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RESEARCH ARTICLE

Claimed effects, outcome variables and methods of measurement for health claims on foods proposed under European Community Regulation 1924/2006 in the area of appetite ratings and weight management

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ABSTRACT

All the requests for authorisation to bear health claims under Articles 13(5) and 14 in the context of appetite ratings and weight management have received a negative opinion by the European Food Safety Authority (EFSA), mainly because of the insufficient substantiation of the claimed effects (CEs). This manuscript results from an investigation aimed to collect, collate and critically analyse the information related to outcome variables (OVs) and methods of measurement (MMs) in the context of appetite ratings and weight management compliant with Regulation 1924/2006. Based on the literature review, the appropriateness of OVs and MMs was evaluated for specific CEs. This work might help EFSA in the development of updated guidance addressed to stakeholders interested in bearing health claims in the area of weight management. Moreover, it could drive the applicants during the design of randomised controlled trials aimed to substantiate such claims.

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1. Introduction

According to an estimation made by the World Health Organisation (WHO) (WHO 2016), more than 600 million people were obese in 2014. This number is expected to increase even more than one billion by 2030 (Kelly et al. 2008). Although there is a certain degree of inter-subject variability, as demonstrated by the existence of a particular phenotype shown by subjects referred to as metabolically healthy obese (Phillips 2013), obesity has been historically associated with an increased risk of cardio-metabolic complications, such as type 2 diabetes mellitus, hypertension, dyslipidaemia, cardiovascular disease (CVD) (Mokdad et al. 2003; Van Gaal et al. 2006). Severe obesity states during adolescence have been also associated with other medical comorbidities including obstructive sleep apnoea syndrome, fatty liver disease, reproductive and musculoskeletal complications (Kelly et al. 2013). Obesity is a multifactorial condition characterised by abnormal or excessive adiposity caused by an imbalance between energy intake and expenditure over a prolonged time leading to a higher adipose tissue (AT) accumulation (Prieto-Hontoria et al. 2011). Genetic predisposition and lifestyle factors, such as sedentary behaviour and deterioration of dietary quality characterised by excess consumption of highly processed and energy-dense foods high in fat, mainly saturated fat, and low in unrefined carbohydrates are important contributors to the obesity epidemic (Popkin et al. 2012). A condition of obesity promotes insulin-resistance and is associated with low-grade inflammation. The development of overweight and obese status is clearly dependent on diet, which plays a key role in inflammation status (Calder et al. 2009) which in turn can predispose to the pathogenesis of metabolic diseases. Therefore, the choice of healthy

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dietetic patterns represents one of the most important strategy of prevention and treatment for overweight and obesity. Food policies at national and international level have promoted educational interventions and informational campaigns toward healthy lifestyles to prevent and contrast the emergency represented by obesity. However, the efforts done in this regard result largely unsatisfactory. Since food industries play an important role actively driving and affecting food choice of the consumers, their contribution is crucial for a complementary approach in that direction. Besides the formulation of foods with nutritional claims (e.g. low energy/sugar/sodium/salt/saturated-fat foodstuff) and foods intended for use in energy-restricted diets for weight reduction, more recently the strategy of food industries includes the formulation of functional foods associated to specific health claims. According to the definition of the International Food Information Council Foundation, a functional food can be defined as a food or food component able to provide benefits beyond basic nutrition and may play a role in reducing or minimising risk of certain diseases and other health conditions (IFIC-Foundation 2011). The interest of food industries to bear health claims in the context of appetite ratings and weight management is progressively increased generating different requests for authorisation. All of them have received a negative opinion from European Food Safety Authority (EFSA) for reasons concerning the formulation of the claim proposed by the Applicants, the insufficient characterisation of the food/food constituent, as well as the design of studies provided for the substantiation of the claimed effects (CEs). The lack of a significant effect of the food/food constituent, observed in many of the requests for authorisation, in the direction of the CEs proposed, is largely due to an improper choice of the primary endpoints evaluated in human intervention studies submitted. The present manuscript reports the results of an investigation aimed to collect, collate and critically analyse the information in relation to CEs, outcome variables (OVs) and methods of measurement (MMs), in the context of appetite ratings and weight management compliant with Regulation 1924/2006.

2. Materials and methods: search strategy

The manuscript refers to CEs, OVs and MMs collected from the relative Guidance document (EFSA NDA Panel 2012), from the Scientific Opinions on the substantiation of health claims under Articles 13.5 and 14 of Regulation 1924/2006 related to appetite ratings and weight management (European Commission 2012), as well as from comments received during public consultations. Following the same search strategy already applied in Martini et al. (2017), 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 in the context of the specific CEs. Starting from a pool of 19 requests for authorisation of health claims, six were not considered because the claim was not defined as a beneficial physiological effect *per se* or because the food/constituent was not sufficiently characterised. The remaining 13 requests were evaluated and referred to four different claims falling under Article 13(5). The critical analysis was performed on 17 different OVs, four of which were assessed for a single CE, whereas eight and five were evaluated in the context of two and three different CEs, respectively. Among the 14 different MMs considered, a half was assessed for a single OV, while two MMs were analysed in relation to five different OVs and five MMs were evaluated in relation to two OVs.

For better understanding of the numbered headings, a detailed number indexing can be found in the Appendix.

3. Results: critical evaluation of outcome variables and methods of measurement

3.1 Claims falling under art. 13(5)

3.1.1 Reduction of body fat/body weight or maintenance of body weight after weight loss

Body composition is known as an important indicator of health and physical fitness. In fact, it has been associated to a number of pathologies, such as obesity, CVD, type 2 diabetes mellitus (T2DM), certain types of cancer, and osteoporosis and osteoarthritis (Must et al. 1999). It is the best long-term indicator of nutritional status and its assessment is useful in both clinical and research settings. Body composition can be presently evaluated at five levels; atomic, molecular, cellular, tissue-organ and whole-body. The five-component "molecular" model, recognised as the reference model of body composition, calculates body weight as the sum of measured fat mass (FM), measured total body water (TBW), measured protein mass, measured mineral mass and measured glycogen. The sum of TBW, protein mass, mineral mass and glycogen makes up the so-called fat-free mass (FFM), which is therefore a highly heterogeneous "compartment" (Wang et al. 1992). Glycogen is usually neglected by body composition studies so that FFM is often simply defined as the sum of TBW, protein mass and mineral

mass. Two-compartment body composition models can be defined under the assumption of a constant physical or chemical property of the FFM. Under the assumption of such constancy, FFM can be estimated from body density (e.g. air displacement plethysmography), TBW (hydrometry) and body potassium (40 K measurement) (Ellis 2000).

The assessment of body composition can be performed at the whole-body or regional level. About 80% of AT is subcutaneous and is located mainly in the abdominal and gluteo-femoral regions (Ibrahim 2010). In detail, subcutaneous AT is commonly defined as the layer of AT located between the dermis and the aponeurosis and fasciae of muscle. Several investigators consider mammary AT a part of subcutaneous fat despite its peculiar function (Shen et al. 2003).

The most interesting regional body composition data available so far are related to abdominal fat and its distribution between subcutaneous and visceral fat. At the abdominal level, adiposity is accumulated around and within the cavity of the abdomen. A clear anatomical demarcation, as well as specific metabolic, endocrine, paracrine and autocrine properties permits to distinguish these two compartments of abdominal fat, represented by abdominal subcutaneous and visceral AT. In the lower abdomen and in the pelvic region, subcutaneous AT can be further separated into superficial, i.e. close to the dermis, and deep, i.e. close to the muscle fasciae. The former is located especially around the abdominal cavity, while the latter is distributed mainly in the posterior abdomen (Tchernof and Despres 2013).

It should be noted that different operational definitions of visceral AT are used in the research literature. Strictly speaking, it is the sum of the AT surrounding thoracic, abdominal and pelvic organs. Nevertheless, some investigators define it as the sum of intra-thoracic, intra-abdominal and intra-pelvic AT. Usually, only intra-abdominal and intra-pelvic AT are measured, with visceral AT defined as the AT within the area delimited by the dome of the liver and the femoral heads. Visceral AT is alternatively defined as intra-abdominal AT, comprised within the superior border of the liver and 5 cm below the 4th or 5th lumbar vertebrae. In the latter case, AT is composed by intraperitoneal (mostly omental and mesenteric) and retroperitoneal AT that surrounds internal organs. Some studies have focussed on intraperitoneal AT and have considered it as visceral AT, with the exclusion of retroperitoneal AT (Shen et al. 2003).

The available data suggest that abdominal AT plays a major role in the pathogenesis of cardiometabolic disease (CMD). An association, even if based on few data, has been shown between an increase of intra-abdominal AT and CMD (e.g. T2DM, myocardial infarction) and all-cause mortality (Tchernof and Despres 2013). Nevertheless, some evidence suggests that abdominal subcutaneous AT might play an important role in the pathophysiology of obesity-related abnormalities, especially insulin resistance (IR) (Patel and Abate 2013). The relative role of visceral and subcutaneous fat in the pathogenesis of CMD is undergoing active investigation.

