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Subclinical Diabetes

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is increasing in prevalence worldwide, and those non-diagnosed or misdiagnosed comprise a significant group compared to those diagnosed. Accumulated scientific evidence indicate that the current diagnostic markers (fasting glycemia, 2h glycemia after an oral glucose load and HbA1c) are indeed late diagnostic criteria when considering the incidence of diabetes-related complications and comorbidities, which are also at high risk in some groups among normoglycemic individuals. Additionally, the earlier identification of future risk of diabetes is desirable since it would allow better adherence to preventive actions such as lifestyle intervention, ultimately avoiding complications and minimizing the economic impact/burden on health care expenses. Insulin resistance and hyperhormonemia (insulin, amylin, glucagon) are non-disputable hallmarks of T2DM, which already takes place among these normoglycemic, otherwise health subjects, characterizing a state of subclinical diabetes. Insulin resistance and hyperinsulinemia can be computed from fasting plasma insulin as an independent variable in normoglycemia. An overview of the current diagnostic criteria, disease onset, complications, comorbidities and perspectives on lifestyle interventions are presented. A proposal for early detection of subclinical diabetes from routine evaluation of fasting plasma insulin, which is affordable and robust and thus applicable for the general population, is further suggested.

Key words: diabetes, diagnosis, insulin, subclinical diabetes, metabolic syndrome.

INTRODUCTION [1]

According to the World Health Organization (WHO), health is defined by a complete state of

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well-being. However, such definition is context and clinically dependent, and not an absolute concept. The perception of apparent physical, mental and social well-being by an individual or by a health care provider is dependent upon the societal context and standards of care for diseases, which are not immutable concepts. Such reference values are typically based on population-based stratification of commonly found values and their correlation with acute markers of the onset of diseases, which might not necessarily presuppose a "complete" health state. In fact, clinical markers might vary widely among societies and ethnically,

and the predisposition to the development, onset and progression of clinically diagnosed chronic diseases. Moreover, disease-related comorbidities has been found to be correlated with the stratification within normal ranges of clinical markers, raising the concern whether a chronic disease would be already in course long before the clinical diagnosis, at a subclinical stage. In light of current scientific knowledge, we discuss below whether the concept of subclinical disease would be applicable to type 2 diabetes mellitus. We further present suggestions for an earlier diagnosis of the asymptomatic pre-disease state and for the prevention of future risk of diabetes, and consequently its complications and comorbidities.

DEFINITION[2]

Diabetes mellitus (DM) is a cluster of chronic metabolic diseases, with many distinct characteristic although having hyperglycemia as a common marker when poorly controlled. They also have in common the malfunctioning of the endocrine pancreas, in particular the pancreatic β-cells, which are highly susceptible to varying factors such as environmental, inflammation, immunogenicity and genetic background (Schwartz et al. 2016). In type 1 DM (T1DM) insufficient production of insulin occurs due to loss in β-cells. The ADA emphasizes that T2DM is "due to a progressive loss of insulin secretion on the background of insulin resistance" (Association 2016). In fact, in type 2 DM (T2DM) the decrease in β-cell mass and insulin production is preceded by a state of hypeinsulinemia, as a compensation for an underlying state of insulin resistance (Yalow and Berson 1960, Association 1998, Garber et al. 2016) (WHO 2015). The resulting inverted-U shape

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[2] *"Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both."* American Diabetes Association (Association 2014)

pattern of the dependence of circulating insulin levels (insulinemia) on glycemia has long been known (Reaven et al. 1967, Kraft 1975), and was defined by DeFronzo as the "Starling's curve of the pancreas" (DeFronzo 1988).

Several studies and organizations have shown an unprecedented increase in the prevalence of diabetes worldwide (NCD-RisC 2016), including T1DM (Harjutsalo et al. 2008, Patterson et al. 2009, Dabelea et al. 2014, Forga Llenas et al. 2015, Lamb et al. 2015), and the estimated number of adults with diabetes has soared to over 380 million (> 8 % world adult population, > 12% in USA) (Menke et al. 2015), with a mean estimation of over 37 % with pre-diabetes and over 45 % undiagnosed according to current diagnostic criteria (Schmidt et al. 2011, Federation 2015, Mechanick 2015, Menke 2015). The prevalence of T2DM in younger individuals has also increased worldwide (Holden et al. 2013, Menke et al. 2015), creating a new class of patients comprising children (Lustig et al. 2016), adolescents and young adults (Weiss et al. 2013, Song 2016). Such changes in phenotypic manifestation of the disease raise concern due to the life expectancy of this segment of the population and the higher cost associated with the treatment of diabetes and related complications (Huo et al. 2016). These non-adult groups in modern society are suffering from T2DM and metabolic syndrome (MetS) (clinically diagnosed by 3 out of 5 measures: elevated waist circumference, elevated triglycerides, reduced HDLc, elevated blood pressure and elevated FBG (Grundy et al. 2005), and consequently it is likely that in the short term they will form a new group of young-adult patients with life-long consequences for their own health, for their families and also for public or private health care provider. In this context, it urge the need for both earlier diagnosis of increased risk for diabetes and related complications and comorbidities (Lima 2017), requiring interventions to revert these conditions, thereby minimizing the impact on their own health and on the health care system.

TABLE I
Current diagnostic criteria for diabetes and impaired glucose metabolism diagnosis.

	ADA	SBD	WHO
Diabetes	- FPG ≥ 126 mg/dL or - 2h-OGTT ≥ 200 mg/dL or - HbA1c ≥ 6.5 ¨% or - Casual glycemia ≥ 200 mg/mL	- FPG ≥ 126 mg/dL - 2h-OGTT ≥ 200 mg/dL - HbA1c – (*) - Casual glycemia ≥ 200 mg/mL	- FPG ≥ 126 mg/dL - 2h-OGTT ≥ 200 mg/dL - HbA1c ≥ 6.5 ¨%
Impaired Glucose Metabolism	**Pre-Diabetes** - FPG 100 mg/dL ~ 125 mg/dL or - 2h-OGTT 140 ~ 199 mg/dL or - HbA1c: 5.7 % - 6.4 %	**TGD** - FPG 100 mg/dL ~ 125 mg/dL - 2h-OGTT 140 mg/dL ~ 200 mg/dL - HbA1c – (*)	**IGT** - FPG < 126 mg/dL and - 2h-OGTT ≥ 140 < 200 mg/dL **IFG** - FPG 110 ~ 125 mg/dL and - 2h-OGTT < 140 mg/dL

FPG – fasting (at least 8 h without caloric intake) plasma glucose. 2h-GTT – 2h - post-load plasma glucose after 75 g oral glucose after at least 8 h fasting. IGT – Impaired glucose tolerance. IFG – Impaired fasting glucose. TGD –reduced glucose tolerance (from Portuguese, "Tolerância à Glucose Diminuida"). * HbA1c – although a reference range is specified in consonance with the ADA criteria, the SBD does not adopt the HbA1c as diabetes diagnostic criteria. Glycemia can be converted from mg/dL to mM by dividing by 18.

