



Claimed effects, outcome variables and methods of measurement for health claims proposed under European Community Regulation 1924/2006 in the area of blood glucose and insulin concentrations

Daniela Martini¹⁰ · Beatrice Biasini¹ · Ivana Zavaroni^{2,3} · Giorgio Bedogni⁴ · Marilena Musci⁵ · Carlo Pruneti⁶ · Giovanni Passeri⁷ · Marco Ventura⁸ · Daniela Galli⁹ · Prisco Mirandola⁹ · Marco Vitale⁹ · Alessandra Dei Cas^{2,3} · Riccardo C. Bonadonna^{2,3} · Daniele Del Rio¹⁰

Received: 20 December 2017 / Accepted: 27 December 2017
© Springer-Verlag Italia S.r.l., part of Springer Nature 2018

Abstract

Most requests for authorization to bear health claims under Articles 13(5) and 14 related to blood glucose and insulin concentration/regulation presented to the European Food Safety Authority (EFSA) receive a negative opinion. Reasons for such decisions are mainly ascribable to poor substantiation of the claimed effects. In this scenario, a project was carried out aiming at critically analysing the outcome variables (OVs) and methods of measurement (MMs) to be used to substantiate health claims, with the final purpose to improve the quality of applications provided by stakeholders to EFSA. This manuscript provides a position statement of the experts involved in the project, reporting the results of an investigation aimed to collect, collate and critically analyse the information relevant to claimed effects (CEs), OVs and MMs related to blood glucose and insulin levels and homeostasis compliant with Regulation 1924/2006. The critical analysis of OVs and MMs was performed with the aid of the pertinent scientific literature and was aimed at defining their appropriateness (alone or in combination with others) to support a specific CE. The results can be used to properly select OVs and MMs in a randomized controlled trial, for an effective substantiation of the claims, using the reference method(s) whenever available. Moreover, results can help EFSA in updating the guidance for the scientific requirements of health claims.

Keywords Claimed effect · Outcome variable · Measurement method · Blood glucose · Insulin · Diabetes

Abbreviations

ADA American Diabetes Association
AUC Area under the curve
AUCi Insulin area under the curve
CEs Claimed effects

CHO Carbohydrates
CMD Cardiometabolic disease
CVD Cardiovascular disease
EFSA European Food Safety Authority
HbA1c Glycated haemoglobin
HOMA Homoeostatic model assessment

Managed by Antonio Secchi.

✉ Daniele Del Rio
daniele.delrio@unipr.it

¹ The Laboratory of Phytochemicals in Physiology, Department of Food Science, University of Parma, Medical School, Building A, Via Volturno 39, 43125 Parma, Italy

² Division of Endocrinology, Department of Medicine and Surgery, University of Parma, Parma, Italy

³ Azienda Ospedaliera Universitaria of Parma, Parma, Italy

⁴ Clinical Epidemiology Unit, Liver Research Center, Basovizza, Trieste, Italy

⁵ Department of Food and Drug, University of Parma, Parma, Italy

⁶ Department of Medicine and Surgery, Clinical Psychology Unit, University of Parma, Medical School Building, Parma, Italy

⁷ Department of Medicine and Surgery, Building Clinica Medica Generale, University of Parma, Parma, Italy

⁸ Laboratory of Probiogenomics, Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy

⁹ Department of Medicine and Surgery, Sport and Exercise Medicine Centre (SEM), University of Parma, Parma, Italy

¹⁰ The Laboratory of Phytochemicals in Physiology, Department of Food and Drug, University of Parma, Medical School, Building A, Via Volturno 39, 43125 Parma, Italy

HPLC	High-pressure liquid chromatography
iAUC	Incremental AUC
IFCC	International Federation of Clinical Chemistry
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
IR	Insulin resistance
IS	Insulin sensitivity
ISI	Insulin sensitivity index
LC-MS	Liquid chromatography mass spectrometry
MMs	Methods of measurement
OGTT	Oral glucose tolerance test
OVs	Outcome variables
QUICKI	Quantitative insulin sensitivity check index
RCT	Randomized controlled trial
T2DM	Type 2 diabetes mellitus

Introduction

Based on an estimation of WHO, diabetes mellitus will be the seventh leading cause of death in 2030. To date, diabetes mellitus affects more than 400 million of people, with an increasing prevalence in low- and middle-income countries [1]. Diabetes can be due to (almost) absent insulin production secondary to autoimmune pancreatic beta cell destruction (type 1 diabetes) or to a combination of reduced beta cell functional mass and insulin resistance (type 2 diabetes). Hyperglycaemia is therefore the hallmark of the disease and is implicated in the pathophysiology of acute and chronic complications affecting almost every tissue/organ. Oxidative stress, endothelial dysfunction and inflammation are included among the main pathways thought to be involved in diabetic complications [2, 3]. The onset of type 2 diabetes can be prevented or delayed by therapeutic lifestyle changes (TLC), including, but not limited to, diet and physical activity. Food and nutrients directly affect glucose metabolism and excursions. Thus, glucose lowering or raising properties of foodstuffs and their bioactive compounds have been receiving increasing attention, as instruments of primary, secondary and tertiary prevention addressed mainly to the public health burdens of diabetes and cardiovascular disease (CVD). As pointed out by Howlett and Ashwell [4], understanding the impact of specific foodstuffs on glucose levels/regulation and selecting the best read-outs of the glucose/insulin system to unveil the (dis)advantages associated with certain food items have fuelled the debate of the scientific community for a long time. In parallel, the media and the grey literature have devoted increasing attention to these topics, contributing to increase consumers' awareness and to improve their skills in food choices. In this scenario, the interest of food industries to declare glucose level modifying properties of their products has been steadily growing, as demonstrated by several requests of authorization to bear

health claims in relation to glucose metabolism. However, most requests for authorization to use health claims under Articles 13(5) and 14 with reference to blood glucose and insulin concentration/regulation presented to European Food Safety Authority (EFSA) have received a negative opinion. In addition to insufficient characterization of the food items and to the choice of non-beneficial effects, reasons for the negative opinion by the Panel on Dietetic Products, Nutrition and Allergies of EFSA are ascribable to the insufficient substantiation of the health claim attributed to the food/food constituent under scrutiny, including inappropriate choice of outcome variables (OVs) and methods of measurement (MMs).

In this scenario, a project was launched aiming to improve the quality of the applications submitted by stakeholders to EFSA [5]. The project consists in the critical review of OVs and MMs proposed so far to substantiate the health claims falling into one out of six main areas of interest, as outlined in the Guidance documents adopted by the Panel on Dietetic Products, Nutrition and Allergies: (1) protection against oxidative damage and cardiovascular health, (2) postprandial blood glucose responses/blood glucose control and weight management, (3) bone, joints, oral and skin health, (4) neurological and physiological functions, (5) gut and immune functions and (6) physical performance.

The present paper is a position statement by the experts involved in the project, which reports the results of the work done to collect, collate and critically analyse the information relevant to claimed effects (CEs), OVs and MMs related to blood glucose and insulin levels and homeostasis compliant with Regulation 1924/2006.

