of newly diagnosed diabetes analyzed by us were overweight or obese (5). In an interesting study by Sims and Horton (6) carried out on normal weight volunteers, they demonstrated that a hypercaloric diet leading to weight gain will show a proportional increase in plasma insulin

levels. However, soon after losing the acquired weight through food restriction, plasma insulin returned to previous basal levels. The relationship between body mass index (BMI) and insulin is now well known (7-11) and can be explained by increased number of insulin dependent cells, rich in insulin receptors. It is obvious that such an enlarged adipose tissue will need higher amounts of insulin. However, such an increasing overload of function will become an important supplementary diabetogenic factor, aside from inherited diabetogenic architecture which can comprise some of the ~50 genes associated with diabetes (12). It is important to know that adipose tissue is the only type of tissue which can increase by 3, 5, or even more times.

The increased insulin secretion observed in obese people has been considered as expressing an insulin resistance. This way of thinking stemmed from the paper published by Yalow and Berson in 1960 (13) in which their results were reported regarding blood glucose and plasma insulin assessment in diabetic patients and in normal (control) subjects, both in fasting state and after stimulation with 100 g or 50 g of glucose intake. Overlooking the plasma glucose levels, evidently higher in diabetic patients than in controls, they suggested that "maturity-onset" diabetics, the actual type 2 diabetes mellitus (T2DM), are "hyperinsulinemic." Of course, after making a correction for insulin plasma levels *versus* blood glucose, such hyperinsulinism does not exist. Rosalyn Yalow (13) was an expert in nuclear physics and Solomon Berson (13) a medical thinker, but not a medical practitioner.

After the Nobel Prize in Physiology of Medicine was awarded in 1978 to Rosalyn Yalow for remarkable achievements in discovering the radioimmunologic method for the assessment of insulin and later for other hormonal biological compounds (Solomon Berson died in 1972), their data referring to the *hyperinsulinism* recorded in newly diagnosed diabetic patients resulted in a way of thinking that went in the wrong direction. This occurred because of a lack of minimal clinical data (age and BMI, for instance), which is an absolute requirement for correct interpretation of insulin levels. In this context, the idea was promoted that the first defect in diabetes is an *insulin resistance* somewhere in the body and the normal β-cell will react by inducing a hypersecretion of insulin (hyperinsulinism) in order to overcome the supposed insulin resistance. During the long-term struggle of the β-cell with this mysterious insulin resistance, β-cells will progressively decline, leading ultimately to total failure. It is amazing to behold today how the wrong interpretation of some abnormal biochemical data can be so ingeniously muddled, based on some logical supposition, and supported mainly by numerous and complicated investigations, such as the "euglycaemic-hyperinsulinemic clamp" of De Fronzo (14), the "minimal model" of Bergman *et al* (15), followed by what we have termed a "mathematical euphoria" (16) which has borne many tens of indices for insulin resistance-sensitivity-disposition index, resulting from elaborate mathematical formulas. Such an "aura of elevated science" is imposing for any practitioner, leaving the impression that this phenomenon does indeed exist. However, some clinicians conclude that similar useful information could be obtained by such sophisticated methods can result simply from abdominal circumference measurements (17).

In 1985, Matthews *et al* (18) put fasting plasma glucose and insulin in a formula, resulting in what is called the Homeostasis Model Assessment (HOMA)-IR (insulin resistance), the most commonly used index of insulin resistance in epidemiological research. Because plasma insulin levels increase with increasing body weight, almost all overweight/obese patients are characterized as insulin resistant. Such a characterization is also made when a β-cell defect results in hyperglycemia. In order to make a distinction between the two components, sensitivity to insulin/ β-cell defects, a new formula called HOMA-B was developed, offering unconvincing information.