CURRENT CRITERIA FOR THE DIAGNOSIS OF DIABETES [3]

The diagnosis criteria of diabetes used by most diabetes societies worldwide has historically been revised in order to include the most up to date evidence correlating with the complications risk. Table I summarizes the current diabetes diagnosis criteria by the American Diabetes Association - ADA (Association 2016) and the World Health Organization - WHO (WHO 2016, Colagiuri et al. 2011), based on reference values of these clinical markers, which comprise HbA1c level over 6.5 %, a fasting plasma glucose level (FPG) over 126 mg/dL, or a plasma glucose over 200 mg/dL 2 hour after an oral load of 75g glucose (named oral glucose tolerance test, OGTT). The use of HbA1c in the diagnostic of diabetes must be regarded with caution, since several factors may affect the result, including erythrocyte lifespan (Simmons and Hlaing, 2014).

Since 2001, a new set of criteria have become commonly used for diagnosis of diabetes. According to these criteria, the overt diabetes (i.e., established, diagnosed) is preceded by two stages of non-diabetes:

i) **pre-diabetes** (also known as frank diabetes): an impaired state (impaired fasting glucose - IFG - or impaired glucose tolerance - IGT) in which these markers are altered toward the level of diabetes, and

ii) **non-diabetic:** within the normal reference range (including normoglycemia FPG < 100 mg/dL, 2h-OGTT<140 mg/mL and HbA1c < 5.7 %).

Fasting plasma insulin (FPI), c-peptide and pro-insulin are not officially adopted as clinical markers for diabetes, although recognized as possible markers of pancreatic function mostly in the form of the homeostatic model assessment (HOMA) in the estimate of β-cell function (HOMA-%B) or insulin resistance (HOMA-IR, the inverse of insulin sensitivity - HOMA-%S = 100/HOMA-IR) (Matthews et al. 1985, Wallace et al. 2004), a much simpler parameter but strongly correlated with the more complex and costly euglycemic clamp method (DeFronzo et al. 1979). Insulin, c-peptide and pro-insulin have half-life of about 5 min, 30 min and 90 min respectively (Zilker et al. 1988, Jones and Hattersley 2013). Despite the longer half-life, only

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[3] *"The definition and diagnostic classification of diabetes and its pre-states should be based on the level of the subsequent risk of cardiovascular complications".* Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary. European Society of Cardiology (ESC) (Rydén et al. 2007)

about 3 % of pro-insulin remains non-cleaved, and thus limiting its use as an analytical marker. Insulin and c-peptide are produced to the same extent since c-peptide is the remaining portion of pro-insulin after cleavage. Taking into account the 6-fold longer half-life of c-peptide compared to insulin and the reduced influence of turnover due to other metabolic factors, measuring c-peptide in the assessment of insulin resistance may be advantageous either fasted of during the OGTT. In the clinical practice, insulin measurements (either FPI or during OGTT) can be prescribed and are considered accessory diagnostic tool (Wu 2006).

Despite the wide use of these established clinical markers, there has been rising concern over whether they would constitute a satisfactory panel for the clinical diagnostic of diabetes, undiagnosed diabetes and pre-diabetes or the early prediction of future diabetes and the cost-effectiveness of implementing as a general population-based approach (Zhang et al. 2005, Toscano et al. 2015).

In 1999 the DECODE group (Diabetes Epidemiology: Collaborative analysis Of Diagnostic criteria in Europe - DECODE - Study Group for the European Diabetes Epidemiology Group), with data gathered from more than 25,000 individuals with a mean follow-up of 7.3 years, that the diagnosis of established diabetes by using a FPG alone was insufficient to predict all-cause mortality, while the 2h glycemia from the OGTT provided a better prognostic information for this outcome (DECODE Study Group 1999).

In order to assess increased risk for diabetes, the ADA recommends testing to all asymptomatic individuals over 45 years old, or overweight or obese (body mass index BMI ≥ 25 kg/m^2 or ≥ 23 kg/m^2 in Asian descendent) with additional risk factors including physical inactivity, first-degree relative with diabetes, hypertension ($\geq$140/90 mmHg or on therapy for hypertension) or history of CVD, HDL <35 mg/dL and/or a triglyceride level >250 mg/dL (2.82 mmol/L or on therapy for dyslipidemia),

among others (Association 2016). However, by the time of their diagnosis, patients with T2DM have already an extensive (about 50 %) reduced pancreatic β-cell function (DeWitt and Hirsch, 2003), as well as signs of the prevalence of diabetes complication markers such as microalbuminuria due to kidney damage (UKPDS 1998a, Stratton et al. 2000, Bash et al. 2008) and retinopathy (Nagi et al. 1997, Kohner et al. 1998, Looker et al. 2012).

In the United Kingdom Prospective Diabetes Study (UKPDS), all microvascular and macrovascular outcomes were increasing related with HbA1c as a positive, linear continuum, with little difference for example between mean 5.6 % and 6.5 % (Stratton et al. 2000). Although not evaluated for normoglycemic non-diabetic individuals, it is unlikely that a drop to risk level equal to zero events would occur in a transition of HbA1c from 5.6 to the 5.0 % level, which suggests that even at this lower level of HbA1c a high rate of events would take place. Also in the UKPDS, the increasing severity of retinopathy was associated with lower fasting serum insulin levels (Kohner et al. 1998), which suggest a late diagnosis of diabetes, and correlates with an extensive loss of β-cell function upon diagnosis (DeWitt and Hirsch 2003).

These data suggest that the advanced complications of diabetes can be seen immediately upon diagnosis, suggesting the current "glucocentric" (Yudkin and Montori 2014) clinical criteria may constitute a late diagnosis, and thus indicating a need for earlier detection of insulin resistance and impaired pancreatic function.

RISK OF FUTURE T2DM WITHIN NORMOGLYCEMIC RANGE [4]

Prospective studies have long been conducted in the search for correlations between glycemic states

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[4] "Insulin resistance is prerequisite for the development of type 2 diabetes and becomes manifest long before hyperglycaemia is evident.". "Insulin-resistant individuals develop type 2

and the prognosis of diabetes and diabetes-related risks.

In a population-based survey of the island of Mauritius conducted with over 3,500 individuals in a follow-up of about 5 years, the incidence of diabetes was shown to progressively correlate with the FPG at baseline within the normoglycemic range (< 100 mg/dL), increasing further for individuals in FPG categories above this level, i.e., IFG (Shaw et al. 2000).

In a retrospective cohort study from the Japanese city of Omiya, conducted with over 11,000 individuals for a mean follow-up of 7 years, the risk of developing diabetes was positively correlated with the FPG at baseline irrespective of gender or age, both in the pre-diabetes range and in the normoglycemic range (Kato et al. 2009). The adjusted hazard ratio for incidence of diabetes with subjects with an FPG of 95 to 99 mg/dL was 2.3 times the risk of those with FPG < 85 mg/dL at baseline.

In an Israeli study conducted with over 13,000 men aged 26 to 45 years, with a mean follow-up of 5.7 years, a significantly augmented risk of developing T2DM was found in groups with FPG in the normoglycemic range of 87 mg/dL to 90 mg/dL (Tirosh et al. 2005). The risk or progression to T2DM was further strongly associated with higher triglyceride levels (< 150 mg/dL or > 150 mg/dL) or body mass index (BMI; <25 kg/m^2, 25-29.9 kg/m^2 or >30 kg/m^2), both showed to be independent risk factors in this same study group.