Materials and methods: search strategy

The manuscript refers to claimed effects (CEs), OVs and MMs collected from the relative Guidance document [6], from the applications for authorization of health claims under Articles 13(5) and 14 of Regulation 1924/2006 related to blood glucose and insulin concentrations (ec.europa.eu/nuhclaims/), as well as from comments received during public consultations. The critical analysis of the OVs and their MMs was performed on the basis of the literature review and was aimed at defining the appropriateness of OVs and MMs with specific reference to the claimed effects [5]. A schematic representation of the strategy applied is shown in Fig. 1. Starting from a pool of 13 requests for authorization of health claims, 2 were not considered because the claim was not defined as a beneficial physiological effect per se. The remaining 11 requests were evaluated. Among these, 10 and 1 were referred to 2 and 1 different claims falling under Article 13(5) and Article 14, respectively. The critical analysis was performed for 10 different OVs, 2 of which

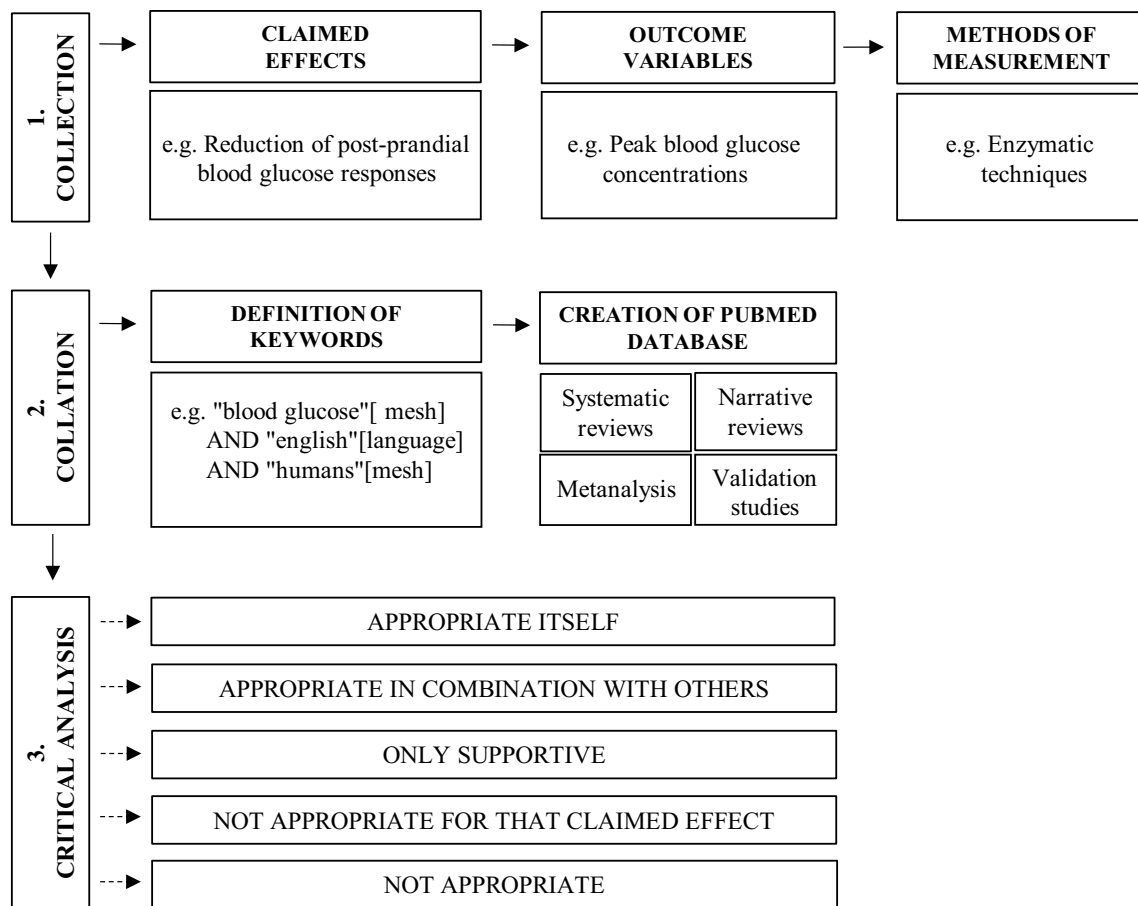


Fig. 1 Schematic representation of the strategy applied to collect, collate and critical analyse the outcome variables and methods of measurement proposed under health claims in the area of blood glucose and insulin concentrations

were assessed in the context of 2 different CEs. Among the 7 different MMs considered, 3 were assessed for the same OV, while 3 MMs were analysed in relation to different OVs and 1 MM was evaluated in relation to only 1 OV.

Results and discussion: critical evaluation of outcome variables and methods of measurement

Function claims falling under art. 13(5)

Reduction in postprandial blood glucose responses

Peak blood glucose concentration The peak of blood glucose after a glucose load or a meal is an outcome variable commonly employed in clinical and research settings. In non-diabetic subjects, the glycaemic peak generally occurs about 60 min after starting to eat and rarely exceeds 140 mg/dL. Blood glucose concentration typically returns to baseline levels within two or three hours. However, the com-

plete absorption of ingested carbohydrates takes 5–6 h after the meal. The quantity, composition and timing of the meal have a crucial role in determining the magnitude and time of the glycaemic peak [7].

To evaluate the appropriateness of peak blood glucose concentration as outcome variable, the literature deriving from database #01 was critically evaluated (see Table 1).

The magnitude of the postprandial peak plasma glucose concentration depends on a variety of factors, mainly the timing, quantity and composition of the meal [7]. Other factors which affect the entity of glucose peaks are the inter-individual and intra-individual variations in glucose transit time across the gut, hormonal response, insulin sensitivity (IS) and glucose effectiveness, which can be defined as the ability of glucose to suppress its own endogenous production and to enhance its own uptake [8]. The blood glucose peak and the glycaemic AUC provide different information. The former represents a single measurement of blood glucose and cannot be used to assess the degree of change of postprandial glucose levels. The latter provides a better assessment of glucose response,

Table 1 Strategies used for retrieving the literature pertinent with outcome variables and methods of measurement under investigation

DB number	Syntax	Total articles	Narrative reviews	Systematic reviews/meta-analyses	Validation studies	Outcome variables
1	“blood glucose”[mesh] AND “english”[language] AND “humans”[mesh]	76,294	6321	1263	248	Peak blood glucose concentration; glucose AUC; blood glucose concentrations and their time-integrated values during OGTT; fasting blood glucose
2	“insulin”[mesh] AND “english”[language] AND “humans”[mesh]	78,407	10,251	911	143	Peak plasma/serum insulin concentration; insulin AUC; fasting plasma/serum insulin
3	“hemoglobin a, glycosylated”[mesh]) AND “english”[language] AND “humans”[mesh]	21,120	1405	692	94	HbA1c
4	(fructosamine [mesh]) AND humans [mesh] AND english [language]	889	37	5	1	Fructosamine
5	(“insulin resistance”[mesh]) AND “english”[language] AND “humans”[mesh]	42,901	9954	793	102	Insulin sensitivity

AUC area under the curve, HbA1c glycated haemoglobin, OGTT oral glucose tolerance test

giving an integrated value of glucose concentrations during a given period of time. However, in the absence of other measurements, such as insulin response to the food challenge, the information provided by the glucose peak is incomplete. Moreover, a lower postprandial peak glucose concentration implies the comparison with a benchmark product or an absolute standard: the test food must be ingested alone, or within a meal, and compared to an established control food/meal. If the standard is glucose in water solution, almost every food product would attain a lower glucose peak level after consumption [9], but at the same time, other reference foods could suffer from poor reproducibility, hampering the comparison. It is advisable to measure postprandial blood glucose at different time points, after an overnight fast, in order to detect the time-point at which the glucose peak will occur. The repeated consumption of a food inducing low blood glucose peaks can be considered beneficial. Replacing food items by others containing the same amount of available carbohydrates (CHO), but able to elicit lower glucose peak, could be considered beneficial on cardiometabolic risk reduction [10–12]. The simultaneous determination of insulin concentrations may provide information about the degree of hyperinsulinaemia to which body tissues are exposed following ingestion of selected foods, in the light of the potential detrimental role that prolonged, repeated overexposure to insulin may play in pathophysiological processes such as atherogenesis [13, 14].

In conclusion, postprandial peak blood glucose is an appropriate outcome variable for the substantiation of health claims regarding the reduction in postprandial blood glucose. However, it should be used in combination with peak plasma/serum insulin concentration to exclude disproportionate insulin values in comparison with the control food/meal.

