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## PASSCLAIM<sup>1</sup> – Body weight regulation, insulin sensitivity and diabetes risk

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■ **Summary** *Background* Insulin sensitivity is a key function in human metabolism because it has a crucial role in the development of disease that are increasingly common in modern society. Impaired insulin sensitivity is an important determinant of type 2 diabetes; moreover, it has been proposed as an independent risk factor for cardiovascular disease. Thus, reduced insulin sensitivity is strongly associated with the metabolic syndrome, which represents a cluster of metabolic abnormalities and cardiovascular risk factor. Insulin sensitivity can be modulated by different environmental factors, in-

cluding dietary habits. Obesity, especially if associated with abdominal adiposity, impairs insulin-sensitivity while physical activity can improve it; however, the composition of the habitual diet is clearly an important regulator of this function. *Aim* To evaluate methodologies and markers that can be used to substantiate existing and potential claims of beneficial effects of foods on relevant functions connected with body fat deposition, insulin sensitivity and blood glucose regulation. *Results* We have reviewed the scientific basis for existing and potential claims, based not only on modifications of the target functions (body fat deposition, insulin sensitivity and blood glucose regulation) but also on modifications of other relevant associated functions (energy intake, energy expenditure, fat storage and oxidation, lipotoxicity, body fat composition, inflammation, oxidative stress, vascular function, glucose production and utilization). In this context we have identified a number of markers and evaluated appropriate method to measure and validate them. *Conclusions* Relevant functions contributing to overweight, the metabolic syndrome and diabetes have been identified. The evidence reviewed

indicates that in this field the link between nutrition, biological responses and diseases is clearly established. Therefore, there is a strong potential to develop functional food science. The major gap

in the evidence continues to be the lack of diet based intervention trials of sufficient duration to be relevant for affecting the natural history of these conditions.

■ **Key words** diet – functional foods – insulin sensitivity – insulin resistance – obesity – metabolic syndrome – type 2 diabetes

## Introduction

Insulin sensitivity is a key function for the human body since it plays a crucial role in the development of diseases that have become very common in modern society and significantly influence duration and quality of life. Its relevance for human health derives from its being at the interface between genetic and environmental factors, and a number of functions and regulatory mechanisms involving intermediary metabolism, body fat deposition, the cardiovascular system, immune function, cell growth and differentiation and many others.

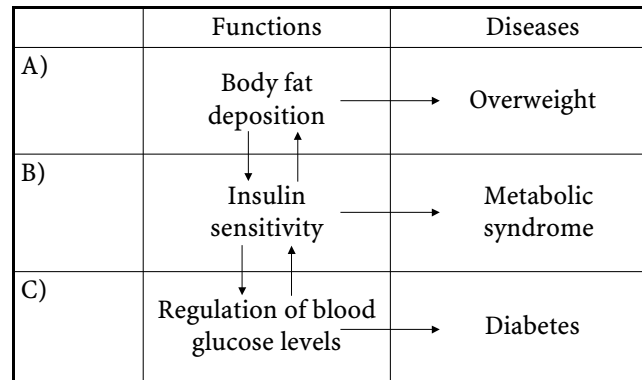
In this context, the relationship among these different functions is complex, and it is not always possible to identify the exact sequence of events in the natural history of the disease. In particular, the derangement of a specific function (i. e. blood glucose regulation) may be the consequence but, at the same time, also the cause of the impairment of a linked function (i. e. insulin sensitivity) that leads to a sort of vicious circle.

We have tried to simplify this picture by identifying a target function for each of the disorders and dysfunctions that are relevant for the topic of this ITG (Fig. 1). Of course, this might be an oversimplification because of the interactions between the metabolic processes; nonetheless our approach is justified by the nature of this exercise which, in order to establish how to support health related claims for foods, has to have a solid scientific basis but, at the same time, needs to be straightforward enough to allow the principles to be applied to the real world.

## Overweight

### ■ Background

Overweight is used to express an excessive accumulation of body fat. As such, it represents the most important risk factor for type 2 diabetes. In addition the distribution of fat (i.e. abdominal) predicts the development of diabetes and of other conditions associated with an impaired insulin sensitivity that cluster in the “metabolic syndrome”. Moreover, overweight represents a major health hazard causing impairments of relevant body functions, which eventually lead to health disorders involving the cardiovascular system, bones



**Fig. 1** Relationship between biological functions and their health consequences in the presence of a derangement

and joints, reproductive system, and the gastrointestinal tract [1].