In a large study using data from the Kaiser Permanente Northwest (KPNW) health organization in Portland, Oregon (USA) a search for correlation between normal FPG and the risk of development of T2DM was conducted (Nichols et al. 2008). Over 46,000 individuals over 40 years of age were

identified with normal FPG at baseline and were followed for up to 10 years (mean follow-up time of 6.8 years) or until diagnosed diabetic. The risk of developing diabetes was positively correlated with FPG in the normal reference value (quartiles categories: < 85 mg/dL, 85 to 89 mg/dL, 90 to 94 mg/dL, 95 to 99 mg/dL) at baseline. The risk of developing diabetes was 49 % higher for those in the 90-94 mg/dL quartile compared with those with FPG below 85 mg/dL. Those subjects who developed diabetes also had some other baseline characteristic distinct from the total study sample, such as higher BMI (mean values ranging from 32.7 to 33.5 kg/m^2 versus 28 to 29.9 kg/m^2), higher mean triglycerides (TG; 209 to 239 mg/dL versus 142 to 164 mg/dL among the quartiles) and slightly lower mean HDLc (47 to 49 mg/dL versus 52 to 57 mg/dL among the quartiles) - which together are a characteristic of dyslipidemia. They also had hypertension (41.4 to 49.6 % versus 19.4 to 21.2 % among the quartiles) and cardiovascular disease (CVD) (8.3 to 11.9 % versus 4.8 to 7.6 % among the quartiles). These baseline characteristic of the subjects who developed diabetes shows altered values in the markers of MetS and increased risk of atherosclerosis, incidence of coronary heart disease (CHD) and mortality (Alberti et al. 2009, Wong et al. 2012). Although the risk of diabetes (4.0 %) was lower in the normoglycemic range group than in the group with pre-diabetes/IFG within the same study setting (11.3 %), both groups (normoglycemic and IFG) had close profiles among the MetS markers (BMI, TG, HDLc, high blood pressure) (Nichols et al. 2007:2). These data not only call for the importance of evaluation of both glycemic indicators, but also for the importance of the trends in MetS and insulin resistance markers. To put this into perspective, a cohort study with 56 normoglycemic individuals showed increased insulin resistance (as assessed by fasting plasma insulin - FPI - and HOMA-IR), dyslipidemia and subclinical inflammation (as evaluated by

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diabetes only if β-cell failure ensues. Thus, measures of β-cell function might be expected to be a key predictor for future type 2 diabetes.". (Abdul-Ghani and DeFronzo 2009)

ultrasensitive C-reactive protein, hsCRP) as independent predictors of coronary artery disease as assessed by coronary artery calcium (CAC) score (Deveci et al. 2009, Budoff et al. 2013, Hecht 2015). In fact, MetS and diabetes increases the likelihood of established CAC (Wong et al. 2003), which is also suggestive of a late diagnosis when considering the risks for progression of CVD. Also, MetS and diabetes are additional risk factors in the progression of CAC (Wong et al. 2012), as shown by the Multiethnic Study of Atherosclerosis Study (MESA) conducted with over 6,810 adults individuals followed for about 4.9 years for incident CHD.

A UK retrospective study with 129 non-pregnant subjects evaluated the ability of the FPG in predicting the outcome of the OGTT (Wiener 1995). The study found that in order to achieve the lowest risk of false positive diagnoses of diabetes it would be necessary to use an upper cut-off limit of 80 mg/dL as normoglycemic range of FPG, a threshold that would rule out the need of further investigation by OGTT or any other clinical test.

Until diagnosis, diabetes can be unnoticed for over a decade under current clinical diagnosis criteria, as seen in the Whitehall II prospective occupational cohort. The participants were classified into two groups according to diagnosis at the end of the follow-up, those who developed and those who did not develop T2DM. During the study with a mean follow-up of 13 years monitored for BMI, FPG, 2h-OGTT, FPI, 2h-OGTT-insulin, HOMA-%S and HOMA-%B, those individuals which did not developed diabetes maintained a low FPG in the lower 80 mg/mL range, individuals which were ultimately diagnosed diabetic continuously increased their FPG within the normoglycemic range. Furthermore, the surge in FPG preceding diabetes diagnostic at endpoint was accompanied by increase in 2h-OGTT, decrease in HOMA-%S and in HOMA-%B, which were continuously altered since baseline throughout the

follow-up trajectories. These data clearly showed a tight relation between the continuum in progression of a clinical marker within the reference normal range and the final onset of diabetes. Moreover, this study shows that the diagnosis of overt diabetes is preceded by more than a decade of asymptomatic progression in metabolic dysfunction, including decreased insulin sensitivity and β-cell function, even within their normal reference range.

The prospective Ansung-Ansan cohort study, part of the Korean Genome and Epidemiology Study, was conducted for 10 years in South Korean involving over 4100 individuals surveyed for chronic diseases. Those who progressed to diabetes and pre-diabetes at endpoint showed at baseline significantly higher BMI, waist circumference, SBP, FPG, 2h-GTT, HbA1c and HOMA-IR, and lower HOMA-%B compared to the group that did not progress, all values within their respective normal reference range (Ohn et al. 2016).

Collectively, these gathered independent studies conducted with varying ethnic groups bring evidence that the risk of future diabetes significantly increases as a function of glycemic markers and β-cell function as a continuum starting within the group of normoglycemic individuals. Below we present further data from studies correlating the risks of complications comorbidities related to diabetes in study groups in the normoglycemic range.

RISK OF DIABETES COMPLICATIONS AND COMORBIDITIES WITHIN THE NORMAL REFERENCE RANGE [5]

DIABETES COMPLICATIONS IN DIABETIC INDIVIDUALS

Several studies have shown decreasing risk of diabetes complications in diabetes-diagnosed individuals that were subjected to a tight glycemic

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[5] *"Those with cardiovascular disease not identified with diabetes are simply undiagnosed".* (Kraft 2008)

control by the use of intensive pharmaceutical therapy protocols. We refer here to two major trials, one with T1DM and other with T2DM.

The Diabetes Control and Complication Trial (DCCT) conducted with 1,441 T1DM individuals with a mean follow-up of 6.5 years showed that the intervention arm under intensive pharmaceutical therapy, with a tight glycemic control close to normal reference values, presented reduction of long-term complications such as retinopathy, nephropathy and neuropathy (DCCT 1993). The rate of progression of retinopathy fell as HbA1c decreased. These benefits observed in the DCCT showed long-term, sustained effects as observed by the lower risk of microvascular (DCCT-EDIC 2000), cardiovascular outcomes and myocardial infarction as seen in the Epidemiology of Diabetes Interventions and Complications study (EDIC), a mean follow-up of 17 years of the DCCT (Nathan et al. 2005). The UK Prospective Diabetes Study (UKPDS) study conducted with T2DM individuals followed for over 10 years showed that a tight glycemic control resulted in decrease of risk of CVD, all-cause mortality, microvascular including nephropathy (UKPDS 1998b) with sulphonylurea of insulin, and macrovascular diseases (myocardial infarction, sudden death, angina, stroke, peripheral disease) with metformin (UKPDS 1998a).

DIABETES COMPLICATIONS ON NON-DIABETIC INDIVIDUALS

The risks of complications of diabetes (such as neuropathy, nephropathy, retinopathy, CVD, vascular, among others) have also been observed as a function of metabolic dysfunction among normoglycemic individuals.