3.1.1.1 Total body fat. To evaluate the appropriateness of total body fat as OV, the literature deriving from database #06 was critically evaluated (see Table 1).

The assessment of total body fat may offer physiologically and clinically relevant information. Fat mass may be a predictor of morbidity and mortality. There is in fact increasing evidence pointing FM as a risk factor for the development of CMD. However, more studies are needed to define the role of the assessment of FM in clinical practice as simpler measures, such as body mass index (BMI) and waist circumference (WC), are easier to obtain and have presently a clearer prognostic significance than FM (Bosy-Westphal et al. 2003). The direct quantification of FM or its measurement from the five-component model is possible (see general introduction) but restricted to few centres worldwide. FM is most commonly estimated from two-compartments models assuming the constancy of a physical or chemical property of the FFM, this latter being simply defined as [body mass (weight) – FM]. Under the assumption of such constancy, FFM can be estimated from body density (e.g. air displacement plethysmography), TBW (hydrometry) and body potassium (40 K measurement) (Ellis 2000). The density of FFM, its hydration and its potassium content however may undergo changes during growth, aging and disease. To properly assess FFM and therefore FM in these conditions, more sophisticated models of body composition are warranted.

In conclusion, total body fat per se is an appropriate OV for the substantiation of health claims in the context of body fat reduction. Provided that it is used in combination with a direct measure of body weight, total body fat is also an appropriate outcome measure for the substantiation of health claims related to body weight reduction or maintenance. Lastly, provided that it is used in combination with a direct measure of abdominal fat, total body fat is an appropriate OV for the substantiation of health claims related to abdominal fat reduction.

3.1.1.1.1 DEXA. Dual energy X-ray absorptiometry (DEXA), the reference method for the assessment of bone mineral content (BMC) is gaining increasing interest for the assessment of whole-body and regional

Table 1. Strategies used for retrieving the literature pertinent with outcome variables and methods of measurement related to appetite rating and weight management.

DB number	Syntax	Total articles	Narrative reviews	Systematic reviews/ meta-analyses	Validation studies	Outcome variables
1	"satiety response"[mesh] AND "english"[language] AND "humans"[mesh]	1164	256	17	10	Satiety
2	"hunger"[mesh] AND "english"[language] AND "humans"[mesh]	6751	1560	84	57	Hunger or appetite
3	"energy intake"[mesh] AND "english"[language] AND "humans"[mesh]	24,407	3715	529	322	Energy intake
4	"body weight"[mesh] AND "english"[language] AND "humans"[mesh]	207,533	26,264	4786	1010	Body weight
5	"body mass index"[mesh] AND "english"[language] AND "humans"[mesh]	80,467	2939	1460	789	Body mass index
6	("fat body"[mesh] OR "body composition"[mesh]) AND "english"[language] AND "humans"[mesh]	28,023	2971	407	415	Body fat Visceral fat Subcutaneous fat Abdominal fat
7	"fat oxidation"[all fields] AND "english"[language] AND "humans"[mesh]	1538	295	9	4	Fat oxidation
8	"lipogenesis"[mesh] AND "english"[language] AND "humans"[mesh]	609	141	4	0	*De-novo* lipogenesis
9	"energy metabolism"[mesh] AND "english"[language] AND "humans"[mesh]	88,693	18,245	538	506	Energy expenditure
10	"waist circumference"[mesh] AND "english"[language] AND "humans"[mesh]	5155	143	102	70	Waist circumference
11	"hip circumference"[all fields] AND "english"[language] AND "humans"[mesh]	1430	31	15	26	Hip circumference
12	"waist-hip ratio"[mesh] AND "english"[language] AND "humans"[mesh]	2761	93	60	31	Waist to hip ratio
13	("lean body mass"[all fields] OR "fat-free mass"[all fields] OR "fat free mass" [all fields]) AND "english"[language] AND "humans"[mesh]	9233	970	145	144	Lean body mass
14	"proteins/metabolism"[mesh] AND "body composition"[mesh] AND "english"[language] AND "humans"[mesh]	5309	417	30	16	Protein metabolism

(arms, legs and trunk) body composition. DEXA employs a three-compartment body composition model, where body mass is calculated as the sum of measured BMC, measured FM and measured lean tissue mass (LTM) (Fosbol and Zerahn 2015). According to the DEXA three-compartment model, FFM is the sum of BMC and LTM. This operational definition of FFM is peculiar to DEXA and is different from that given by the reference five-compartment model of body composition (see general introduction). DEXA performs a whole-body scan using two X-rays of different energies, requiring some analysis time. On the basis of the differential attenuation of such X-rays and under the assumption of some constant properties of the underlying tissues (mostly hydration and shape), DEXA can separate bone tissues, i.e. BMC, from non-bone tissues, i.e. FM and LTM (Fosbol and Zerahn 2015). A very useful feature of DEXA is the ability to evaluate regional body composition together with whole-body composition. Five body regions are generally measured with DEXA: arms (two regions), legs (two regions) and trunk (one region). However, the determination of body composition at trunk level is problematic and has limited accuracy because of the low percentage of bone free-pixels which characterises not only this area but also other body regions, such as neck and head, usually not evaluated by DEXA (Lee and Gallagher 2008). This technique has a minimal radiation exposure (0.1 μGy), is relatively fast (6–7 minutes) and is highly reproducible. These are the main reasons why DEXA is being increasingly used by researchers. On the other hand, DEXA is expensive and requires specific skills. Moreover, validation studies of DEXA against the five-component body composition reference model have given mixed results. Some evidences have reported a possible overestimation of lean body mass provided by DEXA when used for tracking longitudinal changes compared to other techniques, such as computed tomography (CT) or magnetic resonance imaging (MRI). On the basis of these data, DEXA cannot be considered a gold-standard method for the assessment of body composition. Nonetheless, provided that measurements are made appropriately and its limitations are taken into account, there is a general agreement that DEXA is useful to study body composition changes, e.g. those produced by a weight loss programme. Among the limitations of DEXA, it must be kept in mind that it cannot be performed in severely obese subjects because of their higher mass and different body shape (Lohman et al. 2009). Changes of body hydration are especially detrimental to DEXA

estimates of body composition because the underlying algorithms do assume a relatively narrow range of body hydration. Another limitation of DEXA is the use of proprietary algorithms. Data from the same DEXA scanner may give different estimates of body composition when analysed with different versions of the same software. More importantly, the DEXA measurements of body composition obtained by scanners from different manufactures cannot be compared because of the different underlying algorithms (Fosbol and Zerahn 2015).

In summary, provided that its limitations are taken into account, DEXA can be regarded as an appropriate method to be used in human intervention studies to assess total and regional body fat, as well as lean body mass.

3.1.1.1.2 MRI. MRI is a highly sophisticated and costly technique, widely employed for medical application and increasingly used in body composition research. Similarly to CT, MRI is able to measure body composition at tissue and organ level determining whole-body and regional fat, distinguishing between visceral and subcutaneous AT, and skeletal muscle tissue. If compared to cadaver validation studies, MRI has demonstrated an excellent accuracy in skeletal muscle measures. MRI requires a magnet, usually a superconducting one, a magnetic field gradient system for signal localisation and a radio frequency system for signal generation and processing. The array data provided by MRI describe the spatial distribution of physical quantities (Ackland et al. 2012). Using MRI or CT can be reconstructed muscle volumes that multiplied by muscle tissue density (1.04 kg/L) provide muscle masses. The sources of technical errors are irregular borders or shapes set inside tissues blood vessels. MRI obtains whole-body or regional estimates of AT by using single or multiple slices. Even though single-slice imaging is less accurate than multiple-slice imaging, it is commonly used because it is cheap and fast (Shen et al. 2003). Whole-body scans, acquired as a series of stacks and then integrated, are however needed to accurately detect intra-individual changes of AT. The single-slice MRI method presents some limitations, such as the assumption of a similar distribution of visceral fat within the abdomen of the same individual and the inaccurate slice positioning that may produce spurious findings. Even with the multi-slice method, the accuracy of MRI is limited by image distortion and by the pixel size (2 mm × 2 mm) currently employed for total-body scans (Thomas et al. 1998; Ackland et al. 2012). However, the main limitation of MRI is that it cannot be completely automated. A manual or semi-automated analysis of time intensive T1-weighted images is the approach most commonly used. Measurements are operator-dependent in case of manual input. The majority of the semi-automated validated procedures relate to adult subjects. MRI requires small movements and sometimes breath holding so that the assessment of AT in children is more complex. The smaller body mass and fat depots are further reasons of the inaccuracy of MRI in children. This contributes to explain why very limited information is available on AT distribution in children. Furthermore, obese individuals may not fit inside the magnet and scan duration may produce the discomfort of the subject. Together with CT, MRI is considered the most accurate method to assess lean body mass and the amount and distribution of AT and it is thus regarded as a reference method. A clear advantage of MRI over CT is that MRI does not expose subjects to ionising radiation. However, the use of MRI is prioritised to medical applications, and its cost may limit frequent assessments in intervention studies. In virtue of its high accuracy, this technology is an important asset to evaluate body composition in several clinical conditions, such as obesity, sarcopenia and immunological disorders.