The Body Mass Index (BMI) is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is calculated as the weight in kilograms divided by the square of the height in metres ( $\text{kg}/\text{m}^2$ ). BMI provides the most useful, albeit crude measure of overweight and obesity. There is a strong correlation between BMI and total body fat. However, BMI is not always an accurate index of body composition, as it does not account for the wide variation in body fat distribution and a given value may not always correspond to the same amount of body fat. Some subjects can be misclassified as obese, e. g. athletic subjects, who have a large skeletal muscle mass and a high BMI, but are not obese. According to the WHO, a BMI ( $\text{kg}/\text{m}^2$ ; Body Mass Index) of  $\geq 25.0$  is classified as overweight and a BMI of  $\geq 30.0$  as obesity. In the majority of European countries the prevalence of obesity has increased by up to 40% in the past 10 years, and is currently in the range of 10–20% in men and 10–25% in women [2].

Other anthropometric methods for the identification of overweight individuals include skin-fold thickness (a double layer of skin and subcutaneous tissue measured with special callipers) or waist circumference (a good predictor of upper-body fat patterning) [1]. A detailed description of the methods for determining body composition and fat distribution are further discussed later in this paper (“body composition”).

Obesity is now recognised as a chronic disease with a significant impact on quality and duration of people’s

life globally producing major public health problems and thereby contributing significantly to health care costs. Importantly, however, there is strong evidence that weight loss in overweight and obese individuals reduces the risk for diseases and improves life expectancy at all ages.

### ■ Potential claims for health effects of foods

Claims based on modifications of the target function:

- Reduces the risk of body weight gains
- Contributes to body weight reduction
- Decreases body fat
- Reduces abdominal fat

Claims based on modifications of markers of other relevant associated functions:

- Helps to reduce energy/food intake
- Reduces appetite
- Increases satiety
- Increases metabolic rate/energy expenditure
- Increases lipid oxidation (?)

### ■ Target function: body fat deposition

#### Biological meaning

The energy, which the body derives from foods, is required to match that expended not only by physical activity, but also by all body functions essential for growth, survival and reproduction. When energy intake exceeds expenditure, energy is stored in the form of adipose tissue to be utilised in conditions of food scarcity or increased energy demand.

There are no doubts that the complex relationships in the regulation of the energy balance and the control of body weight, involving energy intake, energy expenditure, energy deposition and regulatory mechanisms related to intra- and extracellular signalling molecules and receptors are crucially influenced by a large number of genes. The most recent obesity gene map demonstrated more than 100 genes or marker loci that may have the potential to influence fat deposition [3]. At the metabolic level, the imbalance between energy intake and energy expenditure, which leads to energy deposition in form of adipose tissue, can be seen as an imbalance between fat deposition and fat oxidation. Body tissues able to oxidise fat are those having mitochondria, whereas fat deposition occurs principally in adipose tissue, and, to a lesser extent, liver and skeletal muscles. Finally, fat oxidation is mainly a postabsorptive (i. e. fasting) phenomenon, whereas fat deposition tends to occur during post-prandial (i. e. fed) state.

Overweight and obesity are characterised as out-

comes of a long-term excess of energy intake relative to energy expenditure. It has been shown that nutritional and food-related non-nutritional factors affect the adjustment of energy intake (EI) to energy expenditure (EE) [4]. Managing or preventing overweight and obesity, requires modification of one or both components of the energy balance [5].

As well as dietary approaches the impact of physical activity on energy expenditure and energy balance has to be considered. Physical activity is seen as a critical factor contributing to successful body weight regulation in both lean and obese individuals [6]. 'Physical activity' in the context of physical performance and fitness has been addressed in more detail earlier in the PASSCLAIM Concerted Action [7].

#### Relevance in relation to diseases

Of type 2 diabetic patients 70–80% are overweight or obese; moreover, a number of long-term studies have shown a higher risk of diabetes with increasing body weight [8]. Data from the second National Health and Nutrition Examination Survey [9] show that the prevalence of diabetes is 3.8 times higher in overweight compared with normal weight individuals. This result has been confirmed by other studies in which overweight and obesity were the strongest risk factors for type 2 diabetes mellitus; moreover, the relationship between body weight and the risk of diabetes is continuous and graded [8].