Retinopathy

The trend in increasing of the prevalence of any retinopathy has been seen as a HbA1c dependent variable within the normal reference range in cross-sectional epidemiological studies with the Pima Indians, an Egyptian study, and the third National Health and Nutrition Examination Survey (NHANES) (WHO 2015, Colagiuri et al. 2011).

Cardiovascular disease

In the US San Antonio Heart Study, over 2,560 Mexican-American and non-Hispanic individuals non-diabetic at baseline were followed for 8 years. The risk of CVD was evaluated and the HOMA-IR and FPI were found to be independent risk factor with FPG and 2h-OGTT within the normoglycemic range (Hanley et al. 2002).

A diagnosis of T2DM has been considered an independent risk factor for CVD (Valenti et al. 2016). The prospective DECODE study comprising European 22-cohorts involving over 29,700 individuals in a median follow-up of 11 years reported an increasing risk of all-cause CVD and non-CVD mortality for individuals with 2h-OGTT levels within the normoglycemic range (below 140 mg/dL or 7.7 mM) (DECODE Study Group, European Diabetes Epidemiology Group 2003)

In a longitudinal population-based prospective study conducted in the Japanese town of Hisayama, a total of 2,851 individuals were followed for about 7 years and CVD were found to correlate with HbA1c within the non-diabetic range (Ikeda et al. 2013).

In a 10-year follow-up of the Dutch Hoorn cohort study involving 1,647 non-diabetic subjects (eligible for final data analysis), HbA1c was shown to be an independent predictor of CVD (Van't Riet et al. 2012)

In the Italian Bruneck study conducted with over 910 adults followed for 15 years, the incidence of CVD was increasingly higher in the upper quartiles of HOMA-IR or FPI at baseline even after adjustment for varying risk factors (Bonora et al. 2007).

In the Australian Diabetes, Obesity and Lifestyle (AusDiab) study conducted with over 10,000 subjects over 25 years of age in a follow-up of 7 years, the hazards for all-cause mortality and CVD mortality were independently correlated with 2h-OGTT and HbA1c even within their normal reference range (Barr et al. 2009). Also in the AusDiab cohort, conducted with general non-diabetic population and involving over 8,530 adults over 35 years of age followed for about 5 years the incidence of fatal or non-fatal CVD showed a progression correlated with HOMA-%S, with a hazard ratio of about 2.0 when comparing the first and fifth quintiles (Barr et al. 2010).

In a UK-based prospective population study in which over 10,000 adults were followed for an average of 6 years, an increased risk for fatal and non-fatal coronary heart disease (CHD) cardiovascular events and total mortality associated was found with increasing HbA1c levels in the normal reference range (Khaw et al. 2004).

In the Atherosclerosis Risk in Communities (ARIC) Study, several evidences of diabetes complications have been associated with HbA1c in the normal, non-diabetic reference range (< 5.7 %). In an ARIC prospective case-cohort study conducted for over 8 years of follow-up with over 1,320 non-diabetic adults, a progressive increase in risk of CHD was found as a function of HbA1c in the normal reference range among those in the normoglycemic range (NCD-RisC 2016). Another ARIC study with over 10,880 non-diabetic individuals in a mean follow-up of 8 to 10 years found a correlation of HbA1c within normal reference range with increasing risk of incident ischaemic stroke (Selvin et al. 2005b). A median follow-up of 14 years conducted with over 11,090 non-diabetic adults found a positive correlation between the incidence rate of new diagnosed diabetes, CHD and stroke with HbA1c in its normal range.(Selvin et al. 2010)

In a Canadian prospective study involving over 2100 non-diabetic adult men, the risk of ischemic heart diseases (IHD) was shown to be independently correlated with fasting hyperinsulinemia compared to matched patients with no occurrence of IHD within the 5 years of follow-up (Després et al. 1996). Although the risk of IHD was independent from dyslipidemic state, it showed a synergic effect with increasing triglyceride. In another population-based cohort study (NHANES III) involving over 13,100 non-diabetic individuals over 20 years, c-peptide was found positively correlated with increasing risk of CVD deaths in nondiabetic patients and with decreasing levels of serum HDLc (Li et al. 2015). These studies did not exclude pre-diabetic groups. Although well-known that the risks of CVD is increased in pre-diabetic and diabetic compared to non-diabetic population (Stratton et al. 2000), the risk of first time stroke among normoglycemic Swedish population was positively correlated with fasting pro-insulin and insulin levels, even more pronounced in women (mean OR 13.7) than in men (mean OR 3.4) (Lindahl et al. 2000).

The Spanish cohort from the Canary Islands conducted with over 6,600 adults from the general population showed elevated c-peptide fasting levels associated with increasing risk of coronary disease - acute myocardial infarction (AMI) and coronary artery disease (CAD) - within normoglycemic individuals (Cabrera de León et al. 2015), which also correlated positively with increasing obesity, TG hypertension and HOMA-IR, and lower HDLc.

In a cohort study with 1,073 non-diabetic adult patients in the normoglycemic range (for FPG, HbA1c) which survived AMI, they found an independent association of incident multivessel coronary artery disease (CAD) with HOMA-IR (Karrowni et al. 2013). In this same cohort, the quantiles of HOMA-IR was statistically and positively correlated with increasing FPG, FPI, HbA1c, hsCRP, TG, and BMI.

Cancer

In the Japan Public Health Center-based prospective study (JPHC Study Group) which followed over 29,000 individuals which were cancer-free at baseline. And after a median follow-up of 8.5 years, they found increasing risk of cancer in individuals with higher HbA1c levels within both non-diabetic and diabetic ranges (Goto et al. 2016).

In an Italian prospective case controlled study, involving over 10,000 women followed for over 5 years, FPG within the normal reference value of non-diabetic was found associated with increasing risk of breast cancer (Muti et al. 2002, Sieri et al. 2012).

Dementia

During a median follow-up of 6.8 years of over 2,000 patients, the Adult Changes in Thought (ACT) study found a progressive increasing risk for development and diagnosis of dementia as a function of glycemia at baseline in groups without diabetes (Crane et al. 2013), suggesting insulin resistance and increased microvascular complications in the central nervous system as underlying causes.

In a US cohort from northern Manhattan involving adults over 65 years of age and no sign of dementia at baseline, the odds of Alzheimer disease (AD) and dementia was increased over 100 % in individuals with hyperinsulinemia (Luchsinger et al. 2004).

Renal diseases

In the Atherosclerosis Risk in Communities (ARIC) Study, a community-based cohort with over 1,800 diabetic participants, a positive association between chronic kidney disease (CKD) - in the absence of albuminuria and retinopathy - and a broad HbA1c concentration range, including the normal reference values (<5.7 %)

In conjunction, these data reported above, among others studies reported elsewhere, illustrate that the risks of incidence of diabetes-related complications and comorbidities correlate with markers of glucose metabolism in a dose-dependent manner within the normal reference range for non-diabetic. Consequently, these data indicate the existence of initial manifestation of diabetes-related complications in subclinical conditions not detected by the current diagnostic approach. The missing link relies on the identity of one or more causal agents, or even the identification of a common independent risk factor that would better describe the increased risk of progression to diabetes and related complications and comorbidities among normoglycemic individuals. The clinical markers FPG, OGTT and HbA1c are glycemic proxies highly influenced by a large subset of variables (Rydén et al. 2016), including insulin secretion and insulin resistance, glucagon control, glucose absorption (during the GTT), cortisol, stress hormones, among others. Among these factors, insulin secretion - along with amylin cosecretion - have been shown to be regulated accordingly in order to keep the glucose homeostasis, and thus pancreatic β-cell function is a valuable tool to be investigated.