Enzymatic techniques The determination of blood glucose is one of the most widely used blood chemistry tests and one of the earliest tests available in clinical practice. Comparing the quantification of peripheral to venous blood glucose, the former is preferable. However, the latter can be considered appropriate. In a sample of whole blood, glycolytic enzymes present in both red cells and leucocytes are responsible for decreasing glucose concentration over time. To obtain reliable data, glycolysis can be prevented by adding sodium fluoride to whole blood samples (with the limitation of making the sample not suitable for assessment of other chemistries), by rapidly separating the sample, or by cooling. Generally, a sample is considered adequate in the absence of haemolysis. Moreover, the quantification should be performed on samples obtained within 30 min from whole blood separation [15]. Different methods are available to measure glucose concentrations. They can be classified on the basis of the specific enzymatic reaction or the chemical reactivity of the sugar [16, 17]. Chemical methods, although accurate and with good sensitivity, are poorly specific because hexoses

other than glucose may react. On the contrary, enzymatic methods have higher specificity owing to the selective catalysis of the proteins used. The enzymes currently employed for glucose measurements are hexokinase, glucose oxidase and glucose dehydrogenase. Among these, glucose oxidase is the most specific, reacting only with D-glucose [18]. The method of detection may be colorimetric or electrochemical, depending upon the nature of the enzymatic reaction. The reference method of measurement, which employs hexokinase, requires serum or plasma deproteination [17]. The value obtained can be an underestimation of the true glucose concentration in the presence of haemolysis, or an overestimation due to a positive interference of bilirubin and triglycerides. On the other hand, peroxidase, which acts in the glucose oxidase method, is inhibited by various molecules, including bilirubin and haemoglobin. Depending on the matrix of the specimen, glucose measurements may differ. Compared to serum or plasma, whole blood glucose concentration tends to be up to 10–15% lower [18], because glucose is less concentrated in blood cells. In conclusion, enzymatic techniques represent an appropriate method of measurement for the assessment of postprandial blood glucose concentrations (peak and AUC).

Glucose AUC The glycaemic AUC is used as an overall measure of glucose tolerance in clinical trials and other studies. It is usually calculated by means of a glucose load or after a carbohydrate-rich meal. In the latter case, it can be used to compare the effect of a specific food versus a control food on postprandial glycaemia [19]. The combined measurement of insulin and glucose levels and the calculation of glucose and insulin AUC permit the quantification of body insulin response to dispose the glucose absorbed from a specific food. The AUC can be calculated using different methods, but the most commonly used is the “trapezoidal rule” [20]. The so-called incremental AUC (iAUC) is obtained by subtracting the baseline readings from the AUC [21]. The “positive incremental AUC” considers only the glucose area above the baseline [19, 22]. The choice of the AUC affects the interpretation of glycaemic values [23]. When the glucose curve falls under the baseline value, the iAUC may provide negative values. On the other hand, the positive iAUC has the disadvantage of ignoring the contribution of the readings below the baseline. The calculation of the whole AUC tries to minimize such drawbacks.

To evaluate the appropriateness of glucose AUC as outcome variable, the literature deriving from database #01 was critically evaluated (see Table 1).

Like for postprandial peak glucose concentrations, a lower postprandial AUC can be demonstrated by means of the comparison with a benchmark product or an absolute standard, suffering from the same limitations stated in “[Peak blood glucose concentration](#)” section. However,

different from the glycaemic peak, the AUC provides a time-integrated expression of glucose levels and allows a better assessment of the changes of glucose concentrations after a test food, allowing also the inference of more factors involved in glucose homeostasis, including potential gastrointestinal events (i.e. gastric emptying or starch digestion and glucose uptake by intestinal cells). Nevertheless, also in this case, in the absence of insulin measurements, the glucose AUC provides only partial information, as disproportionate change of insulin concentrations could be missed. For the calculation of glucose AUC, the duration of the examination must be appropriate (at least 2 h) and the sampling of blood glucose is recommended at several time points (e.g. seven), after an overnight fast [24]. As previously mentioned, the ability of food items to determine lower glucose AUC respect to others with a comparable amount of available CHO could be considered beneficial. However, the determination of insulin concentration is required to prove that this hormone is not present in a disproportionate amount (see “[Peak blood glucose concentration](#)” section). In conclusion, glucose AUC is an appropriate outcome variable for the substantiation of health claims regarding the reduction in postprandial blood glucose. However, it should be used in combination with AUC insulin to exclude a disproportionate increase in insulin values in comparison with the control food/meal.

Enzymatic techniques Please refer to “[Enzymatic techniques](#)” section.

Peak plasma/serum insulin concentration The insulin response after a glucose load or a meal, including the plasma/serum insulin peak, is an outcome variable commonly used in research settings. The plasma/serum insulin peak is affected by the increase in blood glucose and by meal composition. Other factors, such as hormones, neurotransmitters, rate of carbohydrate absorption, β -cell function, IS and insulin clearance, also play an important role. Hyperinsulinaemia and/or insulin resistance (IR) have been implicated in the pathogenesis of type 2 diabetes mellitus (T2DM), hypertension and atherosclerosis [25]. However, cut-off values associated with an increased risk of developing cardiometabolic diseases (CMDs) have not yet been established.

To evaluate the appropriateness of peak plasma/serum insulin concentration as outcome variable, the literature deriving from database #02 was critically evaluated (see Table 1).

The estimation of the insulin peak induced by a food/meal in comparison with a reference food/meal containing the same amount of available CHO has many limitations. First, it is a single-point measure, which provides less information and higher variability than a whole insulin

curve (AUC_i). Second, the time needed to reach the insulin peak is highly variable both within and between individuals [26, 27]. This implies that a predetermined sampling time may actually miss the insulin peak, especially when foods/meals with widely different transit times and absorption rates are compared. The measurement of postprandial insulin at different time points, after an overnight fast, is therefore advised in order to better detect the time-point at which the insulin peak occurs. Third, like for all insulin-based indexes, the interpretation of insulin values requires concomitant data on blood glucose. Finally, an important limitation is the fact that there is no worldwide standardization of the insulin assay or a recognized reference interval for insulin AUC [28], which makes difficult the comparison of data generated in different laboratories. In conclusion, postprandial peak insulin response is not an appropriate outcome variable to be used alone for the substantiation of health claims regarding the reduction in postprandial blood glucose. However, it can be used as supportive evidence to ensure that a disproportionate increase in this hormone does not occur when blood glucose concentration decreases, in comparison with the control food/meal.

Chromatographic techniques Three categories of analytical methods can be used to measure insulin and its precursor: immunoassays, HPLC and the stable isotope dilution LC–MS assay. In immunoassays, depending on their specificity, cross-reactions with pro-insulin and its degradation products cannot be excluded. The commercial availability of various insulin immunoassays and the absence of a worldwide standardization make data comparison across laboratories difficult. No international reference method for insulin analysis has yet been established [29]. In this context, isotope dilution LC–MS has been proposed as a reference procedure for serum insulin quantification with immunoassays [30]. In the effort to obtain standardization of insulin immunoassays, this approach can be used to improve the accuracy of their measurement. It implies the addition of a known amount of insulin analogue (labelled with stable isotopes) to the sample to be analysed. The analysis with MS, after the processes of extraction and purification, allows the direct identification of insulin and its analogue, in addition to a precise quantification depending upon the relative intensities of the observed signals.

HPLC can ensure rapid and accurate protein quantification. It is useful for the validation of immunoassays. Among HPLC procedures, reversed phase with an UV detector can be used. This method implies plasma separation followed by insulin extraction. Finally, plasma insulin concentration is determined from the standard curve of insulin. Owing to the small sample volume required, this

procedure is particularly suitable for paediatric microsamples [31].

Therefore, the development of a HPLC method with diode array detection has permitted to obtain reliable data of plasma insulin concentrations with no significant interferences or matrix effects caused by endogenous compounds. This approach includes the advantages of being relatively cheap and the requirement of a single-step extraction procedure [32].

In summary, chromatographic techniques are appropriate methods for the measurement of serum/plasma insulin. **Insulin AUC** Insulin AUC is a useful tool employed as an overall index for insulin levels in clinical trials and research investigations. In controlled studies, it can be calculated comparing the effect on postprandial glucose/insulin responses of a specific food against an established control. The calculation of insulin AUC can be used to quantify the body insulin response to dispose the glucose absorbed from a specific food. Concerning the available methods to calculate the AUC, please refer to "Glucose AUC" section.

To evaluate the appropriateness of insulin AUC as outcome variable, the literature deriving from database #02 was critically evaluated (see Table 1).