In conclusion, MRI is considered an appropriate method to assess total and regional body fat as well as lean body mass.

3.1.1.1.3 CT. CT, now widely employed for medical applications, has been validated for the assessment of body composition by means of *in vivo* studies. It is an excellent but costly method for measuring total and regional adiposity. This technology employs X-ray that are electronically processed to produce tomographic images of given body regions. The subject is scanned supine with the beam of X-ray in a perpendicular plane. A 3D high-resolution image is created by merging multiple cross-sectional images taken around one axis of rotation. Together with MRI, CT provides the most accurate *in vivo* measurements of AT and lean body mass and it is thus regarded as a reference method. However, there is no consensus about a common CT protocol to quantify abdominal AT volume (cm^3). A single-slice imaging method is often employed, although it is less accurate than the multiple-slice method (Shen et al. 2003). Single-slice imaging is commonly performed between the 4th and 5th lumbar vertebrae (L4–L5) and less commonly between the 3rd and 4th (L3–L4) lumbar vertebrae. The main assumption underlying the single-slice CT method is that subcutaneous and visceral AT have the same distribution within the abdomen of the same individual. The multiple-slice approach exposes the subject to higher radiation and cannot therefore be employed in

longitudinal studies requiring repeated scans (Ellis 2000). Moreover, high radiation dose is the reason why total-body scanning in living humans (Ackland et al. 2012) especially in children, is not feasible. Ethnicity-and gender-specific reference data of subcutaneous and visceral AT are available in the literature. As bone, skeletal muscle and AT have different radiological densities, CT offers the possibility to evaluate total and regional body composition. However, despite having the clear advantage of allowing regional body composition assessment (arms, legs and trunk), CT cannot replace the five-component model to assess body composition (see general introduction). Another limitation of CT is that its use is prioritised to medical applications. Moreover, radiation and cost may limit frequent assessments in intervention studies.

In conclusion, CT is considered an appropriate method to assess total and regional body fat, as well as lean body mass.

3.1.1.1.4 Skinfold thickness. The measurement of skinfolds (e.g. biceps, triceps, subscapular, suprailiac, midthigh and calf) allows to directly assess subcutaneous fat, which is a major component (80%) of body fat. By means of predictive equations, skinfolds allow to estimate percent body fat. Such equations estimate body fat directly from skinfolds or from a measure of body density, which has been in turn estimated from skinfolds. As it is true for all indirect body composition techniques, skinfolds may be reasonably accurate to estimate FM at the population level, but do not perform well in single individuals. Skinfolds are measured using skinfold callipers. The type of skinfold calliper, the sites(s) of measurement, and the training of the operator are important factors for the reproducibility of skinfold measurement. Such reproducibility can be improved by adhering to standardised protocols (Mattsson and Thomas 2006). As skinfolds are a direct measure of subcutaneous fat, their association with disease differs from that between this latter and BMI, whose denominator (weight) represents the sum of fat- and fat-free tissues. The reliability of skinfold assessment decreases with increasing weight and the method is not suitable for use in severely obese subjects.

In conclusion, skinfold thickness cannot be considered an appropriate method to be used alone for the measurement of total body fat, mainly in presence of small changes of this variable or if obese subjects are evaluated.

3.1.1.1.5 BIA. Bio-electrical impedance analysis (BIA) is a widely used method for the assessment of body composition. The physical property upon which BIA is based is that the impedance of the body or selected segments of it (arms, legs and trunk) to an alternating electrical current is inversely proportional to their content of water. FM values are obtained from the difference between body weight and FFM. BIA uses prediction equations to estimate TBW or FFM from impedance-based predictors. The most common of such predictors is impedance index, i.e. the ratio between squared height and impedance. Impedance index was devised by assuming that the human body behaves like an ohmic conductor. Although this is certainly false, impedance index has survived many empirical tests as predictor of TBW and FFM and continues to be employed in BIA algorithms because of such empirical evidence (Fosbol and Zerahn 2015). The main BIA techniques can be summarised as follows (Kyle et al. 2004a):

1. SF-BIA is the most commonly employed BIA technique. With this technique, impedance is measured at a single frequency, usually 50 kHz, and TBW or FFM is estimated from an empirical predictive equation.
2. MF-BIA measures impedance at multiple frequencies. When impedance is measured at low frequencies (<50 kHz, usually 1–5 kHz), it provides an indirect assessment of extracellular water (ECW) because most of the electrical current will not cross the cell membranes. On the contrary, when impedance is measured at high frequencies (>50 kHz, usually 100–500 kHz), it provides an indirect assessment of TBW because most of the electrical current will cross the cell membranes. With this technique, TBW, ECW and FFM are estimated by empirical predictive equations.
3. Bio-electrical spectroscopy (BIS) measures impedance at a wider range of frequencies than MF-BIA, e.g. from 1 to 500 kHz with a step of 5 kHz. BIS uses physiological and empirical modelling to predict TBW, ECW and FFM.

BIA can be performed at the whole-body or segmental level. Segmental BIA was developed with the aim of overcoming the problem that the contribution of body limbs to impedance is greater than that of the trunk. Segmental BIA can nonetheless estimate appendicular (arms, legs and possibly trunk) FFM. Despite its simplicity, portability and low-cost, BIA has some important limitations. First, prediction equations are generated using population-specific data. Second, BIA cannot accurately assess TBW and ECW when body water compartments are undergoing acute changes. Third, BIA is not appropriate to estimate small changes in total or regional body composition even when population-specific equations are being used

(Kyle et al. 2004a, 2004b). Fasting, standardisation of body posture before measurement and control for other known confounders (e.g. skin temperature) are required for BIA to be reliable.

In conclusion, BIA is not generally considered an appropriate method to be used alone for the measurement of total or regional body fat, as well as lean body mass, particularly in presence of small changes in these variables or if subjects with morbid obesity/alterations in body water compartments are evaluated.

3.1.1.1.6 Air displacement plethysmography. Air displacement plethysmography is a technique employed to measure body density and to estimate body composition from it. This technique represents a non-invasive alternative method to under water weighing. The two-compartment body composition model employed by air displacement plethysmography considers body mass (weight) as the sum of FM and FFM. Under the assumption of a known and constant density of FFM, it is possible to estimate FFM from body density, i.e. the ratio of body mass (weight) to body volume. However, while FM density remains fairly constant, FFM density changes substantially during growth, aging and disease (Lee and Gallagher 2008).The measurement system of this technique consists of a cabin with two chambers. The reference chamber is separated by the test chamber by a moving diaphragm under computerised control. While the person sits inside the reference chamber, computerised sensors determine the amount of air displaced in the test chamber to calculate body volume. Air displacement plethysmography requires correction for residual lung volume and surface area artefacts. Lung volume is measured by this method during tidal breathing with exhalation against a mechanical obstruction. Intestinal gas cannot be measured and is commonly assumed to be 100 mL (Mattsson and Thomas 2006). To prevent erroneous air displacement due to air pockets and therefore to obtain more precise estimate of body density, it is recommended that the subject wear a synthetic bathing suit and a swim cap (Mattsson and Thomas 2006). Provided that the assumptions about FFM density are met, air displacement plethysmography provides a relatively simple method to assess body volume and therefore body density and body composition. In virtue of its repeatability, quickness and minimal compliance request, this technology can be used in clinical and research settings. Data on FFM density are presently available for most ages of life but most acute and chronic diseases are likely to change the composition and therefore the density of the FFM so that air displacement plethysmography cannot be applied to ill subjects.

The main limitation of the two-component model consists in potential errors in estimating body composition owing to synergy in the potential sources of error listed above.

To minimise the assumption for two-compartment models, a combination of methods can be used to measure lean mass (e.g. going from two-compartment to multi-compartment models) (Fosbol and Zerahn 2015). As an example, it is possible to measure TBW with deuterium and bone mineral mass by DEXA in combination with body volume (by e.g. air displacement plethysmography), instead of assuming the density of the FFM when using air displacement plethysmography alone.

In conclusion, air displacement plethysmography cannot be regarded as an appropriate method to be used alone for the assessment of total body fat, as well as lean body mass, particularly in presence of small changes in these variables or if obese subjects are evaluated.