Not only the grade of overweight but also the distribution of fat is important for the development of diabetes and the metabolic syndrome [10]. An upper body or central distribution of body fat, independent of the absolute level of obesity, is associated with an increased risk of diabetes and the metabolic syndrome. Abdominal obesity (central adiposity, upper body obesity) is defined as a preferential deposition of fat in the abdominal region and often involves accumulation of visceral fat. Patients with central adiposity have higher insulin levels and are more insulin resistant than subjects with similar weight but with a peripheral type of obesity [11, 12].

The importance of overweight in relation to the risk of type 2 diabetes and the metabolic syndrome has been emphasised by intervention studies showing that a reduction of body weight decreases the incidence of diabetes. In addition, in those already affected by the diseases, weight loss reduces postabsorptive rates of hepatic glucose production thereby reducing fasting hyperglycaemia. Furthermore weight loss improves insulin sensitivity in peripheral tissues in particularly increasing the capacity of non-oxidative glucose metabolism [13, 14].

Recently two studies, the Finnish Diabetes Prevention Study and the Diabetes Prevention Program [15, 16] have confirmed the importance of losing weight in the

prevention of type 2 diabetes. These were randomised controlled trials to evaluate the effects of a moderate weight reduction (5–7%) associated with increased physical activity on the prevention of type 2 diabetes in individuals at high risk; both studies achieved a 58% reduction in the incidence of diabetes in the intervention group as compared with the control group.

This represents conclusive evidence that type 2 diabetes can be prevented by reducing body weight in the context of a multifactorial intervention; the average amount of weight loss needed is not large, about 5% of the initial weight, which is much less than the weight loss that used to be considered clinically significant in the past [17].

In these two studies all features of the metabolic syndrome were improved in the intervention group but not in the control group, and this adds further support to the concept that weight reduction and physical exercise exert beneficial effects on insulin sensitivity. In the light of the strong and consistent associations with insulin resistance, diabetes and related diseases, the amount of body fat and fat distribution represent important markers to assess the efficacy of interventions aimed at reducing the risk of these diseases.

### Available evidence on markers and their links with diseases

Determination of body composition covers many different components; ranging from body fat, intra- and extra-cellular water, cell mass to bone minerals. When studying body composition it is fundamental to understand which component is actually measured by the

method employed, which underlying assumptions are made, how accurately the method is measuring the relevant component and what are the factors interfering with the measurement.

In the case of body fat mass (FM), measures can be taken either indirectly (i. e. by subtracting the measured fat free mass (FFM) from total body weight (BW)) or directly, by measuring FM at molecular (triacylglycerols), cellular (adipocyte) or tissue (adipose tissue) level. Different research and field methods have been developed to the aim of measuring body components at different levels of complexity, and a vast literature can be found which systematically reviews the different approaches used (see for example [18]). In the context of this paper we only briefly describe the most common methods used for the estimation of FM, and provide a focus on the quality of the information that can be obtained regarding body fat and fat distribution (Table 1a).

### Total Body Fat: Research methods (Table 1a)

#### – Hydrodensitometry/Plethysmography

*What is measured: body density (direct measure), % body fat and % body fat-free mass (derived measures).* Total body fat has been traditionally measured by underwater weighing, involving the application of Archimedes principle of water displacement. The principle of connecting body density to body composition is the following: the total body volume is the sum of the volumes of all the body components (i. e. the volume of fat and fat-free mass in a two-compartment model). Since the density of

**Table 1a** Characteristics of markers related to body fat deposition (research methods)

Marker/Method	Methodological characteristics	Biological characteristics (relevance to function)	Comments
Hydrodensitometry/ Plethysmography	<ul style="list-style-type: none"> <li>Measures body density which largely depends on body fat</li> </ul>	<ul style="list-style-type: none"> <li>Very reliable for total adiposity; no information on fat distribution</li> </ul>	<ul style="list-style-type: none"> <li>Total error &lt; 5 %</li> <li>May be influenced by hydration and bone density</li> <li>Very cumbersome equipment</li> <li>Total error &lt; 3 %</li> </ul>
DEXA	<ul style="list-style-type: none"> <li>Measures bone mass, fat- and fat-free-soft tissues (derived measures)</li> <li>Rely on equations based on a three-compartment model</li> </ul>	<ul style="list-style-type: none"> <li>Fine measure of total adiposity</li> <li>It can be used for assessing fat distribution</li> </ul>	<ul style="list-style-type: none"> <li>Cheap measure; expensive and non-portable instrument; it requires some analysis time</li> <li>Exposure to a low dose of radiation</li> <li>Total error in measuring body fat around 5 %. If integrated with the measurement of total body water (e. g. by dilution techniques) the error is reduced to less than 0.5 %</li> <li>Differences among instruments and software versions</li> </ul>
Isotope dilution	<ul style="list-style-type: none"> <li>Measures total body water (intracellular and extracellular); body fat is an indirect measure</li> </ul>	<ul style="list-style-type: none"> <li>Very reliable for total adiposity; no information on fat distribution</li> </ul>	<ul style="list-style-type: none"> <li>Allows measurements during everyday activity</li> <li>Influenced by hydration status</li> <li>Expensive and time consuming</li> </ul>



