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

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 authorization 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 randomized controlled trials aimed to substantiate such claims.

Keywords: health claims, claimed effect, outcome variable, method of measurement, appetite rating, weight management

Acronyms:

AT: Adipose tissue

BEE: Basal energy expenditure

BIA: Bio-electrical impedance analysis

BIS: Bio-electrical spectroscopy

BMC: Bone mineral content

BMI: Body mass index

CE: claimed effect

CHD: coronary heart disease

CMD: Cardiometabolic disease

CT: Computed tomography

CVD: Cardiovascular disease

DC: Direct calorimetry

DEXA: Dual energy X-ray absorptiometry

ECW Extracellular water

EI/MS: Electron ionization/mass spectrometry

FFM: Fat-free mass; FFQ: Food frequency questionnaire

FM: Fat mass

HC: hip circumference

IC: Indirect calorimetry

IR: insulin resistance

IR/MS: Isotope ratio/mass spectrometry

LTM: Lean tissue mass

MM: method of measurement

MRI: Magnetic resonance imaging

OV: outcome variable

RQ: Respiratory quotient

T2DM: type 2 diabetes mellitus

TBW: Total body water

TEE: Total energy expenditure

TEF: Thermic effect of food (or diet-induced thermogenesis)

TG: Triglycerides

VAS: Visual analogue scale

VLDL: Very low density lipoprotein

WC: Waist circumference

WHR: Waist to hip ratio

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84 **1 Introduction**

According to an estimation made by the World Health Organization (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 (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 characterized by abnormal or excessive adiposity caused by an imbalance between energy intake and expenditure over a prolonged time leading to a higher adipose tissue accumulation(Prieto-Hontoria et al. 2011). Genetic predisposition and lifestyle factors, such as sedentary behaviour and deterioration of dietary quality characterized 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 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 (2007b) and foods intended for use in energy-restricted diets for weight reduction(2007a), 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 minimizing 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 authorization. All of them have received a negative opinion from European Food Safety Authority for reasons concerning the formulation of the claim proposed by the Applicants, the insufficient characterization 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 authorization, 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(Allergies). 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(Commission), as well as from comments received during public consultations.

Following the same search strategy already applied in Martini et al. (2017)(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 authorization of health claims, 6 were not considered because the claim was not defined as a beneficial physiological effect *per se* or because the food/constituent was not sufficiently characterized(Products 2008a, Products 2008b, Products. 2015, EFSA Panel on Dietetic Products 2012, Products 2011, Products 2014). The remaining 13 requests were evaluated and referred to 4 different claims falling under Article 13(5). The critical analysis was performed on 17 different OVs, 4 of which were assessed for a single CE, whereas 8 and 5 were evaluated in the context of 2 and 3 different CEs respectively. Among the 14 different MMs considered, a half was assessed for a single OV, while 2 MMs were analysed in relation to 5 different OVs and 5 MMs were evaluated in relation to 2 OVs.

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, cardiovascular disease, 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 5-component "molecular" model, recognized as the reference model of body composition, calculates body weight as the sum of measured fat mass, 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. 2-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), total body water (hydrometry), and body potassium (40K measurement)(Ellis 2000).

The assessment of body composition can be performed at the whole-body or regional level. About 80% of adipose tissue (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 adipose tissue 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 permit 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 surround internal organs. Some studies have focused 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(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 outcome variable, the literature deriving from database #06 was critically evaluated (see Table1).