3.1.1.2 Visceral fat. To evaluate the appropriateness of visceral fat as OV, the literature deriving from database #06 was critically evaluated (see Table 1).

The available data suggest that abdominal AT plays a major role in the pathogenesis of CMD. The relative role of visceral and subcutaneous fat in the pathogenesis of CMD is under active investigation. The roots of the interest for visceral vs. subcutaneous AT are in the impressive body of epidemiologic evidence linking WC (or closely related measures of body shape) to CMD (Balkau et al. 2007). Since the late '80s of the last century, this relationship was thought to be underlined by visceral and subcutaneous AT biology, of which waist was reputed to be just an epidemiologically useful proxy. Subcutaneous and visceral AT can be distinguished not only from an anatomical perspective but also from a functional viewpoint as they have different metabolic, endocrine, paracrine and autocrine roles. Compared to adipocytes in subcutaneous AT, visceral adipocytes are more insulin resistant, with higher lipolytic activity (Ibrahim 2010). Visceral adiposity has been associated to impaired glucose homeostasis, as demonstrated by the fact that subjects with higher levels of visceral AT, when compared to those with similar abdominal subcutaneous AT, are more likely to have higher glycaemia after Oral Glucose Tolerance Test and lower glucose disposal rates measured during a euglycemic-hyperinsulinemic clamp (Tchernof and Despres 2013). Moreover, visceral adiposity has been found in association with atherogenic dyslipidaemia (Despres et al. 1990) and proinflammatory profile (Lemieux et al. 2001). Some studies have

suggested thresholds of visceral AT (specially intended as intra-abdominal AT) areas, better associated to cardiometabolic health, similarly to BMI (Williams et al. 1996; Kim et al. 2006). Nevertheless, because of the absence of standardised location of measuring site, validation across studies is challenging. Genes, gender, total body fat, age and ethnicity are major determinants of visceral AT but other factors, such as lifestyle or impaired metabolic function may contribute to its accumulation.

In conclusion, visceral fat is an appropriate OV for the scientific substantiation of health claims related to the reduction of body fat/weight or maintenance of body weight after weight loss only if used in combination with direct measures of total body fat and body weight. However, it is an appropriate OV to be used alone for the substantiation of health claims in the context of reduction of abdominal fat.

3.1.1.2.1 MRI. See section 3.1.1.1.2.

3.1.1.2.2 CT. See section 3.1.1.1.3.

3.1.1.3 Subcutaneous fat. To evaluate the appropriateness of subcutaneous fat as OV, the literature deriving from database #06 was critically evaluated (see Table 1).

Subcutaneous and visceral AT can be distinguished not only from an anatomical perspective but also from a functional viewpoint as they have different metabolic, endocrine, paracrine and autocrine roles. The relative role of visceral and subcutaneous fat in the pathogenesis of CMD is under active investigation. The main function of subcutaneous AT is to buffer the energy excess in the form of fatty acids stored as triacylglycerols (also known as triglycerides (TG)) (Ibrahim 2010). Furthermore, subcutaneous AT, releases a number of active substances ("adipokines") with endocrine and paracrine functions. Several studies have suggested a possible protective role of this compartment against the development of the metabolic syndrome (Lee et al. 2013). Subcutaneous AT contains small adipocytes, which avidly adsorb free fatty acids and TG, preventing their accumulation in non-AT. Moreover, it has a lesser rate of insulin-stimulated glucose uptake as compared to visceral AT which, on the contrary, has a great percentage of large adipocytes resistant to the anti-lipolytic effect of insulin (Ibrahim 2010). Although an increasing of weight loss leads to a greater reduction of VAT, allometric models have suggested a non-linear association between the two variables due to the attenuation of decrease of VAT compared to SAT (Hall and Hallgreen 2008). Visceral obesity is often associated with a dysfunctional subcutaneous AT unable to appropriately expand in the presence of an energy surplus. This condition leads to ectopic TG accumulation (e.g. heart, liver, pancreas, muscles), which is associated with several cardiometabolic risk factors (Tchernof and Despres 2013). As about 80% of AT is subcutaneous and it is located mainly in the abdominal and gluteo-femoral regions, a reduction in subcutaneous AT will nearly always likely occur during an intervention aimed at reducing body fat. Thus, subcutaneous fat is an appropriate OV for the scientific substantiation of health claims related to the reduction of body fat. However, it should be used in combination with body weight for the substantiation of health claims in the context of reduction of body weight or maintenance of body weight after weight loss. Finally, the combined measurement of abdominal subcutaneous fat with visceral fat is preferable for the substantiation of health claims related to the reduction of abdominal fat.

3.1.1.3.1 MRI. See section 3.1.1.1.2.

3.1.1.3.2 CT. See section 3.1.1.1.3.

3.1.1.4 Body weight. Body weight is the main body dimension used to assess nutritional and status in epidemiological, clinical and experimental settings. It is a primary measure in clinical practice and public health policy due to the association of overweight and obesity with an increased risk of CMD and death (Pi-Sunyer 2009). Body weight is the OV employed in weight loss intervention studies to assess the effect of a given treatment during and at the end of follow-up, with the change expressed as both absolute and percent terms. Although the changes of weight and ponderostatural indexes tell very little about the underlying changes in body composition (Okorodudu et al. 2010), they have a clear prognostic significance and this is the reason why they are commonly evaluated as OVs of clinical studies.

To evaluate the appropriateness of body weight as OV, the literature deriving from database #04 was critically evaluated (see Table 1).

Although body weight provides no information on body composition, there is an established association between excess body weight and reduced survival. In fact, excess body weight is a predictor of CMD and death. At the other end of spectrum, low body weight is a predictor of increased mortality (Flegal et al. 2005). Body weight is widely used in clinical practice not only because of its known prognostic significance but also because its measurement is cheap and non-invasive. However, to be useful in practice, weight should always be referred to height, i.e. the second main body dimension. The best use of weight as

prognostic indicator is when it is coupled to height in the form of pondero-statural indexes, the most known and employed of which is BMI (see Section "BMI"). Comparing weight and height between-individual variance, the latter is greatly smaller than the former. Consequently, weight data have the advantage to provide, at very low cost, important information, when employed in large epidemiological studies (Flegal et al. 2013). On the other hand, at individual level, it is an easy and useful tool to monitor interventions for gaining or decreasing weight. In the absence of other information, true health conditions may be neglected, because changes in body weight during weight loss are not able to determine how body composition changes as to fat, proteins and water content. In fact, both FM and LTM decrease during weight loss but the relative proportion is different in case of voluntary (e.g. dietary or pharmaceutical) or involuntary intervention, such as during chronic illness: a major decrease of lean body mass generally occurs in clinical disorders. Alternatively, individuals may manifest weight stability while they are gaining fat and losing lean mass. In spite of this, it should be noted that the quality of the evidence linking the changes in body composition with clinically relevant outcomes is much lower than that of the evidence linking the changes of weight and pondero-statural indexes with the same outcomes. When body weight loss cannot be attributed to a reduction of lean body mass or body water, body weight can be considered a surrogate indicator of total body fat.

In conclusion, body weight is an appropriate OV for the substantiation of health claims related to the reduction of body fat/weight or maintenance of body weight after weight loss only if used in combination with the measurements of total body fat and/or lean body mass and/or body water, to exclude that body weight changes occur mostly at the expense of the latter two compartments. Body weight is not an appropriate OV for the scientific substantiation of health claims related to the reduction of abdominal fat.

3.1.1.5 BMI. BMI or Quetelet's index is calculated as the ratio of weight (kg) to squared height (m) and is the most commonly employed pondero-statural index in epidemiological research and clinical practice. It is used to evaluate undernutrition and overweight/obesity in humans. BMI has a clear prognostic significance, being a predictor of incident CMD and death. The current classification of BMI is based mostly on its association with CMD. The WHO classifies BMI as follows: severe thinness $<16.00\,kg{\cdot}m^{-2}$; moderate thinness $16.00–16.99\,kg{\cdot}m^{-2}$; mild thinness $17.00–18.49\,kg{\cdot}m^{-2}$; normal range $18.50–24.99\,kg{\cdot}m^{-2}$; overweight $\geq 25.00\,kg{\cdot}m^{-2}$; pre-obese $25.00–29.99\,kg{\cdot}m^{-2}$; obese $\geq 30.00\,kg{\cdot}m^{-2}$; obese class I $30.0–34.99\,kg{\cdot}m^{-2}$; obese class II $35.00–39.99\,kg{\cdot}m^{-2}$; obese class III $\geq 40.00\,kg{\cdot}m^{-2}$ (http://apps.who.int/bmi/index.jsp?introPage=intro3.html).

To evaluate the appropriateness of BMI as OV, the literature deriving from database #05 was critically evaluated (see Table 1).