The insulin resistance hypothesis reached its climax in 1988 (19) when Gerald Reaven published his observation leading to the concept of metabolic syndrome. For a short period of time, this syndrome was also called "insulin resistance syndrome" (16). After a re-evaluation of this concept (20-25) in the Editorial in *Diabetologia* (25), the eulogy given by Borch-Johansen wishing insulin resistance syndrome "rest in peace" seems to be rather premature, even if this "phantom concept" reemerges from time to time once in a blue moon. I predict that what is Caesar's will be rendered unto Caesar, and what is attributed to insulin resistance will be given to obesity.

The pancreatic β-cell-adipocyte couple

Now, let us move from the T2DM-obesity couple to the pancreatic β-cell-adipocyte couple. The system which ensures energy homeostasis of the human body is very complex and includes several hypothalamic nuclei and limbic system, with their complex orexigenic and anorexigenic pathways influencing alimentary behavior and food intake. A more complex behavior involving the cerebral cortex also influences the degree of physical activity and levels of energy utilization. The disequilibrium between food/caloric intake (increased) and physical activity (decreased) is promoted by the actual lifestyle which explains stepwise increase in overweight, obesity and T2DM. When such disequilibrium appears, all the components of the energy system react in order to adjust the inactivity according to the new biochemical reality.

The most important subsystem is adipose tissue with its im-

pressive capacity to process and store high to very high amounts of lipids. Its role as a "buffer" system is one of its main metabolic functions. Ancestrally, its secretory function was adapted more to conserve energy. This is why adiponectin secretion mainly in the omento-mesenteric compartment (26) has a role of optimizing the utilization of fatty acids in various organs (liver, skeletal muscle, pancreatic β-cells), maintaining their normal function. Unfortunately, adipocyte function in overweight and obese conditions, our genetic makeup which was not programmed to waste but to conserve energy, and as such, will inherently counteract any attempt in weight reduction, except through the energy consumed by skeletal muscles during physical exercise. Alas, modern imprudent socio-economic patterns which lead to increasingly sedentary lifestyles place the adipocyte in an inescapable dilemma.

What about the pancreatic β-cell? This veritable "metabolic brain" is asked to solve a challenging pathologic condition that is, in fact, unsolvable, in which there exists a large amount of fat accumulation in obese individuals, not only in a protected form (inside adipocytes), but also outside this tissue as ectopic fat deposits, sometimes intracellular and other times extracellular. It must compensate for this additional fatty mass secreting more and more insulin in less and less physiological circumstances.