The assessment of total body fat may offer physiologically and clinically relevant information. Fat mass (FM) 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 fat mass(Bosy-Westphal et al. 2003). The direct quantification of FM or its measurement from the 5-component model is possible (see general introduction) but restricted to few centers worldwide. FM is most commonly estimated from 2-

compartments models assuming the constancy of a physical or chemical property of the FFM, this latter being simply defined as [body mass (weight) – fat mass]. Under the assumption of such constancy, FFM can be estimated from body density (e.g. air displacement plethysmography), total body water (hydrometry), and body potassium (40K 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 (arms, legs and trunk) body composition. DEXA employs a 3-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 3-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 5-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 (2 regions), legs (2 regions) and trunk (1 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 characterizes 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 5-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 program. 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 analyzed 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 localization 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 x 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 ionizing radiation. However, the use of MRI is prioritized 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 (cm3). 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. Because 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 5-component model to assess body composition (see general introduction). Another limitation of CT is that its use is prioritized 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, mid-thigh 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 standardized protocols(Mattsson and Thomas 2006). Because skinfolds are a direct measure of subcutaneous fat, their association with disease differs from that between this latter and body mass index (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 summarized 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 to 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 to 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, Kyle et al. 2004b). Fasting, standardization 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

385 of small changes in these variables or if subjects with morbid obesity/alterations in body water
386 compartments are evaluated.

387 *3.1.1.1.6 Air displacement plethysmography*

388 Air displacement plethysmography is a technique employed to measure body density and to
389 estimate body composition from it. This technique represents a non-invasive alternative method
390 to under water weighing. The 2-compartment body composition model employed by air
391 displacement plethysmography considers body mass (weight) as the sum of FM and FFM.
392 Under the assumption of a known and constant density of FFM, it is possible to estimate FFM
393 from body density, i.e. the ratio of body mass (weight) to body volume. However, while FM
394 density remains fairly constant, FFM density changes substantially during growth, aging and
395 disease(Lee and Gallagher 2008). The measurement system of this technique consists of a
396 cabin with two chambers. The reference chamber is separated by the test chamber by a moving
397 diaphragm under computerized control. While the person sits inside the reference chamber,
398 computerized sensors determine the amount of air displaced in the test chamber to calculate
399 body volume. Air displacement plethysmography requires correction for residual lung volume
400 and surface area artefacts. Lung volume is measured by this method during tidal breathing with
401 exhalation against a mechanical obstruction. Intestinal gas cannot be measured and is
402 commonly assumed to be 100 mL (Mattsson and Thomas 2006). To prevent erroneous air
403 displacement due to air pockets and therefore to obtain more precise estimate of body density,
404 it is recommended that the subject wear a synthetic bathing suit and a swim cap(Mattsson and
405 Thomas 2006). Provided that the assumptions about FFM density are met, air displacement
406 plethysmography provides a relatively simple method to assess body volume and therefore
407 body density and body composition. In virtue of its repeatability, quickness and minimal
408 compliance request, this technology can be used in clinical and research settings. Data on FFM
409 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 minimize the assumption for 2-compartment models, a combination of methods can be used to measure lean mass (e.g. going from 2-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 outcome variable, the literature deriving from database #06 was critically evaluated (see Table1).

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 waist circumference (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 dyslipidemia(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(Kim et al. 2006, Williams et al. 1996). Nevertheless, because of the absence of standardized 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 outcome variable, the literature deriving

460 from database #06 was critically evaluated (see Table1).

461 Subcutaneous and visceral AT can be distinguished not only from an anatomical perspective
462 but also from a functional viewpoint as they have different metabolic, endocrine, paracrine and
463 autocrine roles. The relative role of visceral and subcutaneous fat in the pathogenesis of CMD
464 is under active investigation. The main function of subcutaneous AT is to buffer the energy
465 excess in the form of fatty acids stored as triacylglycerols (also known as triglycerides)(Ibrahim
466 2010). Furthermore, subcutaneous AT, releases a number of active substances ("adipokines")
467 with endocrine and paracrine functions. Several studies have suggested a possible protective
468 role of this compartment against the development of the metabolic syndrome(Lee et al. 2013).
469 Subcutaneous AT contains small adipocytes, which avidly adsorb free fatty acids and
470 triglycerides, preventing their accumulation in non-AT. Moreover, it has a lesser rate of insulin-
471 stimulated glucose uptake as compared to visceral AT which, on the contrary, has a great
472 percentage of large adipocytes resistant to the anti-lipolytic effect of insulin(Ibrahim 2010).
473 Although an increasing of weight loss leads to a greater reduction of VAT, allometric models
474 have suggested a non-linear association between the two variables due to the attenuation of
475 decrease of VAT compared to SAT (Hall KD, et al, 2008). Visceral obesity is often associated
476 with a dysfunctional subcutaneous AT unable to appropriately expand in the presence of an
477 energy surplus. This condition leads to ectopic triglyceride accumulation (e.g. heart, liver,
478 pancreas, muscles), which is associated with several cardiometabolic risk factors(Tchernof and
479 Despres 2013). Because about 80% of AT is subcutaneous and it is located mainly in the
480 abdominal and gluteo-femoral regions, a reduction in subcutaneous AT will nearly always
481 likely occur during an intervention aimed at reducing body fat. Thus, subcutaneous fat is an
482 appropriate OV for the scientific substantiation of health claims related to the reduction of body
483 fat. However, it should be used in combination with body weight for the substantiation of health
484 claims in the context of reduction of body weight or maintenance of body weight after weight