Overweight and obesity, characterised by an excess of body fat, are associated with increased morbidity and mortality (Global BMI Mortality Collaboration et al. 2016). According to the WHO classification, subjects with BMI $\geq 25.0\,kg{\cdot}m^{-2}$ have a "moderately increased" risk and those with a $\geq 30.0\,kg{\cdot}m^{-2}$ have an "increased" risk of CMD. Most studies show a J-shaped relationship between BMI and mortality, with values of BMI $<18.5\,kg{\cdot}m^{-2}$ also associated with higher probability of death (Calle et al. 1999; WHO 2000). BMI is inexpensive and can be easily obtained with minimal subject cooperation. It is associated with the degree of fatness in most individuals, especially when gender is taken into account. However, it is not an accurate indicator of adiposity and this is especially true in children, where the effect of age must be accounted for. BMI is associated with total body fat but its numerator, i.e. weight, is the sum of fat and FFM so by its definition BMI cannot offer an accurate measure of body fat (Flegal et al. 2009). BMI is of course a more accurate measure of body fat at the extremes of its distribution, i.e. in very lean or severely obese individuals. A change in BMI does not discriminate between changes in fat- or fat-free tissues and there is substantial variability in fat content among subjects with the same BMI (Wellens et al. 1996). Moreover, BMI cannot be used to assess fat distribution. As fat- and lean tissues increase physiologically during childhood, the interpretation of BMI variation in this age is more challenging (Centers for Disease Control and Prevention 2015). It must be pointed out that the fact that BMI is not a good surrogate measure of total body fat has nothing to do with its proven prognostic significance, which is the reason why it is commonly employed in clinical practice and epidemiological research. BMI can be considered an appropriate OV of the efficacy of a given treatment in adults, aimed at obtaining weight loss. However, because height is not supposed to change in adults, adults' BMI data have the effect of minimising changes in body weight when intra-subject data are compared.

It should be noted that the quality of the evidence linking the changes in body composition with clinically relevant outcomes is much lower than that of the

evidence linking the changes of weight and ponderostatural indexes with the same outcomes. As a result, in intervention studies, BMI can be used as a recruitment tool and to stratify the results obtained. In contrast to body weight, the use of BMI allows the diagnosis/characterisation of individuals into weight categories (i.e. overweight/obesity).

BMI, even with all above mentioned caveats, can be used as an appropriate OV for the scientific substantiation of health claims in the context of reduction of body fat/weight or maintenance of body weight after weight loss only if used in combination with total body fat and/or lean body mass and/or body water to prove a reduction of total body fat after weight reduction, as already registered for body weight.

3.1.1.6 Energy intake. Food provides energy, as well as macronutrients, vitamins and minerals, water and other substances. Energy intake is controlled by several physiological factors, which regulate energy balance and, consequently, body weight. The pandemic increase in the prevalence of overweight and obesity is likely to have a behavioural aetiology leading to increased energy intake and reduced energy expenditure (physical activity) (Hill 2006). However, body weight is the result of a complex network of genetic, metabolic, biochemical, cultural and psychosocial factors. Retrospective or prospective methods can be applied to evaluate energy intake in individuals. By using food composition tables, it is possible to estimate energy and nutrient intake from food intake.

To evaluate the appropriateness of energy intake as OV, the literature deriving from database #03 was critically evaluated (see Table 1).

Body weight is stable when energy intake equals energy expenditure. Conversely, it increases when energy intake is higher than energy expenditure. As a result, high energy intake, as well as reduced energy expenditure, may lead to increased body weight. Therefore, the reduction of food intake may be pertinent in the context of body weight reduction but its reduction is not necessarily accompanied by a reduction of body weight (Hill et al. 2012).

In conclusion, the measurement of energy intake is not appropriate to be used alone for the substantiation of health claims in the context of reduction/maintenance of body weight after weight loss. However, it can be used to support the postulated mechanisms by which the food/food component exerts the CE.

3.1.1.6.1 FFQs. Several methods are available to estimate energy intake in humans. These methods are based on retrospective or prospective evaluations. Among retrospective methods, food frequency questionnaire (FFQs) are commonly used for the estimation of food and energy intake, and have become a well-accepted method for semi-quantitative assessment of habitual energy intake. A FFQ is typically a questionnaire in which the respondent is presented with a list of foods and is required to report the frequency of consumption and portion size of many items over a defined period of time (e.g. last month or last year). Many FFQs have been developed, mainly varying for the number and the type of foods items (individual or groups of foods) (Willett 1998). The main advantages of this method are its convenience, cost effectiveness and the fact that FFQ provide information on long-term intake. However, similarly to other retrospective methodologies (e.g. dietary history), poor recollection of previous food intake, as well as systematic error due to the underreporting of real intake in obese persons, can occur. These problems are greater if the FFQ is self-administered instead of interviewer-administered (Cade et al. 2004). Although there is no gold standard for directly assessing the validity of FFQs, their validation is crucial to avoid false associations (e.g. regarding the ability of a specific approach to reduce food/energy intake). Such validation should be carried out by paying attention to the study sample (ideally a subgroup of the whole population under study), its size, and the use of an accepted reference method, such as weighed food diary.

In conclusion, the use of validated FFQ is not appropriate for the measurement of energy intake, but it can be used as a useful proxy of a parameter that is otherwise very difficult to assess. However, even when used as a proxy, the food list must be as complete as possible in order to cover the whole diet. The use of weighed food diary to assess food intake remains the best option.

3.1.1.6.2 Weighed food record. The assessment of energy and dietary intake is difficult and the results are strictly affected by the type of method used. Among the available techniques, weighed food record potentially provides quantitative accurate data on food consumption during the recording period. Each item of food and drink needs to be weighed prior to consumption and a detailed description of the food, including its weight, is recorded in a specially designed booklet. Weighed records can be kept for 3–7 days. The records should be reviewed by a trained interviewer at the end of the recording period, in presence of the respondent, in order to clarify entries and to probe for involuntarily omitted foods. Compared to other less detailed and demanding methods, the seven-day weighed food record is often considered the "gold standard" (Willett 1998). As prospective

approach, it is widely used because it does not rely on respondents' memory and omission of food might be minimal. Moreover, it ensures a certain precision of portion sizes resulting more accurate than those obtained by retrospective methods. Nevertheless, its drawbacks include the relatively higher expensiveness to code the information collected than other methods, few data provided on food composition, and misreporting, one of the main sources of error in dietary assessment (Thompson and Subar 2013). In fact, when the values of energy intake obtained by food records of small samples of adults are compared to those of energy expenditure (e.g. estimated using doubly-labelled water), a certain level of underreporting on food records has been found, mainly among individuals with high BMIs, particularly women, and elderly subjects. This effect may be in part explained by demographic or psychological factors, such as education, social desirability and body image. However, to overcome the burden of underreporting and more accurately predict energy intake, the enhancing of respondents' training, the addition of psychosocial questions and the calibration of dietary records to doubly-labelled water have been proposed as possible approaches (Nybacka et al. 2016). Another limitation is that the reliability of records may decrease over time because the validity of the collected information is generally reduced in the later days of a seven-day recording period compared to the earlier days. The record method requires good cooperation on part of the respondents who should be motivated and literate. Thus, it implicates a high participation burden. For this reason, the application of food record may be not appropriate in some populations, such as recent immigrants, children and some elderly groups (Thompson and Subar 2013).

In conclusion, seven-day weighed food record generally represents an appropriate MM to assess energy intake. However, owing to its limitations, the result obtained should be considered with caution when it is applied in particular population subgroups, as detailed above.

3.1.1.7 Energy expenditure. Total energy expenditure (TEE) can be split into three components:

(1) Basal energy expenditure (BEE), corresponding to energy expended in standardised resting conditions, which gives the greatest contribution to TEE;

(2) Diet-induced thermogenesis or thermic effect of food (TEF), corresponding to the energy expended to metabolise food;

(3) Energy expended through physical activity, representing the most variable component of energy expenditure.

However, the classification generally used considers TEE only as the sum of two components: physical activity and REE that includes BEE and TEF (DeLany 2013). In children and adolescents, a fourth voice of energy expenditure is growth-related energy expenditure. Body weight is stable when energy intake equals energy expenditure and changes when energy intake does not equal energy expenditure. Some foods have been proposed to increase energy expenditure, mainly through the activation of the adreno-sympathetic nervous system.

To evaluate the appropriateness of energy expenditure as OV, the literature deriving from database #09 was critically evaluated (see Table 1).