The endoplasmic reticulum stress in pancreatic β-cells and adipocytes

Each nucleated cell, especially secretory cells (such as pancreatic β-cells and adipocytes) have a well developed endoplasmic reticulum (ER)-Golgi apparatus (GA) networks (Fig. 1A). Here take place various post-translational modifications of transcripted preproproteins mediated by polyribosomes attached to ER surface. However, between pancreatic β-cells and adipocytes there are many important differences. The pancreatic islets cells have all together a mass of ~1.5 g. In each islet, there are ~5000 various types of cells (α – glucagon secretion; β – insulin secretion; δ – somatostatin secretion; γ – pancreatic polypeptide secretion;
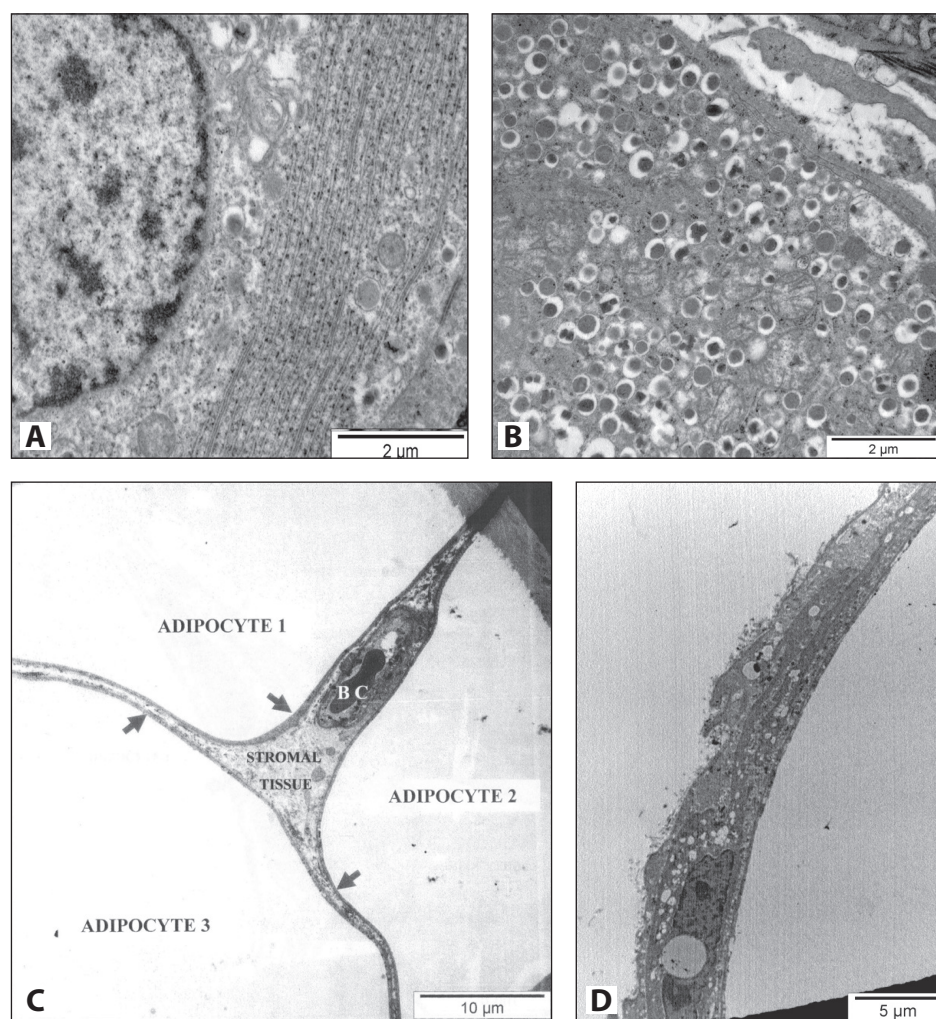
Figure 1. A. An electron microscope image from a section of pancreatic β-cell showing the nucleus (left) and a well developed rough endoplasmic reticulum (right) with a few secretory vesicles present **B.** An electron microscope image from a section of pancreatic β-cell showing numerous secretory vesicles. **C.** Electron microscopy from subcutaneous adipose tissue showing three adipocytes and stromal tissue between them and a capillary vessel (BC). **D.** Section of a large adipocyte showing the nucleus, endoplasmic reticulum and few mitochondria. **Magnification**: A, bar 2 μm; B – 2 μm; C – 10 μm; D – 5 μm.

ε – ghrelin secretion), of which only ~3000 are β-cells, which secrete insulin, C-peptide, and amylin. In contrast, the adipocytes number is enormous (~1.6 billion), weighing ~13 kg at a BMI of 22 kg/m^2 and 35 kg at a BMI of 35 kg/m^2 (27), with possibility to increase manifold in morbid obese subjects (BMI >45).

The ER is a proximal cellular site that is able to sense "biochemical pressure" occasioned by fuel excess in the energy system as well as react in a compensatory manner. To function properly, the ER relies on numerous resident chaperone proteins, higher calcium content, and an oxidative environment. Any perturbation in the function of this system may trigger accumulation of unfolded proteins and activates a specific ER stress response, known as the "unfolded protein response" (UPR) (28). This reaction led either to the reestablishing of physico-chemical derangements or to the triggering of apoptosis of these cells. The same model of reaction appeared in β-cells, where this mechanism was better studied (29,30), but also in adipocytes (31,32), and later in hepatocytes and other insulin-dependent or even non-insulin-dependent cells.