Insulin AUC can be used to comparatively assess the effect of a given food/meal on postprandial insulin. To do so, the test food must be ingested alone, or within a meal, and compared to an established control food [24]. Contrary to the insulin peak, the insulin AUC provides a time-integrated expression of insulin levels and allows a better assessment of the changes of insulin concentrations after a challenge [23]. The lack of a worldwide standardization of the insulin assay [28] could not be considered a limitation per se during an intervention study. However, if different methods of measurement are applied, the comparison of data generated in different laboratories is rather arduous. Furthermore, in the absence of the glucose curve, the interpretation of insulin AUC data becomes difficult, with IR and insulin clearance also playing a relevant role. To calculate insulin AUC, recommendations for test duration and sampling time are those reported in "Glucose AUC" section. In conclusion, postprandial insulin AUC is not an appropriate outcome variable to be used alone for the scientific substantiation of health claims regarding the reduction in postprandial blood glucose. However, it can be used as supportive evidence to ensure that a disproportionate increase in this hormone does not occur when blood glucose concentration decreases, in comparison with the control food/meal.

Chromatographic techniques Please refer to "Chromatographic techniques" section.

(Long-term) maintenance of normal blood glucose concentrations

(Long-term) maintenance of normal blood glucose concentrations is of critical importance to prevent the development of a dysglycaemic status, i.e. impaired fasting glucose/glucose intolerance and diabetes. Failure to maintain normal blood glucose concentrations is a strong predictor for the progression to type 2 diabetes with an annual progression rate ranging from 3 to 14%.

HbA1c Glycated haemoglobin (HbA1c) is a long-term indicator of glucose levels and is currently used for the diagnosis and management of diabetes mellitus. HbA1c is a marker of the average blood glucose concentration in the previous 2–3 months. Over the 120-day lifespan of the erythrocyte, HbA1c is formed through a glycation reaction of haemoglobin A with plasma glucose, so that HbA1c levels reflect the plasma glucose concentration over prolonged periods. The HbA1c test reports the ratio of haemoglobin HbA1c to total haemoglobin A. In 2007, a consensus statement on the worldwide standardization of the HbA1c measurement was issued jointly by the American Diabetes Association (ADA), the European Association for the Study of Diabetes, the International Federation of Clinical Chemistry (IFCC) and Laboratory Medicine, and the International Diabetes Federation [33]. All the statements regarding HbA1c are referred only to HbA1c assayed with methods aligned to the above-mentioned Consensus Statement, i.e. standardized with IFCC reference system for HbA1c and reported in IFCC units (mmol/mol) and derived NGSP units (%). Individuals with normal glucose regulation have HbA1c < 6% (< 5.7% according to ADA), while people with diabetes mellitus in poor glucose control may have levels > 10%. WHO recommends a value of HbA1c \geq 6.5% to diagnose diabetes [34], even though HbA1c < 6.5% does not rule out the presence of diabetes mellitus as demonstrated by its typical clinical picture with a diagnosis associated with fasting glucose or oral glucose tolerance test (OGTT) 2-hour glucose or random glucose.

To evaluate the appropriateness of HbA1c as outcome variable, the literature deriving from database #03 was critically evaluated (see Table 1).

HbA1c is currently used as the reference method to assess glucose medium-term control in patients with diabetes. Compared to single plasma glucose measurements, HbA1c is useful and convenient because it is much less affected by within-day and between-day variability [34]. In addition, HbA1c has minimal diurnal variations and is not affected by food ingestion [35]. Moreover, HbA1c values are strongly positively associated with incident type 2 diabetes mellitus, and it is considered a valid tool to assess the impact of interventions to modify the risk of diabetes [36]. The

measurement of HbA1c may not reflect true glycaemic control in patients with anaemia. An additional source of error is the interference with several haemoglobin variants. Finally, there is evidence that part of the variability of HbA1c is linked to genetic variants unrelated to glucose metabolism.

On the basis of current evidence, HbA1c is an appropriate outcome variable to be used alone for the substantiation of health claims in the context of (long-term) maintenance of normal blood glucose concentrations.

Chromatographic techniques IFCC has developed the current reference method that specifically measures the concentration of one molecular species of glycated haemoglobin and has been used for worldwide standardization of HbA1c assays. “Master equations” have been constructed to correct the results of current methodologies according to the reference method [37]. Many methods are available for the measurement of HbA1c. As reported by Sacks [35], they are generally classified as based on:

- charge differences between glycated and non-glycated haemoglobin (e.g. cation-exchange chromatography and electrophoresis);
- structural differences of glycol groups in haemoglobin (e.g. affinity chromatography and immunoassay);
- chemical reactivity (e.g. electrospray MS).

It should be noted that the latter method can only be used to standardize the HbA1c assay and cannot be used by clinical laboratories, whereas electrophoretic and cation-exchange methods may be influenced by haemoglobin variants [38].

Chromatographic techniques are widely used methods for clinical purposes. The quantification consists of two steps: (1) haemoglobin is cleaved into peptides by a proteolytic enzyme; (2) the specific glycated and non-glycated N-terminal peptides of the β -chain are measured by chromatographic techniques (e.g. HPLC coupled with MS). This method has good within-laboratory repeatability but low between-laboratory repeatability [35].

In general, chromatographic techniques can be considered appropriate as long as they are validated against the IFCC reference method and the results are converted to IFCC equivalents with the use of the “master equations”. The results should also be reported using both the IFCC units (mmol/mol) and the NGSP units (%) [33].

Fructosamine Fructosamine (1-amino-1-deoxy-D-fructose) is a ketoamine formed when glucose reacts non-enzymatically with the N-terminal amino group of proteins. Fructosamine is a biomarker of mid-term glycaemia, far less used than HbA1c. The main component of fructosamine is glycated albumin, which has a faster turnover rate than hae-

moglobin. Fructosamine is a marker of glycaemic control in the mid-term and reflects average glucose levels of the previous 1–3 weeks [39].

To evaluate the appropriateness of fructosamine as outcome variable, the literature deriving from database #04 was critically evaluated (see Table 1).

Fructosamine can replace HbA1c as a marker of glucose control in individuals with a condition that makes HbA1c testing unreliable or when mid-term changes of glucose control are of interest [40]. The measurement of fructosamine is simple and has good intra- and inter-laboratory reproducibility. However, it is still debated whether fructosamine should be corrected for protein/albumin concentration, a fact that makes the measurement not truly standardized. Also, fructosamine is a generic name indicating all glycated proteins, so that it lacks specificity [41]. Because it provides information about glycaemic control in the short term (1–3 weeks), it is not informative in the long term. The main drawback of fructosamine is that no standardization program of its assay has ever been completed. In addition, fructosamine has been less studied than HbA1c as marker of blood glucose control. There is no agreement on the fructosamine goals that should be pursued in various conditions or on the cut-points which could be used to categorize glucose metabolism. In conclusion, fructosamine is not an appropriate outcome variable to be used alone for the scientific substantiation of health claims in the context of (long-term) maintenance of normal blood glucose concentrations. However, it can be used as supportive evidence from shorter-term studies when long-term studies on HbA1c are available.

HPLC Many analytical methods have been proposed for the measurement of fructosamine. These can be classified as immunoassays (involving a specific binding of a reagent to the glycated species) and chromatographic methods (involving modification of the glycated protein), including the furosine/HPLC method. In the furosine/HPLC method, fructosamine is hydrolysed overnight with HCl producing lysine, furosine and pyridoxine. Furosine is quantified by HPLC using a reverse-phase column with UV detection at 254 and 280 nm [42]. This method is quite precise and specific and is currently considered the reference method for the measurement of fructosamine in biologic samples (i.e. serum/plasma). On the basis of the current evidence, HPLC is an appropriate method to measure fructosamine.