It has been hypothesised that low energy expenditure may be a cause of obesity. However, the issue is still controversial, owing to the difficulties in obtaining accurate measurements in humans. When energy expenditure exceeds energy intake, the resulting state of negative energy balance allows a loss of body mass. Therefore, dietary strategies aimed to increase energy expenditure might be beneficial in the context of reduced body weight. However, because the maintenance of a reduced body weight can be associated with compensatory changes in energy expenditure (Leibel et al. 1995; Rosenbaum et al. 2010), which may partly account for the poor long-term efficacy of such strategies, it is crucial to provide evidence on the long term effects in order to exclude adaptation.

In conclusion, the use of energy expenditure is not appropriate to be used alone for the substantiation of health claims in the context of the reduction of body fat/weight or maintenance of body weight after weight loss. However, it can be used in support of the mechanisms by which the food/food component may exert the CE.

3.1.1.7.1 Direct and indirect calorimetry. Both direct calorimetry (DC) and indirect calorimetry (IC) may be employed in human studies to assess energy expenditure and energy requirements. The object of measurement of the direct and indirect approach is different. The former provides a measure of heat emission, whereas the latter gives data of respiratory-gas exchange (Levine 2005).

Although DC is considered the gold standard method for quantifying metabolic rate through the measurement of metabolic heat produced in both metabolically normal and abnormal conditions, its application has several limitations. DC is more expensive than IC and time-consuming. In addition, it does not provide any information about the substrates being burned. Owing to its reduced suitability, it has been widely supplanted by respirometric indirect

technique, relatively easier to be applied in larger-scale studies.

Currently, IC is the reference technique widely used for the assessment of energy expenditure in resting conditions (typically after an 8-h or 12-h fasting) through the analysis of respiratory gas exchange. Consequently, only an estimation of TEE can be provided through IC. IC is based on the concurrent measurement of oxygen consumption (VO_2) and carbon dioxide production (VCO_2). It estimates protein oxidation from urinary nitrogen excretion (Lam and Ravussin 2016). In experienced hands, IC shows good reproducibility and may provide details about individual metabolism thanks to additional information about the oxidised substrate, when coupled with labelled markers. The main drawbacks of IC include the influence of metabolic processes and confounding factors. Among these, some problematic aspects, such as hyperventilation, can be excluded by the measurement protocol.

In conclusion, calorimetry is an appropriate method for the measurement of energy expenditure.

3.1.1.8 Fat oxidation. The human body is able to utilise carbohydrates and lipids to produce energy and to rapidly switch between them. In other terms, the human body is metabolically flexible because it can adjust the oxidation of glucose and fatty acids on the basis of nutrient availability. The failure to match fuel oxidation with changes in nutrient availability can be accompanied by IR and mitochondrial dysfunction. An inability to appropriately oxidise lipids may play a role in the development of obesity and type 2 diabetes (Galgani and Ravussin 2008). To evaluate the appropriateness of fat oxidation as OV, the literature deriving from database #07 was critically evaluated (see Table 1).

There is some evidence that low fat oxidation rates are associated with weight gain and weight regain after weight loss, but the question is still debated. Generally speaking, "metabolic inflexibility", defined as a resilience to rapidly switch from fat to carbohydrate oxidation and vice versa, is a phenotype strongly associated to and thought to be implicated in the pathogenesis of IR and lipotoxicity in human obesity (Kelley 2005). Thus, a dietary strategy aimed at increasing (mitochondrial) fat oxidation may be beneficial in the prevention and treatment of obesity, in that it might reflect an attenuation of lipotoxicity. However, on the basis of current evidence, the measurement of fat oxidation is not appropriate to be used alone for the substantiation of health claims related to the reduction of body fat/weight or maintenance of body weight after weight loss. In spite of this, as already observed for other OVs proposed in claims related to the reduction of body fat/body weight or maintenance of body weight after weight loss, it can be used in support of the mechanisms through which the food/food component may exert the CE.

3.1.1.8.1 Indirect calorimetry. Fat oxidation can be indirectly assessed by IC, which measures oxygen consumption and carbon dioxide production and estimates protein oxidation from urinary nitrogen excretion (Lam and Ravussin 2017). Fat oxidation rate can be calculated by assuming that: (1) all oxygen is used to oxidise degradable fuels; (2) all carbon dioxide is recovered; (3) the respiratory quotient (RQ), i.e. the ratio of O_2 to CO_2 is fixed (0.707 for fat, 1.000 for carbohydrates and 0.809 for proteins). Strictly speaking, IC estimates the net body loss of carbohydrates and fat; since in humans these losses are due to oxidative processes, the terms "fat oxidation" and "carbohydrate oxidation" are used.

IC is the method most commonly employed to estimate fat oxidation and its use is appealing because it is simple and non-invasive (da Rocha et al. 2006). Since its theoretical bases are rooted in thermodynamics, its estimates are relatively robust with regard to potential violations of the assumptions listed above. However, it is time consuming and this partially restricts its use. In conclusion, IC could be considered an appropriate method to measure fat oxidation.

3.1.1.9 De novo lipogenesis. *De novo* lipogenesis is the metabolic pathway for energy storage in which new fatty acids are synthesised from excess carbohydrates and incorporated into TG. In humans, the liver is the primary organ devoted to *de novo* lipogenesis in normal conditions (Ameer et al. 2014). After an overnight fast, serum TG mainly derive from dietary sources, being first stored in fat tissue and subsequently released as non-esterified fatty acids, which are re-esterified and packed in VLDL by the liver. In such conditions, *de novo* lipogenesis is virtually nil. However, hepatic *de novo* lipogenesis could significantly contribute to serum lipids in healthy subjects on high carbohydrate diets (Chong et al. 2007). Enzymatic reactions in cascade produce a flow of carbons from glucose to fatty acids, which is modulated by the lipogenic pathway (Lodhi et al. 2011). The first step of this series of reactions takes place in liver mitochondria where citrate is converted to acetyl-CoA. Insulin promotes the dephosphorylation of acetyl-CoA carboxylase that converts acetyl-CoA into malonyl-CoA, the first committed step in fatty acid synthesis in the liver. The conversion of malonyl-CoA

into palmitate is regulated by the fatty acid synthase that is the key rate-limiting enzyme, and finally palmitate is converted into several fatty acids after a series of reactions. Fatty acids can be further elongated and/or desaturated by enzymes located in the membranes of the endoplasmic reticulum (Lodhi et al. 2011).

To evaluate the appropriateness of *de novo* lipogenesis as OV, the literature deriving from database #08 was critically evaluated (see Table 1).

Alterations in *de novo* lipogenesis are observed not only in a number of pathological conditions, but also in some physiological states. Such alterations may disrupt the usual lipid homeostasis leading to an increased *de novo* lipogenesis and are often observed in obesity, non-alcoholic fatty liver disease, atherogenic dyslipidaemia and the metabolic syndrome (Ameer et al. 2014). In conclusion, the measurement of changes in de-novo lipogenesis as biomarker of body fat metabolism is not appropriate to be used alone for the scientific substantiation of health claims in the context of reduction of body fat/weight or maintenance of body weight after weight loss. However, it can be used in support of the mechanisms through which the food/food component may exert the CE.

3.1.1.9.1 Rate of incorporation of deuterium. The reference method for the assessment of *de novo* lipogenesis is the measurement of triacylglycerol fatty acid synthesis. Such measurement is obtained by evaluating the rate of incorporation of deuterium from the plasma water pool into newly synthesised fatty acids over 24 h (Guo et al. 2000). The portion of newly synthesised triacylglycerol is evaluated as the enrichment of the baseline pool germane to the peak level of achievable enrichment. Plasma or very low density lipoprotein (VLDL)-triglycerides are extracted and samples are analysed for deuterium enrichment using electron ionisation/mass spectrometry (EI/MS) or isotope ratio/mass spectrometry (IR/MS). The fraction of plasma TG derived from *de novo* lipogenesis is estimated on the basis of the time course of deuterium enrichment of TG via appropriate equations (Yuan et al. 2010). On the basis of current evidence, the rate of incorporation of deuterium from the plasma water pool into circulating TG seems to be appropriate for measuring the fractional contribution of *de novo* lipogenesis to circulating TG.

3.1.1.10 Waist circumference. WC is typically used as a surrogate marker of visceral AT. Waist circumference is clinically relevant as is associated with the risk of developing T2DM and CVD (Schutz et al. 2012). Waist circumference is most commonly measured at the midpoint between the lowest rib and the top of the iliac crest (WHO 2008b), using a stretch-resistant tape providing a constant 100 g tension (usually made of fiberglass) (WHO 2008a). However, there are other studies measuring WC just above the iliac crest (National Center for Health Statistics 1996). According to WHO, values of WC should be <102 cm in men and <88 cm in women (Jensen et al. 2014). There is substantial evidence of ethnic and age variations in WC and different ethnic-related cut-points values have been set (Khunti et al. 2012; Reidpath et al. 2013).

To evaluate the appropriateness of WC as OV, the literature deriving from database #10 was critically evaluated (see Table 1).