SUBCLINICAL DIABETES DIAGNOSIS: HYPERHORMONEMIA AND INSULIN RESISTANCE AS THE MISSING PROXIES [6]

After diagnosis, the progression of T2DM is well characterized by the decline in pancreatic β-cell function (Butler et al. 2003, DeWitt and Hirsch 2003, Association 2016). However, the decrease in β-cell function and insulin secretion with the progression of both frank and overt diabetes is preceded by a hyperhormonemia state, in which hormonal

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[6] *"Islet dysfunction is critical for development of IGT and type 2 diabetes and (...) these pathophysiological events already start when subjects are normal glucose tolerant".* (Ahrén, 2009)

(both insulin and amylin) secretion by the β-cells attempts to offset the glucose load and the varying degree of insulin resistance, keeping glycemia in the normoglycemic range (Abdul-Ghani and De-Fronzo 2009). Such observation makes the case for pancreatic hyperhormonemia as a major onto-genic hallmark of diabetes. It follows that evalua-tion of hormonal levels become a natural candidate as a more specific and sensitive clinical diagnostic proxy in normoglycemic individuals, otherwise healthy and non-diabetic according to the current glucocentric diagnostic approach.

FROM THE BIHORMONAL HYPOTHESIS TO THE ISLET MULTIHORMONAL DISFUNCTION

Glucagon

Glucagon is an hormone secreted by the α-cells from pancreatic islets with varying systemic physiologic roles including the maintenance of normoglycemia under fasting, as long recognized by evidences from Unger, Cahill and colleagues (Marliss et al. 1970). The evidences that glucagon levels in individuals with overt diabetes were elevated lead the proposal of the "bihormonal hypothesis" of T2DM in 1975 by Unger and Orci (Dobbs et al. 1975, Unger and Orci 1975), by which hyperglycemia was explained by the lack of insulin regulation of glucose metabolism and paracrine antagonization of excessive secretion of glucagon, currently recognized as a key strategy in the therapeutics of diabetes (Godoy-Matos 2014, Hay et al. 2015, Sisnande et al. 2015, Bower and Hay 2016). In fact, reduced insulin secretion and acute glucagon secretion upon stimulation with arginine is observed in IGT individuals years before diagnosis, as found by Ahrén and colleagues (Larsson et al. 1995, Ahrén 2009). Such traits are found in subjects that were normoglycemic and normoglucagonemic at baseline, but already showing higher FBI at baseline within normal reference range.

Pancreatic Polypeptide (PP)

The pancreatic polypeptide (PP) is secreted by the PP cells and has long been shown to be elevated in T2DM individuals (Floyd et al. 1976). In fact, during an OGTT the insulin levels in pre-diabetic are higher than in non-diabetic individuals, while PP levels and glucagon are similar between these groups. However, in T2DM subjects both insulin, PP and glucagon levels are elevated during an OGTT (Chia et al. 2014), bringing evidences for a further multihormonal hypothesis.
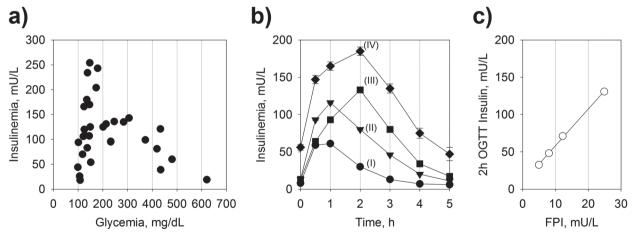
Amylin

Amylin is a 37 amino acid hormone belonging to the calcitonin gene-related peptide (CGRP) family discovered in 1987 (Cooper et al. 1987, Westermark et al. 1987) and is concomitantly released along with insulin by the same secretory granules of the pancreatic β-cells (Guerreiro et al. 2013). Among the physiologic functions, amylin has a central role in the regulation of glucagon secretion (Young, 2005). During OGTT in non-diabetic (30 min OGTT peak <145 mg/dL, 1h OGTT < ~ 130 mg/dL, 2h OGTT < ~90 mg/dL) obese adults, both insulin (1h peak ~ 57 mU/L, ~400 pM) and amylin (1h peak ~ 8 pM) are cosecreted (Thomaseth et al. 1997). They are secreted at an apparent constant ratio in non-diabetic individuals, reaching a serum concentration ratio at 1 h peak of about 50mol:1mol insulin:amylin. In normoglycemic individuals progressing toward pancreatic impairment, a parallel hyperhormonemia (hyperinsulinemia and hyperamylinemia) follows, with an increase in the relative amount of amylin to insulin as a function of progression from NGT to IGT and finally T2DM (Ludvik et al. 1991, Thomaseth et al. 1997, Kahn et al. 1998).

From this islet hormonal panel we notice that insulin and amylin are hormone that may be found elevated in fasting state as well as after an oral glucose load in some normoglycemic non-diabetic

individuals, while glucagon and PP are more likely to be altered in diagnosed diabetic subjects.

Insulin

Yalow, Berson and colleagues have reported a large variability in plasma insulin concentration in normoglycemic patients undergoing OGTT (Yalow and Berson 1960, Yalow et al. 1965), also corroborated by subsequent study by Reaven and colleagues (Reaven et al. 1969). In another study with individuals undergoing GTT, Reaven and colleagues have further found a biphasic dependence of insulinemic response on glycemia, at first reporting a steep increase within the normoglycemic range which preceded a descent phase as a function of increasing glycemia within the IGT and diabetic ranges (Reaven et al. 1967) (Fig. 1a). Kraft has further confirmed the hyperinsulinemic response to an oral glucose load during an extended OGTT - up to 5 hours - among normoglycemic individuals, which anticipates the decline in insulin response observed in the overt diabetes (Kraft 1975, 2008) (Fig. 1b). The varying degrees of insulinemic responses during OGTT were stratified into quintiles (so-called "Pattern")

and correlate with the levels of FPI (Kraft 2008). These varying levels of insulinemic response to OGTT - or even meals - among those otherwise considered healthy individuals, provides evidence for varying degrees of insulin resistance among normoglycemic subjects. These data have also been repeatedly observed by other groups.