Blood glucose concentrations and their time-integrated values during OGTT Blood glucose concentrations (e.g. glucose peak) and their time-integrated values (i.e. glucose AUC) obtained by means of a standard OGTT are outcome variables commonly employed in clinical and research settings. As already mentioned in "[Peak blood glucose concentration](#)" section, in non-diabetic subjects, the glycaemic

peak generally occurs within 60 min after starting to eat and rarely exceeds 140 mg/dL. Blood glucose concentration typically returns to baseline levels within 2 or 3 h [7]. If insulin is measured with glucose, the OGTT also quantifies the body insulin response to dispose the glucose absorbed. Concerning the available methods to calculate the AUC, please refer to "[Glucose AUC](#)" section.

To evaluate the appropriateness of blood glucose concentrations and their time-integrated values during OGTT as outcome variable, the literature deriving from database #01 was critically evaluated (see Table 1).

In order to substantiate health claims related to the long-term maintenance of normal blood glucose concentration, an improvement of blood glucose control may represent an interesting additional insight of the mechanism through which the glucose metabolism has been modulated. Like for HbA1c, this approach should be pursued after a continuous consumption of the food/constituent over at least 12 weeks. Although a better blood glucose control could be exhaustively proved in intervention studies using HbA1c as primary outcome variable, the measurement of plasma glucose concentrations (e.g. glucose peak) and their time-integrated values (i.e. glucose AUC) obtained by means of a standard OGTT before and after the intervention may add valuable information about the ability of the organism to react acutely to a glucose load. However, the acute nature of this measurement makes it inappropriate alone to substantiate long-term maintenance of normal glycaemia. In addition, the simultaneous determination of insulin concentrations is needed to ensure that a disproportionate increasing of this hormone does not occur. In conclusion, blood glucose concentrations and their time-integrated values during a standard OGTT are not appropriate outcome variables to be used alone for the substantiation of health claims in the context of (long-term) maintenance of normal blood glucose concentration. However, they can be used in support of a mechanism by which the food/food constituent could exert the claimed effect.

OGTT OGTT is a non-invasive measurement technique, widely used in research and clinical settings, as a dynamic test. It is the gold-standard test to assess glucose tolerance. However, it can be also used for the assessment of the effect of a specific food on long-term maintenance of normal blood glucose and insulin concentrations. Moreover, it can be used for the diagnosis of diabetes, and it is a good marker of risk for the disease. As a clinical tool to assess glucose tolerance, the OGTT requires only 2 glucose measurements: baseline and 120 min after glucose ingestion [43, 44]. In experimental studies, after an overnight fast, blood samples for determinations of glucose and insulin concentrations are typically taken at 0, 15, 30, 45, 60, 90 and 120 min following the 75-g standard oral glucose load, but different frequency and duration of sampling can be used. OGTT is probably

the most commonly used method to evaluate glucose tolerance because it is the reference method for this purpose and because of its simplicity and low cost. It implies minimal risk, and it is suitable for large-scale studies [43]. In spite of its paramount importance, some studies reported poor reproducibility in specific groups of the population and even within the same individual. This may be partially explained by the variability in the rate of gastric emptying and glucose absorption from the gastrointestinal tract. In addition, gender, adiposity, age and ethnicity affect glucose tolerance (i.e. the OGTT results), so that this test should be used with caution when unbalanced groups for these and other known confounders are compared [45]. Compared to OGTT, using a food/meal testing under the same condition would not guarantee a full standardization due to differences in ingredients and/or preparation and is therefore discouraged. On the basis of current evidence, the use of OGTT at baseline and at the end of the intervention is an appropriate method to obtain blood samples in which glucose and insulin concentrations (e.g. glucose peak), as well as their time-integrated values (i.e. glucose and insulin AUC) can be quantified.

Insulin AUC In intervention studies aimed to demonstrate a long-term maintenance of normal blood glucose concentrations or an improving of glucose tolerance, the insulin AUC can be calculated when performing an OGTT. The insulin AUC quantifies the body insulin response to dispose the glucose absorbed from a specific food. Concerning the available methods to calculate the AUC, please refer to please refer to "[Glucose AUC](#)" section.

To evaluate the appropriateness of insulin AUC as outcome variable, the literature deriving from database #02 was critically evaluated (see Table 1).

The insulin AUC calculated during a standard OGTT provides a time-integrated expression of insulin levels. The key consideration regarding the insulin AUC (and all insulin-based indexes) is that it can be used to assess distinct biologic entities: (1) hyperinsulinaemia; (2) insulin resistance; 3. β -cell function [46, 47]. As to the first issue, it should be taken into account that there is no recognized reference interval for insulin AUC; hence, each laboratory should build its own. Moreover, even though the lack of a worldwide standardization of the insulin assay could not be considered a limitation per se during an intervention study, the comparison of data generated in different laboratories is rather arduous if different methods of measurement are applied. As to the second issue, in addition to the aspects highlighted for the first issue, it should be considered that other two factors, β -cell function and insulin clearance, beyond IR concur in determining the insulin AUC [48, 49]. As to the third issue, the limitations listed for the first issue hold; furthermore, in the absence of the glucose curve, the interpretation becomes difficult, with

IR and insulin clearance also playing a relevant role. In conclusion, postprandial insulin AUC is not an appropriate outcome variable to be use alone for the scientific substantiation of health claims in the context of (long-term) maintenance of normal blood glucose concentrations. However, it can be used as supportive evidence to ensure that a disproportionate increasing of this hormone does not occur.

OGTT Please refer to "[OGTT](#)" section.

Insulin sensitivity IR is defined as the inverse of IS, which in turn is the cellular biologic response to insulin. In current practice and in the present document, IR has a more strict meaning, labelling a condition in which the response of glucose metabolism to insulin is lower than expected. In humans, whole-body IS represents the net result of insulin action on the liver, in which it restrains glucose production, and on the peripheral tissues, primarily skeletal muscle, in which it accelerates glucose utilization. In the presence of IR, blood glucose levels tend to rise, unless there is a full compensation by the pancreatic β -cells with an appropriate release of insulin. Be it fully or partially compensatory, the attempt of the β -cells to cope with IR results in hyperinsulinaemia. The interplay between IR and β -cell function often leads to impaired glucose regulation, which is considered an obligatory phase preceding the development of T2DM. In addition, IS/IR has been implicated in the development of obesity and its complications, such as dyslipidaemia and hypertension [47, 50]. In turn, obesity and essential hypertension are insulin-resistant states on their own. A widespread condition named "metabolic syndrome" (the clinical role of which is still debated) or, more loosely, "cardio-metabolic risk", is the partial, or complete, cluster of central obesity, impaired glucose regulation, elevated BP and atherogenic dyslipidaemia, and it currently recognizes its foremost biologic common denominator in IR. Clinically, individuals at high risk of IR are easy to identify, but IS/IR is not easy to measure. The euglycaemic hyperinsulinaemic clamp technique (reference method) is not suitable for use in clinical practice and epidemiological research. This has led over the decades to the proliferation of surrogate indexes of IS/IR, which have been extensively used for both clinical and research purposes [47].

To evaluate the appropriateness of insulin sensitivity as outcome variable, the literature deriving from database #05 was critically evaluated (see Table 1).

Insulin capacity to stimulate glucose disposal is largely variable (at least sixfold) in apparently healthy individuals with no cut-off values. Reduced IS/IR is not a disease itself, but an alteration in normal physiology that independently increases the likelihood to develop a cluster of abnormalities—i.e. glucose intolerance/T2DM,

hypertension, atherogenic dyslipidaemia—and ultimately increases the risk of CVD.

Several factors related to lifestyle, including regular physical exercise and diet/weight loss, are known to positively affect IS [51, 52]. TLC, which improve IS (i.e. regular physical exercise), have been shown effective in preventing the development of T2DM in high-risk individuals [53].

On the basis of current evidence, a reduction in IR (or an increase in IS) is not an appropriate outcome variable to be used alone for the substantiation of health claims in the context of (long-term) maintenance of normal blood glucose concentrations. However, changes of IS can be used in support of a mechanism by which the food/constituent could exert the claimed effect.