The apoptosis of a cell is accompanied by an inflammatory reaction, which in adipose tissue is very high due to large adipocyte volume. Any supplementary lipogenesis, in a large adipocytes is made with the price of apoptosis. That explained the proinflammatory reaction induced by the signals sent by large adipocytes such as through monocyte chemoattractant protein-1 (MCP-1), serum amyloid A3 (SAA3), retinol binding protein-4 (RBP-4), resistin, visfatin, vaspin, and others. These signal molecules are included in exosome-like structures which are multivesicular body (endosome)-derived microvesicles (50-100 nm), secreted in an oscillatory fashion through an exocytotic machinery acting in adipocytes (33). Under healthy conditions, the human body is in a state of "controlled inflammation" regulated by a delicate balance of pro-inflammatory factors, e.g. tumor necrosis factor-alpha (TNF-α), leptin, interferon-γ, interleukin-1 (IL-1), IL-6, IL-12 and anti-inflammatory/metabotrophic factors, e.g. adiponectin, IL-10, NGF, BDNF, which may also be produced by adipose tissue cells including adipose-associated immune cells (macrophages, mast cells, T-lymphocytes) (reviewed in 34).

The pancreatic β-cell versus the adipocyte

It is worthy of note that many of the current uncertainties concerning the function and dysfunction of these two cell types, the pancreatic β-cell and the adipocyte, are due to the disequilibrium between the information sources available. Many come from studies carried out on animal models, in transgenic mice, in *ex vivo* cultured cells, and very few studies performed on human pancreatic and adipose tissue.

Few people are aware that the diameter of a large adipocyte

(~200 μm) is equal to the total diameter of all pancreatic islets (~250 μm), in which ~5000 of various cells (α, β, γ, δ, and ε) are distributed. We do not have data regarding the cumulated ER for a single pancreatic β-cell and for a single adipocyte, but that for β-cells might be larger and, probably, have a higher activity than their adipocyte counterparts (Fig. 1C, D).

The β-cell mass is generally programmed to produce an amount of insulin covering not only the needs of normal weight subjects, but also supplementary secretory capacity in order to cover the higher insulin needs in some physiological conditions (i.e., pregnancy), and also a higher amount of skeletal muscle cells (hard workers), or excessive fat deposits (in overweight or obese subjects). Why such supplementary secretory overload can't be covered in about 30% of the overweight/obese people is not well known. These are prone to developing T2DM and cardiovascular disorders during their lifetime.

The vulnerability of a marvelous cell

Among the 3 billion pancreatic β-cells, there is a large heterogeneity (35). With its neuronal phenotype, the β-cell can react promptly and efficiently to all biochemical changes in concentrations of various fuels (carbohydrates, amino acids and fatty acids). This refined dose/response reaction depends on the inclusion of insulin (its main secretory product) in small organelles called secretory vesicles (SV). These are, however, complex and stable structures which appear in a nascent form in the ER-GA compartment where nascent vesicles are produced in tight correlation with the amount of exocyted SV. Upon looking at such a secretory β-cell, what is impressive is the high number of such SV (Fig. 1B). In their way, beginning from ER-GA to the last "ready to be released" compartment, the "immature" vesicle became a "mature" vesicle in which up to 99% of proinsulin is cleaved into insulin and C-peptide, and insulin is stored in a concentrated form as hexameric insulin crystals which appears as an electron dense core of such vesicles. Dispersed in the surrounding cytosolic halo can be found, in a very acidic medium (pH 5–5.5), C-peptide, amylin, small amounts of proinsulin and proamylin, and enzymes, chaperones, Ca^{2+}, Zn^{2+}, and other ions, or other small molecules with unknown functions.

From the translation of the preproinsulin gene as a peptide of 110 amino acids, to their appearance as complex three dimensional mature insulin molecule takes approximately 4–5 h. From the ~12 000 SV continuously existing in a β-cell, only a small part are stored in a "ready to be released" compartment, close to the cell membrane.