When comparing groups of normoglycemic non-obese and obese adult individuals, the latter shows both an increased FPI and 24-h insulinemic response, with minor difference in the 24-h glycemic profile (Polonsky et al. 1988). In a study with normoglycemic obese (BMI ~ 35 kg/cm^2) Latino and Afro-American youth, the group was hyperinsulinemic (FPI > 27 U/mL), and consequently insulin resistant (HOMA-OR > 4.3) and also an IGT with high insulinemic profile during OGTT (Lustig et al. 2016). However, even among lean, normoglycemic and normolipidemic (fasting) young individuals a subgroup with elevated FPI can be found - yet "normal" (< 13 mU/mL) - showing a hyperinsulinemic and dyslipidemic 24h profile - in particular high TG and de-novo hepatic lipogenesis (Petersen et al. 2007). In another study, Finnish individuals were

Figure 1 - Insulinemic response following an oral glucose load. **a)** Insulinemic response in individuals following an oral load of glucose. Data from (Reaven et al. 1967). **b)** Insulinemic response in normoglycemic individuals following an oral glucose load. Each curves (I, II, III and IV) represent a Pattern (quantile) with average of the insulinemic responses from a large subset of individuals following an oral glucose load. Data from (Kraft 2008). **c)** Correlation between FPI and 2h insulinemia following an oral glucose load. Data form (Johnson et al. 2010).
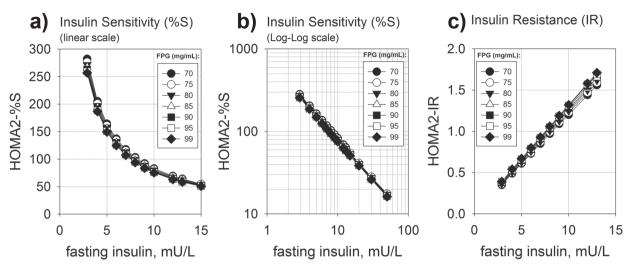
screened for their degrees of insulin resistance using euglycemic hyperinsulinemic clamp, and the results were compared with their FPI (Laakso 1993). In the group with NGT, the prevalence of insulin resistance increased as a function of their FPI, matching similar prevalence of those in the frank (IGT) or overt (diagnosed) diabetic groups at a FPI of 13 mU/L and above. In the San Antonio Metabolism (SAM) study conducted with 388 obese and non-obese subjects (NGT, IGT and T2DM), the insulin secretion during OGTT showed a typical inverted-U shape when plotted as a function of FPG, showing the decline in insulin secretion preceded by an hypersinsulinemic phase in the normoglycemic range (Gastaldelli et al. 2004). This study also found that the insulin secretion along the OGTT correlated with the degree of insulin resistance as assessed by euglycemic insulin clamp. It also found that the decline in β-cell function is an exponential continuum as a function of FPG (or 2h-OGTT), beginning in the normoglycemic range. In a retrospective US study a group of over 675 subject with FPG in the normoglycemic range, FPI and HOMA-IR positively correlated with 2h-OGTT insulin (Johnson et al. 2010) (Fig. 1c).

INSULIN RESISTANCE, HYPERINSULINEMIA AND HEPATIC MANIFESTATION OF METABOLIC SYNDROME

We recall that HOMA is a measure calculated from results of measurements of fasting glycemia and insulinemia during routine blood work. HOMA is well accepted due to its simplicity and an analytic power comparable to clamps, golden standards for evaluation of insulin resistance but a complex test not suitable for routine clinical diagnostic. Although well known as a suitable proxy for evaluation of β-cell function and insulin resistance in individuals diagnosed with frank or overt diabetes, HOMA can also be a direct evidence for β-cell function in the normal reference range, and thus an appropriate - and affordable - proxy. In order to keep a tight

glycemic control, individuals in the normoglycemic range show increasing insulin resistance, which manifests itself through increasing insulinemia both fasting and in response to an oral glycemic load. This increasing insulin resistance is phenotypically manifested as an increasing insulinemia, which is reflected as a steep decline in HOMA-%S (Figs. 2a, b) and a linear increase in HOMA-IR (Fig. 2c). According to this model, it is noted that an increase from 2 mU/L to about 15 mU/L in FPI is followed by an 8 fold decline in HOMA-%S, from 400 to 50 (as interpolated from Fig. 2b). These modeled data are confirmed by experimental results, showing the extensive increase in prevalence of insulin resistance within the normal reference range of FPI (< 13 mU/L) and normoglycemic range (Laakso 1993).

From decades of independent studies it becomes evident that an early multihormonal disfunction takes place in as an attempt to control glycemia in the context of the metabolic degeneration. More importantly, this analysis shows that FPI is a direct and reliable indicator of insulin resistance in otherwise healthy non-diabetic normoglycemic individuals. In fact, the importance for the assay of FPI in the evaluation of insulin resistance before the onset of the clinical diabetes has long been recognized by the ADA for almost 20 years, as stated in their official statement "*Consensus Development Conference on Insulin Resistance*" (Association 1998). In that report, the non-recommendation for the routine screening of fasting insulin relied for the most part in analytical limitations, in the absence of clear cut-point criteria, and in the lack of correlational evidence between insulin resistance and outcomes.

The present body of evidence indicates that the glucocentric approach (FPG, HbA1c and OGTT) does not conclusively predict future risk of diabetes and related complications/comorbidities. Given that insulin resistance is the underlying basis for glucose intolerance leading to rise in glycemia and all others diagnostic markers for diabetes and

Figure 2 - Insulin sensitivity as a function of fasting plasma insulin according to HOMA2 model. The HOMA2 model as implemented in the HOMA calculator (https://www.dtu.ox.ac.uk/homacalculator/) (Wallace et al. 2004) was used to diagram the insulin sensitivity (HOMA2-%S, the inverse of insulin resistance) and insulin resistance (HOMA2-IR) as a function of fasting plasma insulin. **a)** HOMA2-%S, linear scale; **b)** HOMA2-%S, log-log scale. c) HOMA2-IR. Numbers in the legends correspond to the FPG levels used in simulation, within the normoglycemic range (from 70 to 99 mg/L). Notice that insulin sensitivity shows a steep dependence on the fasting insulin **(a)** behaving as a double exponential decay function and thus linear in log-log scale **(b)**. Interpolation of this data allows the observation of a decrease in half of insulin sensitivity when FPI rises from 2 to 5 mU/L FPI, and a further decrease in half from 5 to 10 mU/L. Even within the normal reference range of FPI (up to 13 mU/L), a shift from 2 to 10 mU/L in FPI corresponds to a decrease to about 25 % of initial insulin sensitivity, a clear sign of advanced insulin resistance **(c)** and thus subclinical diabetes in the normoglycemic range.

pre-diabetes and that insulin resistance may builds up even in normoglycemic individuals, the direct assessment of insulin resistance should constitute the diagnostic panel in the screening of diabetes, requiring solely a FPI and/or a confirmatory OGTT with insulin measurement.

The insulin resistance index (HOMA-IR) by means of FPI may be evaluated along with a broader metabolic panel prospecting the intraorgan origin of insulin resistance, in particular hepatic by means of gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST) (Unwin et al. 2015, Oniki et al. 2016) and ferritin (Brudevold et al. 2008). Non-alcoholic fatty liver disease (NAFLD) spectrum has been considered an hepatic manifestation and/or precursor of MetS (Smits et al. 2013, Lonardo et al. 2015) and even elevated levels within the normal reference range have been associated with markers of MetS, NAFLD and

T2DM (Sanyal et al. 2015), and thus a subclinical stage of MetS. Furthermore, other anthropometric markers associated to MetS should be evaluated since accumulation of MetS markers are indicative of increased insulin resistance (Garg et al. 2011). These MetS markers include subcutaneous, abdominal and intravisceral fat (BMI, waist to hip ratio), cardiovascular and atherosclerotic outcomes (such as carotid intima-media thickness - CIMT (Pais et al. 2016), coronary artery calcium - CAC, also known as calcium score (Valenti et al. 2015)), blood pressure, inflammation (hsCRP) and atherogenic dyslipidemia - high circulating triglycerides, low HDLc, high apolipoprotein B or high LDL particle number (Leroux et al. 2000, Pourfarzib et al. 2014), high small LDL particles and oxidized LDLc (Boizel et al. 2000), some of them assessed as the lipoprotein insulin resistance index - LPIR score, which shows strong association with HOMA-IR (Shalaurova et al. 2014) (Fig. 3).