Euglycaemic hyperinsulinaemic clamp The euglycaemic hyperinsulinaemic clamp technique provides a direct measure of IS. After an overnight fast, insulin is infused intravenously to raise plasma insulin above the fasting level (hyperinsulinaemia) to the point that glucose disposal in skeletal muscle and adipose tissue is increased and hepatic glucose production is inhibited. Blood glucose levels are constantly measured using a bedside glucose analyser, while dextrose is infused intravenously at a variable rate to maintain euglycaemia [47]. The amount of glucose infused to maintain euglycaemia, after correcting for changes in the glucose pool, is a measure of whole-body (liver plus peripheral tissues) IS. In order to discriminate between IS in the liver and in the peripheral tissues, the euglycaemic insulin clamp must be combined with the glucose tracer dilution technique [54]. To determine the extent of compensatory β -cell hypersensitivity, one reference method is the hyperglycaemic clamp, in which glucose is infused intravenously to almost instantaneously raise and maintain plasma glucose up to a predetermined level above the baseline values. The β -cells, when stimulated by square wave hyperglycaemia, respond with a characteristic biphasic pattern of insulin secretion, the so-called first and second phases of insulin secretion [55]. The data generated with the hyperglycaemic clamp can be used to yield also a good index of IS. The validity of clamp-based techniques depends on the achievement of steady-state conditions. They are gold-standard techniques as to accuracy and show fairly good within-subject reproducibility. On the other hand, clamp-based techniques are invasive and require intravenous access, are time-consuming (i.e. the standard duration of a glucose clamp is 120 min but takes about 4 h to perform it), quite expensive, and require 1–2 well-trained operator(s) to manage the sessions [50]. In conclusion, euglycaemic hyperinsulinaemic clamp is an appropriate method to be used alone for the measurement of IS.

ISI-OGTT In the quest for non-invasive measurement techniques for IS, several models have been proposed as an alternative to the euglycaemic hyperinsulinaemic clamp technique [50]. The OGTT is a dynamic test, widely used in clinical practice and research. It is the gold-standard test to assess glucose tolerance and can be used to diagnose IGT and diabetes mellitus. However, the OGTT is also often used to evaluate IR, considering that plasma glucose and insulin responses during OGTT reflect the ability of pancreatic β -cells to safeguard normal glucose regulation. A large number of formulas to compute an ISI on the basis of OGTT (ISI-OGTT) glucose/insulin data have been developed. All of them should be considered surrogate indexes of IS, because they reflect not only whole-body (liver plus peripheral tissues) IS, but also, to variable extent, the roles played by glucose absorption in the gut, insulin clearance, glucose effectiveness (i.e. glucose-mediated glucose metabolism) and, if present, glycosuria. One possible protocol to perform an OGTT with the goal of deriving an ISI-OGTT entails, after an overnight fast, a 75-g standard oral glucose load and drawing blood samples at 0, 30, 60, 90 and 120 min to measure plasma glucose and insulin concentrations. In spite of the above-mentioned limitations, the whole-body IS derived from the OGTT is attractive because it represents a composite of both hepatic and peripheral tissue sensitivity to insulin. ISI-OGTT is expected to perform better in longitudinal studies, in which changes are monitored within the same subject. When compared vis-à-vis, the ISI-OGTT proposed by Matsuda and De Fronzo in 1999 usually is more closely related to IS, as measured by the euglycaemic insulin clamp, than indexes which consider only fasting glucose and insulin values (e.g. HOMA-IR) [56]. The Matsuda's ISI-OGTT has been widely tested and has performed well in a number of conditions, including diabetes mellitus and related conditions. However, also for the Matsuda's ISI-OGTT a standardized reference range is missing.

On the basis of current evidence, ISI-OGTT to estimate IR, especially in the version proposed by Matsuda and De Fronzo, is an appropriate method to measure IS since it incorporates both peripheral and hepatic IS and show the strongest correlations with the reference method (clamp techniques).

QUICKI The homeostatic model assessment (HOMA), the fasting glucose/insulin ratio, and the quantitative IS check index (QUICKI) methods are probably the most frequently used techniques in clinical investigations. QUICKI, developed in 2000, provides a quite accurate index of IS thanks to the use of a derived mathematical transformation of fasting blood glucose and plasma insulin concentrations. It can be considered a variation of the HOMA equation, with the difference consisting in a logarithmic transformation of the insulin glucose product. QUICKI employs fasting insulin

and glucose as HOMA does, but it transforms data by taking the inverse of the sum of their logarithms [QUICKI = $1/[\log(\text{Insulin } \mu\text{U/mL}) + \log(\text{Glucose mg/dL})]$] [57]. As a consequence, QUICKI and HOMA-IR should be considered equivalent to estimate IS/IR, and all the considerations which are valid for one are also valid for the other method. QUICKI is a surrogate index of IS, employed because convenient and minimally invasive. Its simplicity makes QUICKI very attractive in large population studies [58]. However, it should be recalled that QUICKI is a sort of compact index influenced not only by liver IS, but also by glucose effectiveness (i.e. glucose-mediated glucose metabolism), and insulin clearance. QUICKI is derived from fasting measures, which primarily reflects the role of liver in glucose regulation. Similarly to the HOMA, QUICKI does not provide information on the stimulated glucose and insulin system, but only about the function of homeostatic mechanisms in the fasting state. This primarily reflects the effects of insulin on hepatic glucose production, not on peripheral glucose uptake.

There are conditions in which QUICKI performance is notoriously misleading, primarily impaired fasting glucose (IFG). Moreover, its application appears to be limited due to a lack of standardized reference range for QUICKI as well as to many assumptions (e.g. equivalence of hepatic and peripheral IS) that limit its use [50].

In conclusion, the information concerning insulin action provided by QUICKI appears somewhat limited, although it could be used to monitor longitudinal changes in IS in the same individual. Altogether, the use of QUICKI does not appear an appropriate method to be used alone for the measurement of IS.

Claims on disease risk reduction art 14(a)

Type 2 diabetes mellitus

T2DM is a widespread chronic disease characterized by hyperglycaemia as a result of impaired insulin secretion and/or action which reflects β -cell dysfunction and/or IR. As a consequence, insulin absolute or relative deficient action leads to abnormalities in carbohydrate, fat, and protein metabolism on target tissues. Chronic hyperglycaemia is associated with long-term microvascular—renal, retinal and nerve—and macro-vascular—cardiovascular—complications.

T2DM is diagnosed whenever even only one of the four following criteria is fulfilled [43]:

- (a) Fasting plasma glucose ≥ 126 mg/dL in two different occasions, based on the relationship between fasting plasma glucose and incidence of retinopathy;
- (b) Blood glucose concentrations ≥ 200 mg/dL at 2 h after an OGTT with a standard glucose load (75 g) after an overnight fast [44], based on the relationship between 2-h glucose and incidence of diabetic retinopathy;
- (c) Random plasma glucose ≥ 200 mg/dL together with the clinical picture of diabetes (polyuria, polydipsia, weight loss);
- (d) HbA1c $\geq 6.5\%$ based on the relationship between HbA1c and incidence of diabetic retinopathy.

The rate of undiagnosed T2DM is high due to the lack of specific symptoms, which become manifest (e.g. polyuria, polydipsia, weight loss) in the presence of blood glucose levels above 180–200 mg/dL.

An intermediate group of subjects may display higher than normal glucose levels—which do not meet the diagnostic criteria for diabetes but are associated with higher cardio-metabolic risk. These pre-diabetic states are impaired fasting glucose and impaired glucose tolerance defined as fasting plasma glucose 100–125 mg/dL and 2-h post-load glucose 140–199 mg/dL (when OGTT is used), respectively [43].

The aetiology of T2DM is ascribable to the interaction between genetic predisposition, and behavioural and environmental risk factors. Family history of diabetes and certain ethnicities are predictive of T2DM, whereas the most important modifiable risk factors are obesity and physical inactivity. Pre-diabetes, diagnosed as IFG if fasting glucose is between 100 and 125 mg/dL or as IGT if blood glucose at 2 h after OGTT is between 140 and 199 mg/dL, is associated with an increased risk for developing type 2 diabetes.

Fasting blood glucose Fasting plasma glucose is commonly used to assess glucose tolerance. Plasma glucose should be assessed in the morning following an overnight (at least 8 h) fasting [43].

To evaluate the appropriateness of fasting blood glucose as outcome variable, the literature deriving from database #01 was critically evaluated (see Table 1).