In fact, the syntagma used by Orci 30 years ago, for β-cell as an "insulin factory" (36), can be better characterized as a "secretory vesicle factory". No regulated secretion of insulin can be possible

without the inclusion of insulin in these fascinating organelles, in which each contain about 300 000 insulin molecules. There is, however, a high price for this refined structure and function. In adults, these cells are post-mitotic cells, and neogenesis is either very low or (especially in diabetic patients) completely absent (37). In such a condition, we can state that the onset of diabetes is the moment when an apoptosed β-cell cannot be replaced with a new one. Fortunately, β-cell loss occurs very slowly, in that for the decompensation of blood glucose regulation (onset of clinical diabetes) this process may take years, even tens of years. This is why the highest incidence of T2DM is around the age of 60 years. By the time diabetes is diagnosed, about 50% of β-cell mass/function has already been irreversibly lost. It is obvious that diabetes starts earlier, and when hyperglycaemia appears, what we must do is to prevent the severe chronic complications of diabetes, to nip them in the bud ahead of time.

The diabetogenic role of adipose tissue

The good news is that not all obese people develop diabetes, only 30%. The bad news is that in our days, in an increasing number of individuals, diabetes onset appears at younger ages than before (27). This higher number of new cases of diabetes is recruited especially from overweight and obese children, adolescents or young adults (27). If our aim is to stop this diabetic trend, we must prevent obesity, acting in each growth and developmental period, with preventable lifestyle interventions. So, nowadays, the main goal is to detect diabetes when the first diabetogenic mechanisms begin. Hyperglycemia must remain an important parameter, but we must replace it with new and earlier indicators of the functional disequilibrium between pancreatic β-cells and the adipocytes. Recent data demonstrate that the neurotrophins nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are produced by peripancreatic adipose tissue and might be involved in the regulation of insulin secretion as well as the pathogenesis of metabolic syndrome and T2DM (38, also see Hiriart *et al* in this issue of *Adipobiology*).

We will attempt to demonstrate that β-cell and adipose tissue dysfunction must be reflected in the level of plasma proinsulin and adiponectin.

Visceral versus subcutaneous obesity

Classically, visceral obesity was considered much more pathogenic than subcutaneous obesity (39-42). Is the visceral (omento-mesenteric) compartment more pathogenic than subcutaneous adipose tissue? A conclusive response to this question is impossible in the present time. Contrary to what is currently accepted that visceral adipose tissue is pathogenic and subcutaneous adipose tissue is protective, we can provide some physi-

ological arguments in favor of normal function of omento-mesenteric adipose tissue. First, the visceral adipose tissue expresses more adiponectin, a potent anti-inflammatory and anti-diabetic adipokine, than any other adipose compartment (26) and expresses less pro-inflammatory adipokines (26). Also, it is possible that adipocytes from omento-mesenteric compartment have a lower turnover than adipocytes from abdominal subcutaneous tissue. From this point of view, the visceral adipose tissue could have a greater role as an endocrine, adiponectin-secreting organ compared to a metabolic role (as a buffer system for fatty acids) of subcutaneous adipose tissue; adiponectin secretion decrease reflects its severe dysfunction.

Silent, restless, and aggressive adipose tissue

In the classification recently proposed by one of us (16), adipocytes can pass through three different functional/dysfunctional phases: the *silent (quiet)* adipocyte; the *restless* adipocyte; and finally, the *aggressive* adipocyte. The notion of the *silent* adipocyte corresponds the physiological activity which the adipocyte carries out in a clinically unnoticeable way. The crosstalk of adypocites with all other cells belonging to the "energy administration system" of the human body which works precisely.

The notion of the *restless* adipocytes corresponds to the production of many helper signals sent to circulating monocytes (via MCP-1), which accumulate in dysfunctional adipose tissue (e.g., in hypoxia due to the discordance between angiogenesis and adipogenesis), or by the increasing diameter of hypertrophied adipocytes, exceeding the oxygen diffusion capacity in their deeper stratum.