Waist circumference is an effective surrogate measure of central/visceral AT, which has been linked to metabolic abnormalities, including decreased glucose tolerance, reduced IS and adverse lipid profile (Schutz et al. 2012). Waist circumference is the best anthropometric measure for identifying children (and, over a certain value, people at any age) with IR and hypertriglyceridaemia, and it is one of the key criteria to recognise subjects with the metabolic syndrome (NHANES-ATPIII definitions). It represents an independent cardiovascular risk factor, with a higher predicting value compared to BMI, which is more a measure of total adiposity (Reidpath et al. 2013; Cerhan et al. 2014). Waist circumference as a measure of abdominal obesity appears to be more strongly associated with the risk of CVD compared to hip circumference (HC), which represents mainly a measure of subcutaneous fat (gluteo-femoral obesity) and which shows an independent, often protective, effect on cardiovascular risk (WHO 2000). The waist-to-hip ratio measure is an attempt to incorporate the risk associated with both waist and HCs, which might, however, be related to risk profiles that operate in opposite directions. Although the waist-to-hip ratio shows strong associations with CVD, it has not typically been markedly superior to the use of WC alone. Waist circumference provides a simple and very low cost, important information, when employed in large epidemiological studies but could also be used in nutritional interventions as a surrogate measure of visceral fat (Contardo Ayala et al. 2014). Nevertheless, body weight remains the primary measure to be considered in claims of weight reduction.

In conclusion, the measurement of WC appears to be appropriate for the substantiation of health claims in the context of the reduction of body fat/weight or maintenance of body weight after weight loss only if accompanied by the measures of total body fat and

body weight. On the other hand, it is an appropriate OV to be used for the scientific substantiation of health claims related to the reduction of abdominal fat if the reduction is sufficiently large so that it could not be attributed to a reduction in lean body mass/body water.

3.1.1.11 Hip circumference. Hip circumference is most commonly measured at the widest portion of the buttocks, using a stretch-resistant tape that provides a constant 100 g tension (usually made of fiberglass), with the tape parallel to the floor. Some studies reported inverse associations between HC, mortality and heart disease (Heitmann and Lissner 2011). Such associations persist after correction for WC (WHO 2000). HC reflects different body compartments in the gluteo-femoral region, i.e. muscle mass, bone mass and FM (Molarius et al. 1999).

To evaluate the appropriateness of HC as OV, the literature deriving from database #11 was critically evaluated (see Table 1).

Differences in measurement protocols across studies might account for inconsistencies or discrepant findings in the association with risk factors, CVD or mortality outcomes. Hip circumference is inversely related to the incidence of diabetes and coronary heart diseases (CHD), after adjusting for BMI and WC (Snijder et al. 2003; Bigaard et al. 2004). On the contrary, without these adjustments, HC is positively associated with diabetes and no associated with CHD (Snijder et al. 2003). There is substantial evidence of ethnic, gender and age variations in HC (WHO 2000). A large HC seems to offer strong and independent protection against development of CVD or early mortality in women but not in men, even though the question is still debated (Heitmann and Lissner 2011).

In conclusion, the measurement of HC does not appear to be appropriate for the substantiation of health claims in the context of reduction of body fat/weight or maintenance of body weight after weight loss.

3.1.1.12 Waist to hip ratio. WC is most commonly measured at the midpoint between the lowest rib and the top of the iliac crest whereas HC at the widest portion of the buttocks, using a stretch-resistant tape that provides a constant 100 g tension (usually made of fiberglass). Waist to hip ratio (WHR) is calculated from the equation: WHR = WC (cm)/HC (cm). Waist to hip ratio values of $\leq$0.90 and $\leq$0.80 are considered normal for men and women, respectively (WHO 2008a). Waist to hip ratio, although its use is not still suggested by present guideline, is a simple anthropometric index, a useful measure of obesity and it is related to a wide range of risk factors (Akpinar et al. 2007; de Koning et al. 2007; Carmienke et al. 2013).

To evaluate the appropriateness of WHR as OV, the literature deriving from database #12 was critically evaluated (see Table 1).

Differences in measurements protocols across studies limit direct comparisons and could be responsible for variation in the association of these measures with risk factors, disease or mortality outcomes (Czernichow et al. 2011). As risks are greater for a given WC and/or WHR in different ethnic groups, different cut-offs may be needed (Lear et al. 2010). Moreover, there is substantial evidence of gender and age variations in WHR (Seidell 2010). Waist circumference in some studies is more closely correlated with the level of visceral abdominal AT than WHR. Although a greater WHR is associated with an increased risk for CVD, diabetes and all-cause mortality, it has not been markedly superior to the use of WC alone (Vazquez et al. 2007).

In conclusion, the measurement of WHR does not appear an appropriate OV to be used alone for the substantiation of health claims in the context of reduction of body fat/weight or maintenance of body weight after weight loss. However, it can be used as supportive evidence in addition to other appropriate OVs (e.g. body fat, body weight).

3.1.1.13 Satiety. Several studies suggest that a variety of factors, both intrinsic and extrinsic to foods, may influence the quantity of food needed to induce satiety (Woods 2009). Considering that a high energy/food intake can be linked to an increased in body weight, strategies able to induce satiety during the meal time and/or in the postprandial phase may represent an effective tool to discourage excessive energy intake. Increasing satiety might be one of the strategy possibly reducing energy intake, with a consequent effect on weight management. In this regard, it is useful to distinguish between satiation and satiety, despite both have to do with the inhibition of eating. People usually eat until they are comfortably full (satiation, also called intra-meal satiety), after which they do not eat for a certain time (post-prandial satiety).

To evaluate the appropriateness of satiety as OV, the literature deriving from database #01 was critically evaluated (see Table 1).

Inappropriate high energy intake is causally linked to overweight/obesity. Therefore, strategies to reduce energy intake, such as acting on the postprandial feeling of hunger and/or satiety, may be effective in

preventing or curbing or counteracting body weight increase. The increase in satiety (e.g. by reducing the energy density of foods) may be particularly relevant in body weight control, but changes in satiety eventually inducing changes in energy intake might not necessarily translate into actual weight loss. The reason behind this inconsistency is linked to the multifactorial intrinsic nature of body metabolism, where satiety and hunger play only a partial role (Moehlecke et al. 2016). Moreover, only few validated and reproducible methods are available to assess satiety. Furthermore, recording sensations after a single meal is not sufficient and the effects of repeated consumption of a specific food should be taken into consideration to rule out adaptive mechanisms.

In conclusion, the measurement of satiety is not an appropriate OV to be used alone for the substantiation of health claims related to the modulation of appetite ratings in the context of reduction/maintenance of body weight. However, it can be used as supportive of the mechanisms by which the food/constituent could exert the CE.

3.1.1.13.1 VAS. Appetite is often assessed using visual analogue scale (VAS) recording the subjective sensations of hunger and satiety. The most common VAS is represented by a horizontal line of fixed length (typically 100 mm), anchored at either end with the extreme limits of the parameter to be measured (e.g. satiety or appetite), and typically orientated from the left ("None") to the right ("Extreme") (Blundell et al. 2010). VAS can be presented in a number of ways, including a scale with a middle point, numerical rating scales or curvilinear scales, with a good agreement between different scales.

The main advantages of VAS are their ease of use and simple interpretation. Several studies suggest that VAS are the best choice in within-subjects and repeated-measures designs, which are useful to compare different treatments under similar conditions. This is due to the fact that the perceived satiety differs among individuals in a given situation and even within the same individual in different situations.

The objective validation of satiety measurements is difficult because the underlying measure is subjective and there are no accepted surrogate biological markers. However, an indirect validation can be performed by considering the capacity of the scale to predict the behaviour that is being assessed (i.e. *ad libitum* food intake) and by assessing reproducibility (e.g. test–retest procedure). VAS have been shown to be highly reliable and reproducible in measuring appetite and in predicting subsequent food intake, especially in young subjects (Flint et al. 2000). On the other hand, in older people, the relation between appetite measured by VAS and food intake has not been fully assessed, also because of the weaker inverse relation between hunger and fullness in this type of population.

In conclusion, a validated VAS appears appropriate for the measurement of satiety and hunger or appetite, and more generally of behavioural assessment.

3.1.1.14 Hunger or appetite. Appetite, considered as the sum of processes influencing eating, can be defined as the internal driving force for the search, choice and ingestion of food. Although they are often considered as synonyms, the terms "appetite" and "hunger" are rather different, the latter generally referring to a conscious sensation reflecting a mental urge to eat (Blundell et al. 2013). Both appetite and hunger are driven by factors related to energy needs (e.g. low blood glucose), but also, and more often, by extrinsic factors such as habits, time of day or stress levels.

The control of food intake may play an important role in managing energy balance, insofar as reduction of hunger and appetite might be a strategy to discourage excessive energy intake.