Fasting plasma glucose represents a key criterion to assess glucose tolerance and diagnose type 2 diabetes. A condition of dysglycaemia—IFG or IGT—represents a robust risk factor for development and progression of type 2 diabetes [59]. Moreover, with pre-diabetes developing from normal fasting glucose levels as a continuous process in which hyperglycaemia gradually grows then playing an important role on the genesis of acute and chronic complications (e.g. micro- and macro-vascular), the risk to develop diabetes based on fasting or 2-h post-load glycaemia is comparable to that defined by HbA1c levels [59]. However, this might be particularly true for subjects whose plasma glucose levels fall above the IFG threshold. For this reason, changes in categories of glucose tolerance (i.e. from glucose intolerance to normo-tolerance)

rather than the punctual fasting glucose values within the same glucose category may be considered as an appropriate indicator of risk reduction to be used alone for the scientific substantiation of health claims regarding T2DM risk reduction.

Enzymatic techniques Please refer to "[Enzymatic techniques](#)" section.

Fasting plasma/serum insulin Like fasting plasma glucose, fasting plasma/serum insulin is measured after an overnight fast, with the same recommendations stated in "[Fasting blood glucose](#)" section. Insulin is the main anabolic hormone involved in the regulation of glucose homeostasis, promoting glucose uptake and glycogenesis, besides lipogenesis and protein synthesis in fat tissue and muscle. Different from glycaemia, there is no consensus on the insulin levels [28], which may be associated with the development of CMD.

To evaluate the appropriateness of fasting blood glucose as outcome variable, the literature deriving from database #02 was critically evaluated (see Table 1).

Fasting plasma/serum insulin levels can only be considered as a surrogate marker for IR, which is a key defect in the pathogenesis of T2DM [47]. In epidemiological prospective studies, hyperinsulinaemia may predict the onset of T2DM. However, if considered for use in health claims, insulin assessment is fraught with the lack of a cut-off value to define normality and/or low-/high-risk conditions and of a worldwide standardization of the insulin assay, making rather difficult to compare data from different laboratories/work.

Moreover, all insulin-based IR surrogate indexes require concomitant glucose data for interpretation [60].

In conclusion, for the above-mentioned limitations, although fasting/plasma serum insulin may be associated and/or predictive the development of T2DM, it cannot be used alone to scientifically substantiate health claims regarding T2DM risk reduction. However, it can be used as supportive of a mechanism through which the food/constituent could exert the claimed effect, in addition to the measurement of blood glucose levels.

Chromatographic techniques Please refer to "[Chromatographic techniques](#)" section.

HbA1c As stated in "[HbA1c](#)" section, part of the variability of HbA1c seems linked to genetic variants unrelated to glucose metabolism. HbA1c is also linearly related to the risk of CVD and diabetes in non-diabetic individuals [61, 62]. The mortality curve associated with HbA1c levels is U-shaped, with the lowest death rates for values between 5.0 and 5.5% [63]. No intervention studies have targeted HbA1c

for the prevention of cardiovascular morbidity/mortality in people with normal glucose regulation.

In conclusion, HbA1c, if provided by a certified and standardized method, represents one of the criteria to diagnose T2DM. HbA1c, after careful control for the potential confounders listed above, can be considered as an appropriate risk factor to be used alone for the substantiation of health claims regarding T2DM risk reduction.

Chromatographic techniques Please refer to "[Chromatographic techniques](#)" section.

Conclusions

Currently, most applications to use health claims with regard to blood glucose and insulin concentration/regulation under Articles 13(5) and 14 are not being approved by the Panel on Dietetic Products, Nutrition and Allergies of EFSA. Rejections are due not only to selecting poorly characterized food/food constituents and/or non-beneficial effects, but also to providing inappropriate OVs as primary endpoints and to using inappropriate MMs. As a result of the collecting, collating and critical analysing the OVs and MMs considered under beneficial claims with regard to blood glucose and insulin concentrations compliant with the European Regulation, the present position statement defines their appropriateness level, showing that different or even opposite assessments could be attributed to the same OV in relation to different CEs. For instance, during the performance of randomized controlled trials (RCTs), postprandial blood glucose concentrations (glycaemic peak) and their time-integrated values (glucose AUC) are defined to be appropriate as primary endpoints to substantiate health claims regarding the reduction in postprandial blood glucose, whereas they can be used only as supportive evidence to substantiate health claims in the context of (long-term) maintenance of normal glucose regulation. Accordingly, the choice of MMs should be restricted to gold-standard methods or the best available one for each OV. The present work, therefore, could be a useful roadmap to support an appropriateness-driven selection process of OVs and MMs to be used in RCTs. Furthermore, the information provided in this position statement could be of help to EFSA during the update of the guidance on the scientific requirements for health claims regarding blood glucose and insulin concentration/regulation.

Acknowledgements This project has received financial support from the European Food Safety Authority (EFSA), Grant GP/EFSA/NUTRI/2014/01. The present article, however, is under the sole responsibility of the authors. The positions and opinions presented in this article are those of the authors alone and do not necessarily represent the views/any official position or scientific works of EFSA. To know

about EFSA guidance documents and other scientific outputs of EFSA, please consult its website at <http://www.efsa.europa.eu>.

Authors' contribution DM was the developer of the project and wrote the manuscript together with BB who helped in carrying out the project; GB generated the databases of literature; IZ, CP, MV1, DG, PM, MV2, ADC, RCB and GP helped secure the funding and critically read and revised the manuscript; DDR was the Principal Investigator of the project, critically revised the manuscript and had primary responsibility for final content together with RCB and ADC.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants performed by any of the authors.

Informed consent For this type of study formal consent is not required.