The adipocytes (in fact, adipose tissue) become *aggressive* in subjects with a higher degree of obesity (BMI >40), and many apoptosed adipocytes attract a high number of macrophages which surround the dead adipocytes, giving a "crown-like" appearance. These macrophages also produce numerous adipokines such as TNF-α, IL-6, IL-10, RBP-4, resistin, visfatin, vaspin, which through the systemic circulation induce the so-called low-grade inflammation status. In this condition, adiponectin production decreased, and that heralded the onset of T2DM and cardiovascular disease.

The importance of this pathophysiological classification of adipose tissue, whose characteristics are listed in Table 1, have two goals: (*i*) it permits a better understanding of the etiopathogenesis of T2DM associated with obesity, and (*ii*) to use some of the adipocyte's molecular markers for earlier identification of active diabetogenic mechanisms, which sometimes precede from years to decades the irreversible decompensation of blood glucose regulation.

An important factor in the progression from the silent to the

Table 1. The main characteristics of silent, restless and aggressive adipocytes

| Indicators of adipocyte secretory function/dysfunction*	Silent	Restless	Aggressive
Adiponectin	Normal	Decreased ↓	Decreased ↓↓
Leptin	Normal or ↑	Increased ↑	Increased ↑
RBP-4	Normal	Increased ↑	Increased ↑↑
IL-10	Normal	Decreased ↓	Decreased ↓↓
TNF-α	Normal	Increased ↑↑	Increased ↑↑↑
IL-6	Normal	Increased ↑	Increased ↑↑
Resistin	Normal	Increased ↑	Increased ↑
Visfatin	Normal	Increased ↑	Increased ↑↑
Progranulin	Normal	Increased ↑	Increased ↑↑

*Based on information from various published sources.
RBP-4, retinol binding protein-4; TNF-α, tumor necrosis factor-alpha; IL-10, interleukin-10.

restless stage is the increased volume of adipocytes from ~300 picoliters to ~750 and then to 1000 picoliters (43-45), which corresponds to an increase in adipocyte diameter from 90 μm (in silent adipocytes) to 120 μm and then over this value ("giant adipocytes"), in *restless* or *aggressive* adipocytes.

Diagnostic markers for early stage of diabetes

Following our previous studies, we proposed for the detection of early phase of diabetes the *proinsulin-to-insulin ratio* (46-51). The latter is increased in all phenotypes of diabetes (Fig. 2), even in prehyperglycaemic stages and in obesity, heralding the future decompensation of blood glucose regulation. However, both of these parameters, insulin and proinsulin, reflect the same defect in β-cell function following the above mentioned defect in the cleavage of proinsulin to insulin and C-peptide, indicating a defect in maturation of the SV (52).

Recently, Giannini *et al* (53) performed an interesting study on adolescent obese subjects including 1600 Caucasians, Afro-Americans and Hispanic youths. All subjects underwent a single oral glucose tolerance test (OGTT) and 80.2% were normal glucose tolerant (NGT) according to classical definition of normality: fasting plasma glucose <100 mg/dl and 2-h glucose <140 mg/dl; 18.8% were IGT (>139 mg/dl but <200 mg/dl at 2h). Then, 60 NGT subjects were divided in two subgroups according to the 2-h blood glucose value: <100 mg/dl; 100-119 mg/dl and
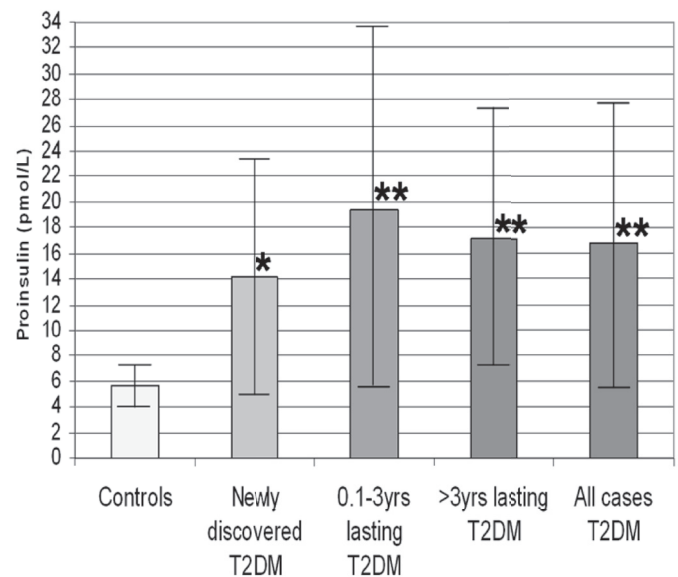
Figure 2. Plasma proinsulin levels in various subgroups of T2DM patients versus controls. NS, not significant, *p<0.05, **p<0.01 *versus* controls (from ref. 49).