fat is  $0.9 \text{ g/cm}^3$  and the weighed average density of the components of fat-free mass, according to human cadaver analyses, is  $1.1 \text{ g/cm}^3$ , the density of the whole body obtained by underwater weighing (after taking into account the residual air content in the lung and intestine) can be used to obtain the proportion of body fat. However, differences in hydration and bone density, for example in old age, could affect FFM density and thus the derived measures. In this case, correction for Total Body Water (TBW) using dilution techniques (see below) can improve the measure and reduce the error. Typically, the error linked to BF measure is in the order of 5% for a two-compartment model and it is reduced to <1% when corrections for TBW in a three-compartment model are applied [19].

A similar approach to measure body density is the displacement of air. Plethysmography, which uses gas dilution to assess body volume in a special confined capsule. This technique may overcome many of the practical problems of underwater weighing, mainly related to patient cooperation, but it still needs validation in a wide variety of subjects.

#### – Dual-energy X-ray absorptiometry

*What is measured: X-ray attenuation (direct measure), % total bone mineral mass and bone density, % fat-free soft tissue, % body fat (derived measures).* Total body fat (BF) may be assessed accurately in most individuals by dual-energy X-ray absorptiometry (DEXA), an X-ray technique at low radiation exposure commonly used to monitor total bone mass (TBM) and bone density. This method is now more available than the traditional hydrodensitometry technique and is less demanding on the subject. The subject is exposed to a whole-body scan and approximately 10000 pixels are collected by the detector as the result of X-ray energy traversing the body. A number of pixels (40–45%) contain both bone and soft tissue, the rest contain soft tissue alone. The attenuations of X-rays by fat and fat-free soft tissues are different and related to the X-ray energy employed, allowing quantitative calculation of the respective masses in each pixel without bone. These data are then extrapolated by the software to cover the whole body. The approach is therefore a three-compartment model with mineral, fat-free and fat soft-tissues as the body components. The repeatability of the technique is excellent, with a CV% of about 1% for bone and 2% for fat mass measurements. The total error for BF estimation is in the order of 5% [19]. However, when applied to estimate TBM in a four-compartment model [20, 21] which includes body density (by hydrodensitometry) and TBW (by dilution techniques) the total error for BF estimation is as low as 0.5%.

#### – Isotope dilution

*What is measured: total body water volume, extra cellular water volume, intracellular water volume (direct measures), total cell mass, % body fat-free mass (derived measures), % body fat (indirect measure).* Body fat can also be measured indirectly by assessing FFM with a variety of techniques and subtracting it from total body weight. The most used approaches are the dilution techniques that measure body water either as total body water or in the two main water compartments (intra-cellular and extra-cellular) [22]. TBW is normally measured by deuterium dilution. The tracer is administered as  $^2\text{H}_2\text{O}$  in a known amount and after some equilibration time (usually 10 hours) the %  $^2\text{H}_2\text{O}$  enrichment is measured in saliva, urine or blood by mass spectrometry allowing measurement of the total water distribution volume and space, which for deuterium is both intra- and extra-cellular. Once the TBW is known it can be used to calculate the BF% by assuming that water occupies a constant fraction (73.2%) of the FFM [23].

The technique is precise in assessing TBW (1–2 CV%) but the derived measure of FFM and the indirect measure of BF are strongly dependent on the degree of hydration of the subject (which can vary even in healthy subjects from 70 to 76%). Furthermore, the hydration may acutely be changed after exercise, dieting and in several diseases. At the minimum, correction should be made by applying a three-compartment model that allows for measurement of FFM hydration.