485 loss. Finally, the combined measurement of abdominal subcutaneous fat with visceral fat is
486 preferable for the substantiation of health claims related to the reduction of abdominal fat.

487 *3.1.1.3.1 MRI*

488 *See Section 3.1.1.1.2*

489 *3.1.1.3.2 CT*

490 *See Section 3.1.1.1.3*

491 *3.1.1.4 Body weight*

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

501 To evaluate the appropriateness of body weight as outcome variable, the literature deriving
502 from database #04 was critically evaluated (see Table1).

503 Although body weight provides no information on body composition, there is an established
504 association between excess body weight and reduced survival. In fact, excess body weight is a
505 predictor of CMD and death. At the other end of spectrum, low body weight is a predictor of
506 increased mortality(Flegal et al. 2005). Body weight is widely used in clinical practice not only
507 because of its known prognostic significance but also because its measurement is cheap and
508 non-invasive. However, to be useful in practice, weight should always be referred to height, i.e.
509 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 3.1.1.5). 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·m-2; moderate thinness 16.00 to 16.99 kg·m-2; mild thinness 17.00 to 18.49 kg·m-2; normal range 18.50 to 24.99 kg·m-2; overweight ≥ 25.00 kg·m-2; pre-obese 25.00 to 29.99 kg·m-2; obese ≥ 30.00 kg·m-2; obese class I 30.0 to 34.99 kg·m-2; obese class II 35.00 - 39.99 kg·m-2; obese class III ≥40.00 kg·m-2 (http://apps.who.int/bmi/index.jsp?introPage=intro 3.html)

To evaluate the appropriateness of BMI as outcome variable, the literature deriving from database #05 was critically evaluated (see Table1).

Overweight and obesity, characterised by an excess of body fat, are associated with increased morbidity and mortality(Global et al. 2016). According to the WHO classification, subjects with BMI ≥ 25.0 kg·m-2 have a "moderately increased" risk and those with a ≥ 30.0 kg·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·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. Because fat- and lean tissues increase physiologically during childhood, the interpretation of BMI variation in this age is more challenging(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 in adults height is not supposed to change, adults' BMI data have the effect of minimizing 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 pondero-statural 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/characterization 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

585 intake and reduced energy expenditure (physical activity)(Hill 2006). However, body weight is
586 the result of a complex network of genetic, metabolic, biochemical, cultural and psychosocial
587 factors. Retrospective or prospective methods can be applied to evaluate energy intake in
588 individuals. By using food composition tables, it is possible to estimate energy and nutrient
589 intake from food intake.

590 To evaluate the appropriateness of energy intake as outcome variable, the literature deriving
591 from database #03 was critically evaluated (see Table1).