120-139 mg/dl. An additional 21 IGT group was also included. These 4 groups were also evaluated two years later. The authors sought to determine whether obese adolescents with high "normal" 2-h post OGTT present or not some pathologic signals. A very careful investigation was made with both *basal/static* methods (determining many careful anthropometric and biochemical parameters, including proinsulin and adiponectin levels) and *dynamic* state-of-art techniques (hyperglycaemic-euglycaemic clamp and hyperglycaemic clamp) in order to detect earliest defects in insulin secretion and sensitivity to insulin. They found that first phase insulin secretion decreased across NGT categories, but second phase insulin secretion was similar across all NGT and IGT groups. Also, the *disposition index* ($_c$DI, which results from glucose disposal x sensitivity first phase insulin secretion) decrease across categories. This last parameter can be a summing up of the fundamental defects involved in T2DM: *insulin secretory defect* and a decrease in *peripheral glucose uptake.*

The same information referring to the both dysfunction (in the β-cells and adipocyte cells) results from the ratio between plasma proinsulin (an indicator of β-cell dysfunction) and plasma adiponectin (an indicator of adipocyte dysfunction). From Figure 3, on the base of our 300 studied cases (172 M/188 F, divided in normal weight controls, overweight, non-diabetic obese and obese diabetic) result a clear increasing ratio starting from normal weight subjects to normoglycaemic overweight and to a highest ratio in hyperglycaemic obese patients.

In order to check if our data are valuable, in Figure 4 we had showed the proinsulin-to-adiponectin ratio using the data presented by Geannini's group in their paper (53). The proinsulin-to-adiponectin ratio appears in the upper part (A) of the figure, whereas in the bottom part (B) is given the composite cDI index. Indeed the ratio proinsulin-to-adiponectin mirrors cDI and indicates a dysfunction in both β-cell and peripheral tissues. The clearer information obtained from proinsulin-to-adiponectin ratio in our patients (Figure 3) vs. the same ratio in Geannini's paper (Figure 4A) can be explained by the higher patient's age in our groups than their group including adolescent subjects

In fact, this state-of-the-art technique supports the validity of the proinsulin-to-insulin ratio. The contribution of an increased ratio (an increase in proinsulin, or a decrease in adiponectin) might give us a clue for better understanding the contributions of the individual dysfunction of these two important cell types.