In this case, as well as to study FFM composition (for example in the calculation of body cell mass (BCM) metabolic rate), TBW can be divided into intra- and extra-cellular water by using bromide dilution. Bromide, administered as NaBr, is a tracer that does not pass the cell membrane and thus equilibrates in the extra-cellular water compartment (ECW) only. It can be measured relatively easily by chromatography or fluorescence. The precision of the technique in measuring ECW is around 1%. Bromide dilution, coupled with deuterium dilution, allows the measurement of intracellular water (ICW) by subtracting ECW from TBW [24].

The precision of calculation of ICW is worse than that of TBW and ECW alone, since it depends on the combined error of two methods. If, for example, TBW and ECW are measured with a 1.5% relative precision, the precision of ICW will be in the order of 2.5%.

Fat free mass can be also measured by  $^{40}\text{K}$  whole-body counting [24, 25]. The assumptions hereby made are that potassium is distributed mainly intra-cellularly and is not present in the triacylglycerol stores, that a fixed fraction of 0.012% of potassium is present as the radioisotope  $^{40}\text{K}$  and that total potassium is present at concentrations of 2.5–2.66 g/kg FFM. By using a whole body counter (a shielded room containing a  $\gamma$ -counter partially or completely surrounding the sub-

ject) total body cell mass and FFM can be calculated within 3% accuracy, comparable to that of isotope dilution.

### Total Body Fat: Field methods (Table 1b)

Currently, there is no body composition method used in field studies able to assess body fat mass directly (Table 1b). Surrogates are obtained by anthropometric measurements, such as calculation of BMI from height and weight or measuring a varying number of skinfolds and circumferences. BF can also be measured indirectly by assessing FFM using bioelectrical impedance analysis (BIA) and by applying prediction equations that are population specific.

#### – Anthropometry

*What is measured: weight to height ratio, thickness of skin plus subcutaneous tissues, girth measurements (direct measures), % body fat (predicted measure).* When gender is taken into account, BMI correlates well with fat mass at an epidemiological level in healthy subjects, but does not accurately reflect BF in athletes or in individuals with diseases affecting nutritional status. Percent body fat can be calculated from BMI using different prediction equations [26].

Skinfold thickness is the thickness of a double fold of

skin and subcutaneous adipose tissue taken by special spring-calipers at specific and well-described sites of the body, usually biceps, triceps, sub-scapula and supra-iliac regions.

The sum of the above four skinfolds is placed into gender- and age-specific regression equations that allow the estimation of body density [27–29]. The body density can then be used to calculate BF% as for hydrodensitometry (see above). When compared to a four-compartment model as the reference method, skinfold thickness shows a total error ranging from 7 to 10% in estimating BF [19]. However, single (truncal) skinfold thickness has been found better related to insulin sensitivity and to 2-h post OGTT plasma glucose than waist-to-hip ratio in a group of individuals with a wide range of body fat [30].

In addition to their use in assessing fat distribution (see below) waist and hip circumferences have been used to develop equations to assess BF validated against hydrodensitometry [31, 32]. Their total error in measuring BF is around 7% [19].

#### – Bioelectrical impedance

*What is measured: electrical resistance (direct measure), total body water volume, extra cellular water volume, intracellular water volume (predicted measures), % body fat (indirect measure).* Bioelectrical impedance (BIA)