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

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

601 *3.1.1.6.1 FFQs*

602 Several methods are available to estimate energy intake in humans. These methods are based
603 on retrospective or prospective evaluations. Among retrospective methods, food frequency
604 questionnaire (FFQs) are commonly used for the estimation of food and energy intake, and have
605 become a well-accepted method for semi-quantitative assessment of habitual energy intake. A
606 FFQ is typically a questionnaire in which the respondent is presented with a list of foods and is
607 required to report the frequency of consumption and portion size of many items over a defined
608 period of time (e.g. last month or last year). Many FFQs have been developed, mainly varying
609 for the number and the type of foods items (individual or groups of foods)(Willett 1998). The

610 main advantages of this method are its convenience, cost effectiveness and the fact that FFQ
611 provide information on long-term intake. However, similarly to other retrospective
612 methodologies (e.g. dietary history), poor recollection of previous food intake, as well as
613 systematic error due to the underreporting of real intake in obese persons, can occur. These
614 problems are greater if the FFQ is self-administered instead of interviewer-administered(Cade
615 et al. 2004). Although there is no gold standard for directly assessing the validity of FFQs, their
616 validation is crucial to avoid false associations (e.g. regarding the ability of a specific approach
617 to reduce food/energy intake). Such validation should be carried out by paying attention to the
618 study sample (ideally a subgroup of the whole population under study), its size, and the use of
619 an accepted reference method, such as weighed food diary.
620 In conclusion, the use of validated FFQ is not appropriate for the measurement of energy intake,
621 but it can be used as a useful proxy of a parameter that is otherwise very difficult to assess.
622 However, even when used as a proxy, the food list must be as complete as possible in order to
623 cover the whole diet. The use of weighed food diary to assess food intake remains the best
624 option.

625 *3.1.1.6.2 Weighed food record*

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

635 approach, it is widely used because it does not rely on respondents' memory and omission of
636 food might be minimal. Moreover, it ensures a certain precision of portion sizes resulting more
637 accurate than those obtained by retrospective methods. Nevertheless, its drawbacks include the
638 relatively higher expensiveness to code the information collected than other methods, few data
639 provided on food composition, and misreporting, one of the main sources of error in dietary
640 assessment(Thompson and Subar 2013). In fact, when the values of energy intake obtained by
641 food records of small samples of adults are compared to those of energy expenditure (e.g.
642 estimated using doubly-labelled water), a certain level of underreporting on food records has
643 been found, mainly among individuals with high BMIs, particularly women, and elderly
644 subjects. This effect may be in part explained by demographic or psychological factors, such as
645 education, social desirability and body image. However, to overcome the burden of
646 underreporting and more accurately predict energy intake, the enhancing of respondents'
647 training, the addition of psychosocial questions and the calibration of dietary records to doubly-
648 labelled water have been proposed as possible approaches(Nybacka et al. 2016). Another
649 limitation is that the reliability of records may decrease over time because the validity of the
650 collected information is generally reduced in the later days of a 7-day recording period
651 compared to the earlier days. The record method requires good cooperation on part of the
652 respondents who should be motivated and literate. Thus, it implicates a high participation
653 burden. For this reason, the application of food record may be not appropriate in some
654 populations, such as recent immigrants, children and some elderly groups(Thompson and Subar
655 2013).
656 In conclusion, 7-day weighed food record generally represents an appropriate MM to assess
657 energy intake. However, owing to its limitations, the result obtained should be considered with
658 caution when it is applied in particular population subgroups, as detailed above.
659 *3.1.1.7 Energy expenditure*

660 Total energy expenditure (TEE) can be split into three components:

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

663 2) diet-induced thermogenesis or thermic effect of food (TEF), corresponding to the energy
664 expended to metabolize food;

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

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

673 To evaluate the appropriateness of energy expenditure as outcome variable, the literature
674 deriving from database #09 was critically evaluated (see Table1).

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

684 In conclusion, the use of energy expenditure is not appropriate to be used alone for the

685 substantiation of health claims in the context of the reduction of body fat/weight or maintenance
686 of body weight after weight loss. However, it can be used in support of the mechanisms by
687 which the food/food component may exert the CE.