INTERVENTION IN THE PREVENTION OF DIABETES[7,8]

We present below a brief perspective on some of them to shed light on the manageable aspect of the metabolic dysfunction by means of lifestyle interventions.

MEDITERRANEAN DIET APPROACH

Lifestyle intervention showed effective in the prevention of T2DM in non-diabetic individuals at high risk of CVD who followed a Mediterranean diet approach (high in vegetables, olive oil, fish, nuts, fiber, polyphenos, α-linoleic acid, and minimally processed *in natura* products, low linoleic acid and ultraprocessed food items) for a mean follow-up of 5 years (Salas-Salvadó et al. 2011, Estruch et al. 2013, 2016, Babio et al. 2014).

The Diabetes Prevention Program (DPP) study (DPP 2002) conducted with high risk group showed a 58 % reduction in the risk of diabetes by lifestyle intervention, which included the National Cholesterol Education Program Step 1 (NCEP Step 1) diet, similar to a Mediterranean Diet (Kris-Etherton et al. 2001). The lifestyle intervention in the DPP proved to be superior to metformin (850 mg b.i.d.) intervention, both compared to placebo.
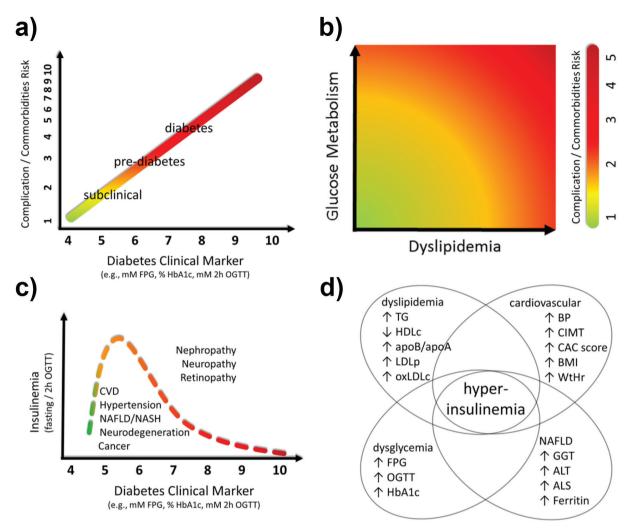
CALORIC RESTRICTION

Another approach in the control of diabetes markers is caloric restriction. This approach has been used

for over 100 years, long before the availability of insulin and other drugs for diabetes (Mazur 2011). Modern studies have been confirming the caloric restriction protocol and the reversal of organ dysfunction and the reversal of diabetes markers (Steven et al. 2016) and has been also recognized by the ADA as an intervention in the improvement of insulin resistance (Association 1998). According to this protocol, not only is total caloric intake decreased, but also the glycemic load, which introduces confounders, making it difficult to extrapolate the individual benefits of each component in a multivariate analysis.

Although some authors argue that isoenergetic meals varying in content of macronutrient may behave similarly in the short term (Hall et al. 2015), metabolic markers and the long term impact of a large set of variables influences considerably the metabolism according to the macronutrient distribution (Gardner et al. 2007), insulin resistance status (Ebbeling et al. 2007, McClain et al. 2013), the content of resistant starch (Leeman et al. 2005) and the use of seasoning components such as vinegar (Ostman et al. 2005), fats as olive oil and butter (Bozzetto et al. 2016), among others variables. Given that the post-prandial insulin spike may take about 5 h to reach the baseline level (Gannon and Nuttall, 2004), the meal distribution over the course of the day should be taken into account since it is likely to impact the 24 h insulinemic profile (Kahleova et al. 2014).

It is also recognized the existence of large population variability in glycemic response to similar meals (Zeevi et al. 2015), the intra-individual variability in glycemic response to different meals with equivalent macronutrient load (e.g., carbohydrate: starch + sugar) (Hätönen et al. 2006), which poses further uncertainty on the adequacy of total caloric restriction protocols, the validity of the calorie-in-calorie-out model (Hall et al. 2015), and the usefulness of the glycemic index (Jenkins et al. 1981, Atkinson et al. 2008) model.

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[7] "Rather than screening whole populations for diabetes, primary care teams should focus efforts on earlier detection, lifestyle advice and intensive treatment of risk factors among individuals at high risk of diabetes and cardiovascular disease." Forouhi and Wareham 2014

[8] "First line "treatment" for pre-diabetes by whatever definition is lifestyle advice. And because the risk factors overlap with those of other non-communicable diseases, the question is why focus attention on a specific group of people with a diagnosis of pre-diabetes while ignoring the remainder of the healthy population who would benefit from the same advice.". Yudkin and Montori 2014

Figure 3 - Schematic diagram of the continuum of risk of common diabetes complications as a function of glucose metabolism markers. A continuous color gradient is depicted ranging from lower (green, lower left side) to higher risks (red, upper right side). **a)** Schematic representation of the progression of risks (log scale) of future complications and/or comorbidities related to currently adopted official diabetes clinical markers (FPG and/or HbA1c and/or 2h-OGTT) and at normoglycemic range (subclinical diabetes). Diagram inspired on the UKPDS (Stratton et al. 2000) and ARCS (Selvin et al. 2005a) studies. **b)** The progression of complications / comorbidities of diabetes in the subclinical range (within the normal reference ranges, without established diabetes symptoms) of glycemic and lipid markers. Glucose metabolism state for FPG, FPI/c-peptide/pro-insulin, HbA1c, 2h-OGTT glucose. Dyslipidemia states for TG, HDL, TG/HDL ratio, oxLDL. Diagram inspired on the study of (Tirosh et al. 2005). **c)** Schematic representation of the biphasic behavior of insulin secretion as a function of progression of diabetes. At low, normoglycemic reference values of glucose indicators, insulin secretion is increased in order to compensate for building insulin resistance. Diagram inspired by Reaven (Reaven et al. 1967), Kraft (Kraft 1975), and DeFronzo (Gastaldelli et al. 2004) studies. **d)** Schematic representation of the clinical markers associated with hyperinsulinemia within normal reference ranges.

CARBOHYDRATE /GLUCOSE LOAD RESTRICTION

Taking into account the needs for decreasing in the risk of future diabetes and complications thereof, a lifestyle interventional approach that decreases endogenous insulin requirements, improve insulin sensitivity, pancreatic function and improves evident risk markers such as TG, HDL, insulinemia and glycemia should be considered.

A direct correlation between glycemic load from dietary carbohydrate and physiologic insulin requirement - currently known as food insulin

index (FII) - is well-established. It is well-known that glucose load induces expressive increase in glycemia and insulin, while other macronutrients loads do not significantly change both markers (Robertson et al. 2002, Bao et al. 2009). Prioritization of meal preparations with low FII and thus with low bioavailable glucose from total carbohydrate contents such as starch and sugars along with the preferred use non ultra-processed food items (Canella et al. 2014) is likely to compose a strategy to decrease the excessive demand upon pancreatic function, to overcome long-term hyperinsulinemic status, reduction in insulin resistance and in the likely of development of MetS-related outcomes.