References

- WHO (2016) Global report on diabetes. World Health Organization, Geneva
- Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* 414(6865):813–820
- Brownlee M (2005) The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 54(6):1615–1625
- Howlett J, Ashwell M (2008) Glycemic response and health: summary of a workshop. *Am J Clin Nutr* 87(1):212S–216S
- Martini D, Rossi S, Biasini B et al (2017) Claimed effects, outcome variables and methods of measurement for health claims proposed under European Community Regulation 1924/2006 in the framework of protection against oxidative damage and cardiovascular health. *Nutr Metab Cardiovasc Dis* 27(6):473–503
- EFSA (2012) Guidance on the scientific requirements for health claims related to appetite ratings, weight management, and blood glucose concentrations. *EFSA J* 10(3):2817
- American Diabetes Association (2001) Postprandial blood glucose. *Diabetes Care* 24(4):775–778
- Best JD, Kahn SE, Ader M et al (1996) Role of glucose effectiveness in the determination of glucose tolerance. *Diabetes Care* 19(9):1018–1030
- Atkinson FS, Foster-Powell K, Brand-Miller JC (2008) International tables of glycemic index and glycemic load values: 2008. *Diabetes Care* 31(12):2281–2283
- Evans CE, Greenwood DC, Threapleton DE et al (2017) Glycemic index, glycemic load, and blood pressure: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr* 105(5):1176–1190
- Feliciano Pereira P, das Gracas de Almeida C, Alfenas RC (2014) Glycemic index role on visceral obesity, subclinical inflammation and associated chronic diseases. *Nutr Hosp* 30(2):237–243
- Schwingshackl L, Hoffmann G (2013) Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. *Nutr Metab Cardiovasc Dis* 23(8):699–706
- Bonadonna RC (2004) The syndrome of insulin resistance and its links to atherosclerosis. In: DeFronzo RA, Ferrannini E, Keen H, Zimmet P (eds) *International textbook of diabetes mellitus*, vol 2, 3rd edn. Wiley, Chichester, pp 1379–1394
- Scazzina F, Dei Cas A, Del Rio D et al (2016) The beta-cell burden index of food: a proposal. *Nutr Metab Cardiovasc Dis* 26(10):872–878
- Sacks DB, Arnold M, Bakris GL et al (2011) Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Diabetes Care* 34(6):e61–e99
- Galant AL, Kaufman RC, Wilson JD (2015) Glucose: detection and analysis. *Food Chem* 188:149–160
- Moodley N, Ngxamngxa U, Turzyniecka MJ et al (2015) Historical perspectives in clinical pathology: a history of glucose measurement. *J Clin Pathol* 68(4):258–264
- McMillin J (1990) Blood glucose. In: Walker HK, Hall WD, Hurst JW (eds) *Clinical methods: the history, physical, and laboratory examinations*. Butterworths, Boston
- Wolever TM, Jenkins DJ, Jenkins AL et al (1991) The glycemic index: methodology and clinical implications. *Am J Clin Nutr* 54(5):846–854
- Tai MM (1994) A mathematical model for the determination of total area under glucose tolerance and other metabolic curves. *Diabetes Care* 17(2):152–154
- Lefloch JP, Escuyer P, Baudin E et al (1990) Blood–glucose area under the curve—methodological aspects. *Diabetes Care* 13(2):172–175
- Wolever TMS, Jenkins DJA (1986) The use of the glycemic index in predicting the blood–glucose response to mixed meals. *Am J Clin Nutr* 43(1):167–172
- Allison DB, Paulre F, Maggio C et al (1995) The use of areas under curves in diabetes research. *Diabetes Care* 18(2):245–250
- Brouns F, Bjorck I, Frayn KN et al (2005) Glycaemic index methodology. *Nutr Res Rev* 18(1):145–171
- Wilcox G (2005) Insulin and insulin resistance. *Clin Biochem Rev* 26(2):19–39
- Widjaja A, Morris RJ, Levy JC et al (1999) Within- and between-subject variation in commonly measured anthropometric and biochemical variables. *Clin Chem* 45(4):561–566
- Hirsch S, Barrera G, Leiva L et al (2013) Variability of glycemic and insulin response to a standard meal, within and between healthy subjects. *Nutr Hosp* 28(2):541–544
- Staten MA, Stern MP, Miller WG et al (2010) Insulin assay standardization leading to measures of insulin sensitivity and secretion for practical clinical care. *Diabetes Care* 33(1):205–206
- Chen Z, Caulfield MP, McPhaul MJ et al (2013) Quantitative insulin analysis using liquid chromatography–tandem mass spectrometry in a high-throughput clinical laboratory. *Clin Chem* 59(9):1349–1356
- Rodriguez-Cabaleiro D, Van Uytanghe K, Stove V et al (2007) Pilot study for the standardization of insulin immunoassays with isotope dilution liquid chromatography/tandem mass spectrometry. *Clin Chem* 53(8):1462–1469
- Khaksa G, Nalini K, Bhat M et al (1998) High-performance liquid chromatographic determination of insulin in rat and human plasma. *Anal Biochem* 260(1):92–95
- Yilmaz B, Kadioglu Y, Capoglu I (2012) Determination of insulin in humans with insulin-dependent diabetes mellitus patients by HPLC with diode array detection. *J Chromatogr Sci* 50(7):586–590
- Committee Consensus (2007) Consensus statement on the worldwide standardization of the hemoglobin A1C measurement: the American Diabetes Association, European Association for the Study of Diabetes, International Federation of Clinical Chemistry and Laboratory Medicine, and the International Diabetes Federation. *Diabetes Care* 30(9):2399–2400
- WHO (2011) Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. World Health Organization, Geneva

35. Sacks DB (2012) Measurement of Hemoglobin A1c: a new twist on the path to harmony. *Diabetes Care* 35(12):2674–2680
36. Ruijgrok C, Dekker JM, Beulens JW et al (2017) Size and shape of the associations of glucose, HbA1c, insulin and HOMA-IR with incident type 2 diabetes: the Hoorn Study. *Diabetologia*. <https://doi.org/10.1007/s00125-017-4452-7>
37. Hanas R, John G (2010) 2010 consensus statement on the worldwide standardization of the hemoglobin A1c measurement. *Diabetes Med* 27(7):737–738
38. Sacks DB, Arnold M, Bakris GL et al (2011) Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clin Chem* 57(6):e1–e47
39. Armbruster DA (1987) Fructosamine—structure, analysis, and clinical usefulness. *Clin Chem* 33(12):2153–2163
40. True MW (2009) Circulating biomarkers of glycemia in diabetes management and implications for personalized medicine. *J Diabetes Sci Technol* 3(4):743–747
41. Koga M (2014) Glycated albumin; clinical usefulness. *Clin Chim Acta* 433:96–104
42. Schleicher E, Wieland OH (1981) Specific quantitation by HPLC of protein (lysine) bound glucose in human serum albumin and other glycosylated proteins. *J Clin Chem Clin Biochem* 19(2):81–87
43. ADA (2010) Diagnosis and classification of diabetes mellitus. *Diabetes Care* 33(Suppl. 1):S62–S69
44. WHO (2006) Definition and diagnosis of diabetes mellitus and intermediate hyperglycaemia: report of a WHO/IDF consultation. World Health Organization, Geneva
45. American Diabetes Association (2010) Standards of medical care in diabetes. *Diabetes Care* 33(Suppl. 1):S11–S61
46. Ferrannini E, Mari A (2014) β -Cell function in type 2 diabetes. *Metabolism* 63(10):1217–1227
47. Muniyappa R, Lee S, Chen H et al (2008) Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *Am J Physiol Endocrinol Metab* 294(1):E15–E26
48. Ferrannini E, Cobelli C (1987) The kinetics of insulin in man. I. General aspects. *Diabetes Metab Rev* 3(2):335–363
49. Wolever T, Campbell J, Pupovac J et al (2003) High fiber cereal reduces postprandial insulin responses in obese, hyperinsulinemic, but not in lean, normo-insulinemic subjects. *Obes Res* 11:A57–A57
50. Singh B (2010) Surrogate markers of insulin resistance: a review. *World J Diabetes* 1(2):36
51. Bird SR, Hawley JA (2017) Update on the effects of physical activity on insulin sensitivity in humans. *BMJ Open Sport Exerc Med*. <https://doi.org/10.1136/bmjsem-2016-000143>
52. Mason C, Foster-Schubert KE, Imayama I et al (2011) Dietary weight loss and exercise effects on insulin resistance in postmenopausal women. *Am J Prev Med* 41(4):366–375
53. Colberg SR, Sigal RJ, Fernhall B et al (2010) Exercise and type 2 diabetes: the American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. *Diabetes Care* 33(12):2692–2696
54. DeFronzo RA, Tobin JD, Andres R (1979) Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 237(3):E214–E223
55. Del Prato S, Marchetti P, Bonadonna RC (2002) Phasic insulin release and metabolic regulation in type 2 diabetes. *Diabetes* 51(Suppl 1):S109–S116
56. Matsuda M, DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22(9):1462–1470
57. Katz A, Nambi SS, Mather K et al (2000) Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab* 85(7):2402–2410
58. Abbasi F, Reaven GM (2002) Evaluation of the quantitative insulin sensitivity check index as an estimate of insulin sensitivity in humans. *Metab Clin Exp* 51(2):235–237
59. Tabák AG, Herder C, Rathmann W et al (2012) Prediabetes: a high-risk state for diabetes development. *Lancet* 379(9833):2279–2290
60. Patarrão RS, Wayne Lutt W, Paula Macedo M (2014) Assessment of methods and indexes of insulin sensitivity. *Revista Portuguesa de Endocrinologia, Diabetes e Metabolismo* 9(1):65–73. <https://doi.org/10.1016/j.rpedm.2013.10.004>
61. Ikeda F, Doi Y, Ninomiya T et al (2013) Haemoglobin A1c even within non-diabetic level is a predictor of cardiovascular disease in a general Japanese population: the Hisayama Study. *Cardiovasc Diabetol* 12:164
62. Pai JK, Cahill LE, Hu FB et al (2013) Hemoglobin A1c is associated with increased risk of incident coronary heart disease among apparently healthy, nondiabetic men and women. *J Am Heart Assoc* 2(2):e000077
63. Paprott R, Schaffrath Rosario A, Busch MA et al (2015) Association between hemoglobin A1c and all-cause mortality: results of the mortality follow-up of the German National Health Interview and Examination Survey 1998. *Diabetes Care* 38(2):249–256