688 *3.1.1.7.1 Direct and indirect calorimetry*

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

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

699 Currently, IC is the reference technique widely used for the assessment of energy expenditure
700 in resting conditions (typically after an 8-hour or 12-h fasting) through the analysis of
701 respiratory gas exchange. Consequently, only an estimation of TEE can be provided through
702 IC. IC is based on the concurrent measurement of oxygen consumption (VO2) and carbon
703 dioxide production (VCO2). It estimates protein oxidation from urinary nitrogen excretion(Lam
704 and Ravussin 2016). In experienced hands, IC shows good reproducibility and may provide
705 details about individual metabolism thanks to additional information about the oxidized
706 substrate, when coupled with labelled markers. The main drawbacks of IC include the influence
707 of metabolic processes and confounding factors. Among these, some problematic aspects, such
708 as hyperventilation, can be excluded by the measurement protocol.

709 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 utilize 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 insulin resistance (IR) and mitochondrial dysfunction. An inability to appropriately oxidize 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 outcome variable, the literature deriving from database #07 was critically evaluated (see Table1).

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 outcome variables 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

734 Fat oxidation can be indirectly assessed by IC, which measures oxygen consumption and carbon
735 dioxide production and estimates protein oxidation from urinary nitrogen excretion(Lam and
736 Ravussin 2017). Fat oxidation rate can be calculated by assuming that: 1) all oxygen is used to
737 oxidize degradable fuels; 2) all carbon dioxide is recovered; 3) the respiratory quotient (RQ),
738 i.e. the ratio of O2 to CO2 is fixed (0.707 for fat, 1.000 for carbohydrates and 0.809 for
739 proteins). Strictly speaking, IC estimates the net body loss of carbohydrates and fat; since in
740 humans these losses are due to oxidative processes, the terms "fat oxidation" and "carbohydrate
741 oxidation" are used.
742 IC is the method most commonly employed to estimate fat oxidation and its use is appealing
743 because it is simple and non-invasive(da Rocha et al. 2006). Since its theoretical bases are
744 rooted in thermodynamics, its estimates are relatively robust with regard to potential violations
745 of the assumptions listed above. However, it is time consuming and this partially restricts its
746 use. In conclusion, IC could be considered an appropriate method to measure fat oxidation.

747 *3.1.1.9 De-novo lipogenesis*

748 De novo lipogenesis is the metabolic pathway for energy storage in which new fatty acids are
749 synthesized from excess carbohydrates and incorporated into triglycerides (TG). In humans, the
750 liver is the primary organ devoted to de novo lipogenesis in normal conditions(Ameer et al.
751 2014). After an overnight fast, serum TG mainly derive from dietary sources, being first stored
752 in fat tissue and subsequently released as non-esterified fatty acids, which are re-esterified and
753 packed in VLDL by the liver. In such conditions, de novo lipogenesis is virtually nil. However,
754 hepatic de novo lipogenesis could significantly contribute to serum lipids in healthy subjects
755 on high carbohydrate diets(Chong et al. 2007). Enzymatic reactions in cascade produce a flow
756 of carbons from glucose to fatty acids, which is modulated by the lipogenic pathway(Lodhi et
757 al. 2011). The first step of this series of reactions takes place in liver mitochondria where citrate
758 is converted to acetyl-CoA. Insulin promotes the dephosphorylation of acetyl-CoA carboxylase

759 that converts acetyl-CoA into malonyl-CoA, the first committed step in fatty acid synthesis in
760 the liver. The conversion of malonyl-CoA into palmitate is regulated by the fatty acid synthase
761 that is the key rate-limiting enzyme, and finally palmitate is converted into several fatty acids
762 after a series of reactions. Fatty acids can be further elongated and/or desaturated by enzymes
763 located in the membranes of the endoplasmic reticulum(Lodhi et al. 2011).
764 To evaluate the appropriateness of *de-novo* lipogenesis as outcome variable, the literature
765 deriving from database #08 was critically evaluated (see Table1).
766 Alterations in de novo lipogenesis are observed not only in a number of pathological conditions,
767 but also in some physiological states. Such alterations may disrupt the usual lipid homeostasis
768 leading to an increased de novo lipogenesis and are often observed in obesity, non-alcoholic
769 fatty liver disease, atherogenic dyslipidemia and the metabolic syndrome(Ameer et al. 2014).
770 In conclusion, the measurement of changes in de-novo lipogenesis as biomarker of body fat
771 metabolism is not appropriate to be used alone for the scientific substantiation of health claims
772 in the context of reduction of body fat/weight or maintenance of body weight after weight loss.
773 However, it can be used in support of the mechanisms through which the food/food component
774 may exert the CE.