To evaluate the appropriateness of hunger or appetite as OV, the literature deriving from database #02 was critically evaluated (see Table 1).

As previously mentioned (see Section "Satiety" under the section "Reduction of body fat/body weight or maintenance of body weight after weight loss"), an excessive energy intake may result in increased body weight, thus acting on the postprandial feeling of hunger and/or satiety to reduce energy intake may be quite effective in preventing or curbing or counteracting body weight increase. However, it is still debated whether changes in hunger associated with changes in energy intake eventually translate into weight loss. In addition, to exclude adaptation through compensatory mechanisms, repeated tastings meal of the food of interest are warranted. Hunger and appetite may be assessed in several ways, and the reliability and validity of their measurement is usually higher under laboratory conditions (Blundell et al. 2010).

In conclusion, hunger or appetite are not appropriate OVs to be used alone for the scientific substantiation of health claims related to the modulation of appetite ratings in the context of reduction/maintenance of body weight. However, they can be used as an ancillary support of the mechanisms by which the food/food component may exert the CE.

3.1.1.14.1 VAS. See section 3.1.1.13.1.

3.1.2 Reduction of abdominal fat

3.1.2.1 Abdominal fat. To evaluate the appropriateness of abdominal fat as OV, the literature deriving from database #06 was critically evaluated (see Table 1).

The available data suggest that abdominal AT plays a major role in the pathogenesis of CMD. Several cohort studies have shown that excess abdominal fat is associated with incident CVD as well as with glucose and lipid disturbances. The relative role of visceral and subcutaneous fat in the pathogenesis of CMD is under active investigation (see also Sections "Visceral fat" and "Subcutaneous fat"). The amount of fat within the abdominal cavity has been associated with a higher risk of CMD, e.g. T2DM, stroke, hypertension and dyslipidaemia (Tchernof and Despres 2013). As a surrogate measure of abdominal or "central" fat, WC can be used in intervention studies aimed to reduce abdominal fat. However, it does not distinguish between abdominal subcutaneous AT and intra-abdominal (referred also as visceral) AT. In fact, in spite of an elevated intra-abdominal AT, an individual may thus have acceptable values of WC. Abdominal fat, considered as the sum of abdominal subcutaneous and visceral fat, is an appropriate OV to be used alone for the scientific substantiation of health claims related to the reduction of abdominal fat.

3.1.2.1.1 MRI. See section 3.1.1.1.2.
3.1.2.1.2 CT. section 3.1.1.1.3.

3.1.2.2 Visceral fat. See section 3.1.1.2.
3.1.2.2.1 MRI. See section 3.1.1.1.2.
3.1.2.2.2 CT. See section 3.1.1.1.3.

3.1.2.3 Subcutaneous fat. See section 3.1.1.3.
3.1.2.3.1 MRI. See section 3.1.1.1.2.
3.1.2.3.2 CT. See section 3.1.1.1.3.

3.1.2.4 Waist circumference. See section 3.1.1.10.

3.1.2.5 Body weight. See Section 3.1.1.4.

3.1.2.6 Total body fat.
3.1.2.6.1 Methods for body fat assessment. See sections from 3.1.1.1.1 to 3.1.1.1.6.

3.1.3 Increase/maintenance of lean body mass

3.1.3.1 Lean body mass. Lean body mass is often used as a synonym of FFM, which, summed to FM, makes the simplest body composition model, i.e. the two-compartment model.

However, when a three-compartment model is considered, the two terms (FFM and lean body mass) are not equivalent because lean body mass does not include bone minerals, whereas FFM includes them. Thus, bone mass may be distinct or not based on the method used to assess body composition (Mattsson and Thomas 2006).

To evaluate the appropriateness of lean body mass as OV, the literature deriving from database #13 was critically evaluated (see Table 1).

The terms lean body mass/FFM (muscle and bone mass) are frequently used for the standardisation of physiological measures, such as resting metabolic rate and the amount of muscle mass. Sarcopenia is a clinically important reduction of lean body mass with clinically negative prognostic implications. FFM is a heterogeneous compartment including water, bone, proteins and glycogen. The estimation of FFM requires that the assumptions regarding the two-compartment model are met (Ellis 2000). In conclusion, lean body mass is an appropriate OV to be used alone for the scientific substantiation of health claims related to the maintenance or increase of lean body mass. However, the use of appropriate techniques enabling the determination of a three-compartment model is required for correct measurements.

3.1.3.1.1 DEXA. See section 3.1.1.1.1.
3.1.3.1.2 MRI. See section 3.1.1.1.2.
3.1.3.1.3 CT. See section 3.1.1.1.3.
3.1.3.1.4 BIA. See section 3.1.1.1.5.
3.1.3.1.5 Air displacement plethysmography. See section 3.1.1.1.6.

3.1.3.1 Protein metabolism (synthesis and breakdown). Body protein content is determined by the balance between protein synthesis and degradation. As there is no protein storage pool, proteins that serve vital roles are continuously synthesised and catabolized. Hormonal, nutritional and other factors regulate protein metabolism. Alterations of both the synthesis and degradation of proteins may cause disorders leading to increased morbidity and mortality, e.g. sarcopenia. Whole body protein balance is thus the difference between protein synthesis and breakdown in all tissues and organs (Arnal et al. 1987).

To evaluate the appropriateness of protein metabolism as OV, the literature deriving from database #14 was critically evaluated (see Table 1).

Most adults (97.5% of the reference population) will be in protein balance by consuming 0.8 g/kg body weight/day (Institute of Medicine of the National Academies 2005). However, the protein needs may be increased in the presence of physiological conditions

(growth, pregnancy and lactation) or diseases (SINU 2014), even if no evidence is available showing that a higher protein intake improves clinical outcomes in elderly people and in other diseases. In conclusion, protein metabolism is not an appropriate OV to be used alone for the substantiation of health claims in the context of maintenance or increase of lean body mass. However, it can be used as supportive evidence of the mechanisms by which the food/food component may exert the CE.

3.1.3.1.1 Aminoacid tracer dilution techniques. Many attempts have been made to quantify either whole-body- or tissue-specific protein metabolism *in vivo*. Such studies have employed almost exclusively tracer measurements, i.e. the dilution of a continuously infused labelled amino acid at isotopic steady state (Holm and Kjaer 2010). Essential amino acids are infused under the assumption that, in the fasting state, the amino acid turnover rate reflects whole body protein breakdown. The most commonly employed amino acids are L-[1-*C]leucine and L-[*H5]phenylalanine (Wagenmakers 1999), labelled with either radioactive or stable isotopes and usually administered by a prime-continuous intravenous infusion which reaches a steady state in about two hours. When the steady state is reached, the rate of appearance (Ra) of the labelled amino acid provides an estimate of the rate of protein degradation (plus dietary intake, in the fed state). The rate of amino acid disappearance (Rd) is the sum of oxidation plus protein synthesis; thus, a measure of one of these two pathways is required to correctly interpret the amino acid rate of disappearance. With this method, it is also possible to "perturb" the steady state and to estimate dynamic changes to specific interventions. In order to dissect out amino acid fluxes, sophisticated mathematical modelling is required in case of absence of steady state, although this tool cannot compensate for it having to assume many unknown quantities. Despite the fact that this method requires substantial modelling assumptions, it is the most accurate technique available to simultaneously estimate protein synthesis and breakdown. However, this technique, unless extensive use of mathematical modelling is employed, underestimates the total turnover of tissue proteins as measured other methods (Reeds and Davis 1999; Davis and Reeds 2001).

On the basis of current evidence, isotopic tracer kinetic seems to be appropriate to measure protein metabolism.

4. Conclusions

To date, the totality of the requests for authorisation of health claims in the context of appetite ratings and weight management pursuant to Article 13(5) and 14 of Regulation (EC) No 1924/2006 has received a negative opinion from the EFSA. The prevalent reason is an insufficient substantiation of the health claim proposed, also because of an improper selection of OVs (and in some cases of MMs) as primary endpoints. In randomised controlled trials, they should be selected according to their appropriateness in the framework of the specific claim proposed, taking into account that a different, or even opposite, assessments could be attributed at the same OV for different CEs. For instance, on the basis of the considered CE, total body fat is appropriate to be used alone for the substantiation of health claims in the context of reduction of body fat but it cannot be used alone to substantiate the health claims related to the reduction of abdominal fat. Similarly, also the MMs applied should be chosen adequately, applying when possible the gold standard method, such as MRI or CT for the measurement of body fat, or the best available for each OV.

The information provided by the present manuscript may represent an important tool to be used for the choice of the most appropriate OVs and MMs in human randomised controlled trials. At the same time, the information deriving from this work might help EFSA in the development of updated guidance to applicants for the preparation of applications for authorisation of health claims in the context of appetite ratings and weight management.

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Appendix