It is obvious that such an elegant investigation carried out by Giannini *et al* (53) which are laborious, costly and time-consuming is not destined for clinical practice. Instead what we propose is to obtain in a single blood glucose sample as much diagnostic information as possible from several essential determinations such as glucose and HbA1c (routinely used) and, additionally,
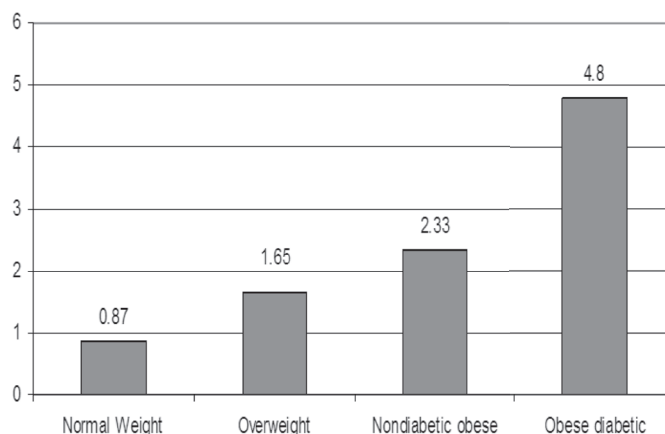
Figure 3. Cumulative data regarding proinsulin-to-adiponectin ratio obtained in various categories of nondiabetic and diabetic overweight and obese subjects: 60 normal weight; 80 overweight; 75 nondiabetic obese; 120 obese diabetic.
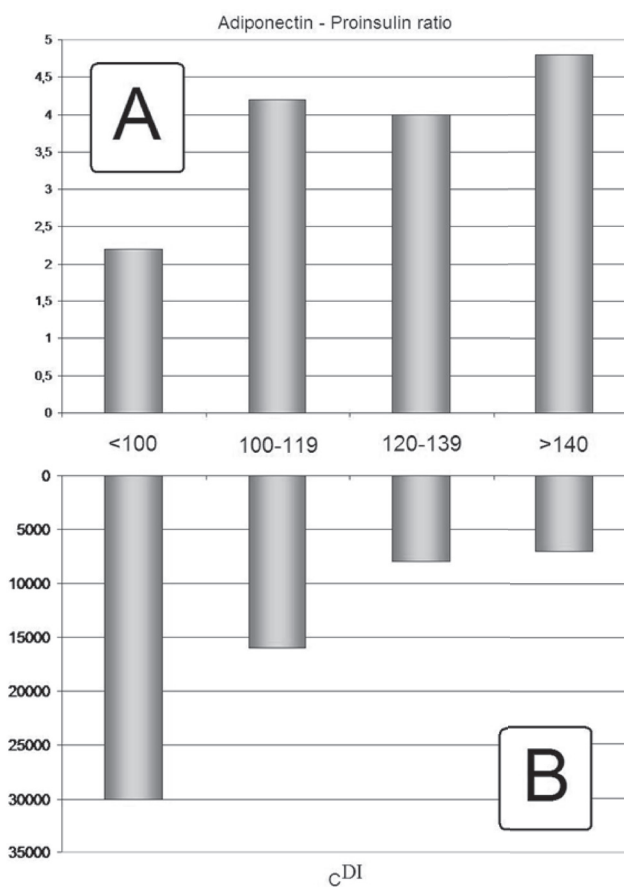
Figure 4. The parallels between information given by the proinsulin-to-adiponectin ratio (**A**) and from the $_c$DI (resulting from glucose disposal x sensitivity first phase insulin secretion) (**B**). See text for explanation.

plasma insulin, proinsulin and adiponectin. Otherwise in a recent commentary published in Diabetes, Kulkarni (53) underlines the urgent need for identification of useful biomarkers for early phases of diabetes. Our efforts, is inline with this purpose. Using the information obtained through individual analysis of few hormonal parameters, we can also obtain not only diagnostic, but supplementary pathogenic information, such as HOMA-IR and HOMA-B, as well as the proinsulin-to-insulin (48-51) and proinsulin-to-adiponectin ratios.

For a large clinical utilization of such parameters, the main current barrier is the cost of proinsulin and adiponectin determination. However, by a large usage in clinical practice their cost may rapidly decrease. If the value of our ratios will be confirmed in prospective populational studies, then very soon, a new classification of diabetes (regarding their staging) will be necessary. Such a classification requires a change in the criteria for definition of diabetes, replacing hyperglycaemia (the current diagnostic criteria) with earlier indicators of β-cell/adipocyte dysfunction. In fact, we are trying to change the *epiphenomenon* hyperglycaemia with *phenomenon* itself which is the progressive decrease in β-cell mass/function. In present, diagnosis of diabetes based on hyperglycaemia detects only those cases in which > 50% of β-cell mass/function has already been irreversibly affected (54, 55).

Acknowledgments

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