775 *3.1.1.9.1 Rate of incorporation of deuterium*

776 The reference method for the assessment of de novo lipogenesis is the measurement of
777 triacylglycerol fatty acid synthesis. Such measurement is obtained by evaluating the rate of
778 incorporation of deuterium from the plasma water pool into newly synthesized fatty acids over
779 24 h(Guo et al. 2000). The portion of newly synthesized triacylglycerol is evaluated as the
780 enrichment of the baseline pool germane to the peak level of achievable enrichment. Plasma or
781 very low density lipoprotein (VLDL)-triglicerydes are extracted and samples are analysed for
782 deuterium enrichment using electron ionization/mass spectrometry (EI/MS) or isotope
783 ratio/mass spectrometry (IR/MS). The fraction of plasma TG derived from de novo lipogenesis

784 is estimated on the basis of the time course of deuterium enrichment of TG via appropriate
785 equations(Yuan et al. 2010). On the basis of current evidence, the rate of incorporation of
786 deuterium from the plasma water pool into circulating TG seems to be appropriate for
787 measuring the fractional contribution of de novo lipogenesis to circulating TG.

788 *3.1.1.10 Waist circumference*

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

799 To evaluate the appropriateness of waist circumference as outcome variable, the literature
800 deriving from database #10 was critically evaluated (see Table1).

801 Waist circumference is an effective surrogate measure of central/visceral adipose tissue, which
802 has been linked to metabolic abnormalities, including decreased glucose tolerance, reduced IS
803 and adverse lipid profile(Schutz et al. 2012). Waist circumference is the best anthropometric
804 measure for identifying children (and, over a certain value, people at any age) with IR and
805 hypertriglyceridemia, and it is one of the key criteria to recognize subjects with the metabolic
806 syndrome (NHANES-ATPIII definitions). It represents an independent cardiovascular risk
807 factor, with a higher predicting value compared to BMI, which is more a measure of total
808 adiposity(Cerhan et al. 2014, Reidpath et al. 2013). Waist circumference as a measure of

809 abdominal obesity appears to be more strongly associated with the risk of CVD compared to
810 hip circumference, which represents mainly a measure of subcutaneous fat (gluteo-femoral
811 obesity) and which shows an independent, often protective, effect on cardiovascular risk (WHO
812 2000). The waist-to-hip ratio measure is an attempt to incorporate the risk associated with both
813 waist and hip circumferences, which might, however, be related to risk profiles that operate in
814 opposite directions. Although the waist-to-hip ratio shows strong associations with CVD, it has
815 not typically been markedly superior to the use of waist circumference alone. Waist
816 circumference provides a simple and very low cost, important information, when employed in
817 large epidemiological studies but could also be used in nutritional interventions as a surrogate
818 measure of visceral fat(Contardo Ayala et al. 2014). Nevertheless, body weight remains the
819 primary measure to be considered in claims of weight reduction.
820 In conclusion, the measurement of waist circumference appears to be appropriate for the
821 substantiation of health claims in the context of the reduction of body fat/weight or maintenance
822 of body weight after weight loss only if accompanied by the measures of total body fat and
823 body weight. On the other hand, it is an appropriate OV to be used for the scientific
824 substantiation of health claims related to the reduction of abdominal fat if the reduction is
825 sufficiently large so that it could not be attributed to a reduction in lean body mass/body water.
826 *3.1.1.11 Hip circumference*
827 Hip circumference (HC) is most commonly measured at the widest portion of the buttocks,
828 using a stretch-resistant tape that provides a constant 100 g tension (usually made of fiberglass),
829 with the tape parallel to the floor. Some studies reported inverse associations between hip
830 circumference, mortality and heart disease(Heitmann and Lissner 2011). Such associations
831 persist after correction for waist circumference(WHO 2000). HC reflects different body
832 compartments in the gluteo-femoral region, i.e. muscle mass, bone mass and fat mass(Molarius
833 et al. 1999).