For several decades now, the role of glycemic load in the long term rise of triglycerides has been known, and it is worsened according to the individual insulin responses (Reaven et al. 1967). The hypertriglyceridemic effect of fructose from sugars and its impact on markers of MetS is also well documented (Lim et al. 2010), and its dietary reduction has been shown to be associated with improvement in MetS markers (Schwarz et al. 2015, Lustig et al. 2016)
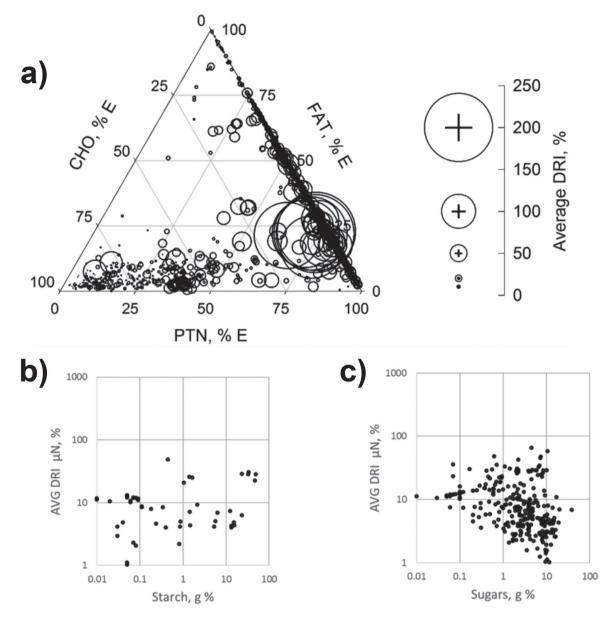
Moreover, it has been postulated that most metabolic diseases show increased risk under an hyperinsulinemic condition (Cordain et al. 2003, Kopp 2003). In fact, a study with over 4,150 participants from Northern China followed for over 4.2 years suggested that even when participants in a cohort were normalized for similar baseline characteristics (BMI, age, age, smoking, waist circumference - lower limit of 81 cm - SBP, DBP, normoglycemic range (about 4.8 mM = 87 mg/dL), fasting plasma insulin (about 7 mU/L), total cholesterol, HDLc (about 50 mg/dL), obesity, hypertension and hyperlipidemia, but with SBP and triglyceride (in the upper normal range),the study found correlation of increasing consumption of starchy foods with the probability of MetS, indicating the existence of the disease and their probable complications and comorbidities.

The average load of essential micronutrients expressed as percentage of the dietary reference intake (DRI) show a positive correlation with the overall content of protein in unprocessed or minimally processed food items (Fig. 4a), while displaying a lower prevalence in starch- and sugar-rich food items (Fig. 4b and Fig. 4c, respectively), which are the main origin of exogenous glucose (USDA 2014). Limiting the intake of essential micronutrients can pose an increasing risk for the prevalence of diabetes and other metabolic disorders (Ames 2006, Miao et al. 2013, Kaur and Henry 2014). Given that the insulin demand is directly related to exogenous glucose (Bao et al. 2011), decreasing the consumption of starch and sugar - rich food items is a better strategy for the control of both established diabetes and prediabetes (Feinman et al. 2015, Tóth and Clemens 2015) as well as subclinical diabetes (Lima 2017). There is no minimum human requirement for exogenous glucose since the human body can produce glucose (Institute of Medicine 2005, Cahill 2006) and use it along with ketone bodies as main energetic source in many organs including brain (Cunnane and Crawford 2003, Institute of Medicine 2006), provided adequate amounts of nutrient-rich protein and good-quality lipids are consumed.

CONCLUSIONS [9]

There has been intense debate whether the current thresholds for diagnosis of diabetes should be decreased and if HbA1c and other additional metrics could compose a more complete risk assessments panel for diabetes and its complications (Cefalu 2016, Yudkin 2016). The extensive scientific

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9 *"From a clinical perspective, the most practical way of assessing insulin resistance would seem to be the measurement of insulin concentration in plasma". "Plasma insulin levels whether measured in the fasting state or after a glucose load are a powerful predictor for the risk of type 2 diabetes".* Consensus Development Conference on Insulin Resistance American Diabetes Association (Association 1998)

Figure 4 - Correlation between the average content of essential micronutrients and macronutrients load. The average load of 28 essential micronutrients in non-processed food items (",raw" keyword filter, resulting in 1.325 food items) was calculated from the whole USDA database (SR27) in the basis of % of the *Dietary Reference Intake* (DRI; males, 31-50 years old) for each 100 g product and reported here. As a quick reference for high micronutrient load, consider the distribution above 50 % DRI. Essential amino acids were not included in order to avoid bias by skewing data toward high micronutrient in protein. **a)** Ternary plot representing the proportion of macronutrient (PTN = protein, CHO = carbohydrate, FAT = lipids) as % energy. Each symbol represent one non-processed (",raw") food item and the size is proportional to the average load of the 28 essential micronutrients expressed as % of the DRI. **b)** Average load of 28 essential micronutrients (% DRI, male, 31-50 years old) in 100 g unprocessed food item as a function of sugar content. **c)** Average load of 28 essential micronutrients (% DRI, male, 31-50 years old) in 100 g unprocessed food item as a function of starch content. Notice the LOG-LOG scale. Data from USDA National Nutrient Database for Standard Reference, Release 27 (USDA, 2014:27) (SR27; http://www.ars.usda.gov/) and Dietary Reference Intake (DRI) for adult male 31-50 years-old (http://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx). Micronutrients: Ca, Fe, Mg, P, K, Na, Zn, Cu, F, Mn, Se, VitA, VitE, Vit D (D2 + D3), Vit. C, Thiamin, Riboflavin, Niacin, Pantothenic Acid, Vit B6, Folate (total), Vit. B12, Choline (total), Vit. K (phylloquinone), Folate (food), Linoleic Acid (LA), α-linolenic acid, and also included EPA+DHA (not in sr27, but known for its limited endogenous production and requirement from exogenous source (Mozaffarian and Wu 2012).

literature cited above displays sound evidence for the strict dependence of markers of metabolic disorder as a continuum of insulinemia and as a direct indicator of the trajectory of insulin resistance taking place among normoglycemic individuals.

It is time to reappraise the diagnosis criteria beyond the sole use of the glucocentric markers, and to evaluate the levels of insulin resistance (through insulinemia) and hepatic manifestation of metabolic syndrome (by means of lipid metabolism, even within the normolipidemic range but at outer percentiles), which comprise a set of cost-accessible diagnostic parameters of direct markers of the underlying basis of the disease. Under the patient perspective, it would allow the health care provider to propose early non-pharmacological lifestyle intervention once identified a subclinical diabetes pattern, aiming to improve overall health and reducing the risks of future diabetes and the evident risks of diabetes-related complication and comorbidities. From the public health perspective, adopting policies aimed at the promotion of a healthy nutrition and lifestyle and the consequent minimization of the burden of diabetes, complications and comorbidities would reduce the economic impact on the health care system and society overall.

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