To evaluate the appropriateness of hip circumference as outcome variable, the literature deriving from database #11 was critically evaluated (see Table1).

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 waist circumference (Bigaard et al. 2004, Snijder et al. 2003). On the contrary, without these adjustments, hip circumference 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 hip circumference(WHO 2000). A large hip circumference 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 hip circumference 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 ≤ 0.90 and ≤ 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, Carmienke et al. 2013, de Koning et al. 2007).

To evaluate the appropriateness of waist to hip ratio as outcome variable, the literature deriving

from database #12 was critically evaluated (see Table1).

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). Because risks are greater for a given waist circumference and/or waist to hip ratio 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 waist to hip ratio(Seidell 2010). Waist circumference in some studies is more closely correlated with the level of visceral abdominal AT than waist to hip ratio. Although a greater waist to hip ratio is associated with an increased risk for CVD, diabetes and all-cause mortality, it has not been markedly superior to the use of waist circumference alone (Vazquez et al. 2007).

In conclusion, the measurement of waist to hip ratio 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 outcome variables (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 outcome variable, the literature deriving from

884 database #01 was critically evaluated (see Table1).

885 Inappropriate high energy intake is causally linked to overweight/obesity. Therefore, strategies
886 to reduce energy intake, such as acting on the postprandial feeling of hunger and/or satiety, may
887 be effective in preventing or curbing or counteracting body weight increase. The increase in
888 satiety (e.g. by reducing the energy density of foods) may be particularly relevant in body
889 weight control, but changes in satiety eventually inducing changes in energy intake might not
890 necessarily translate into actual weight loss. The reason behind this inconsistency is linked to
891 the multifactorial intrinsic nature of body metabolism, where satiety and hunger play only a
892 partial role(Moehlecke et al. 2016). Moreover, only few validated and reproducible methods
893 are available to assess satiety. Furthermore, recording sensations after a single meal is not
894 sufficient and the effects of repeated consumption of a specific food should be taken into
895 consideration to rule out adaptive mechanisms.

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

900 *3.1.1.13.1 VAS*

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

908 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 outcome variable, the literature

deriving from database #02 was critically evaluated (see Table1).

As previously mentioned (See Section 3.1.14.1), 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 outcome variables 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 outcome variable, the literature deriving from database #06 was critically evaluated (see Table1).

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 3.1.1.2 and 3.1.1.3). The amount of fat within the abdominal cavity has been associated with a higher risk

of CMD, e.g. T2DM, stroke, hypertension and dyslipidemia(Tchernof and Despres 2013). As a surrogate measure of abdominal or "central" fat, waist circumference 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 waist circumference. 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

See 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 2-compartment model.

However, when a 3-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 outcome variable, the literature deriving from database #13 was critically evaluated (see Table1).

The terms lean body mass/FFM (muscle and bone mass) are frequently used for the standardization 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 2-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 3-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. Because there is no protein storage pool, proteins that serve vital roles are continuously synthesized 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 outcome variable, the literature deriving from database #14 was critically evaluated (see Table1).

Most adults (97.5% of the reference population) will be in protein balance by consuming 0.8 g/kg body weight/day(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 2 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(Davis and Reeds 2001, Reeds and Davis 1999).

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 authorization 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 European Food Safety Authority. 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 randomized 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 randomized 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 authorization of health claims in the context of appetite ratings and weight management.

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Disclosure of interest

The authors report no conflicts of interest.

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