**Table 1b** Characteristics of markers related to body fat deposition (field methods)

Marker/Method	Methodological characteristics	Biological characteristics (relevance to function)	Comments
Body weight	<ul style="list-style-type: none"> <li>• can be easily assessed with a scale</li> <li>• serial weights should be measured on the same or a carefully calibrated scale</li> </ul>	<ul style="list-style-type: none"> <li>• does not distinguish between weight associated with muscle and fat</li> <li>• rough measure of total body energy stores</li> <li>• changes in weight parallel energy balance</li> </ul>	<ul style="list-style-type: none"> <li>• cheap and easy field measure</li> </ul>
BMI	<ul style="list-style-type: none"> <li>• index of weight for height</li> <li>• most useful, albeit crude measure of overweight/obesity</li> <li>• large interindividual variation</li> </ul>	<ul style="list-style-type: none"> <li>• crude measure of obesity</li> <li>• gender-specific</li> <li>• does not distinguish between muscle and fat</li> <li>• associated to disease outcome at epidemiological level</li> </ul>	<ul style="list-style-type: none"> <li>• cheap and easy field measure</li> <li>• should be integrated with girth measures to assess fat distribution</li> </ul>
Skinfold thickness	<ul style="list-style-type: none"> <li>• index of subcutaneous fat</li> <li>• rely on equations validated against two-component models (body density)</li> <li>• requires skilled operator(s)</li> </ul>	<ul style="list-style-type: none"> <li>• measure of body density</li> <li>• gender-specific</li> <li>• it partly accounts for variation in body fat distribution (trunk skinfold)</li> <li>• related to insulin resistance</li> </ul>	<ul style="list-style-type: none"> <li>• cheap field measure</li> <li>• total error in measuring body fat around 10%</li> </ul>
Waist/waist-to-hip ratio	<ul style="list-style-type: none"> <li>• index of fat distribution in the abdominal region</li> <li>• can be easily assessed with an inextensible tape</li> </ul>	<ul style="list-style-type: none"> <li>• associated to visceral fat</li> <li>• related to intermediate markers of insulin resistance</li> <li>• cut offs are specific for age, gender and ethnicity</li> </ul>	<ul style="list-style-type: none"> <li>• cheap and easy field measure</li> <li>• total error in measuring body fat around 7%</li> </ul>
Bioelectrical impedance	<ul style="list-style-type: none"> <li>• measures electrical resistance; body fat is an indirect measurement</li> </ul>	<ul style="list-style-type: none"> <li>• related to markers of insulin resistance</li> </ul>	<ul style="list-style-type: none"> <li>• influenced by hydration status and equations employed</li> <li>• good within-subject reproducibility</li> <li>• differences among instruments and software versions</li> </ul>

methods are commonly used for body composition assessment, even at the research level [33]. However, it must be kept in mind that BIA does not measure BF but it is mainly a descriptive method of measuring TBW based on a molecular model. The principle of BIA is that the electrical conductance of the body is related to the water compartments (and their solutes, which are considered constant). By applying an alternating current of known intensity (500–800 mA at 50 kHz) through the body and by measuring the difference of potential (V) between two extremities (usually wrist and ankle) the resistance (R) can be calculated by Ohm's law as  $R = V/I$ . Assuming that the current flows into a cylinder representing the water compartment of the body, the resistance is related to the length and radius of the cylinder and to a coefficient which is the specific resistivity of the fluid. By taking height as L,  $L^2/R$  is the resistivity index of the individual under study and is directly related to TBW in prediction equations that must be validated against direct methods to measure TBW, such as deuterium dilution. As a matter of fact, prediction equations employed by commercial softwares often include BMI, age, sex and sometimes skinfolds, which are per se related to adiposity and therefore obscure the relationship between the resistance measured and FFM. Moreover, as for dilution techniques, the hydration status of the subject is crucial and strongly affects the results unless highly standardised protocols regarding drinking, eating, temperature and physical exercise are applied.

Multi-frequency bio-impedance is an evolution of the BIA technique that measures R at different frequencies. Since cell membranes let high frequency current pass, whereas they act like capacitors blocking direct current, it is possible to discriminate between ECW (extrapolation of the resistance of the direct current) and TBW (extrapolation of the resistance of a current at infinite frequency). The multi-frequency BIA seems to represent a better technique than BIA, especially when measures of changes in the water compartments are needed, although it is affected by the same assumptions regarding the hydration of FFM.

### Regional fat distribution

Regarding research methods to assess fat distribution, the predominant location (subcutaneous vs. visceral) of abdominal fat is usually quantified by computerised tomography (CT) or by nuclear magnetic resonance (NMR) with a single cross sectional slice between the second and fourth lumbar vertebrae, whereas fat distribution in the legs relative to the muscular tissue (inter- vs. intra-muscular) is necessarily assessed by NMR, CT or by histochemistry (perivascular or interfibrillar fat vs. intramyocellular fat). Although the precision of CT is very good, the levels of ionising radiations, technical difficulties and costs involved are high, thus practically

limiting the use of this technique in routine body composition analysis [34, 35].

Regarding field methods, they mainly rely on the measurement of body circumferences, such as leg, arm, waist and hip. The waist-to-hip and waist-to-height ratio are commonly used to stratify individuals regarding the central distribution of fat, which is related at an epidemiological level to insulin resistance, but give no information about the subcutaneous or the peri-visceral deposition of abdominal adipose tissue. Waist circumference (WC) has been indicated as a better marker of abdominal adiposity than either waist-to-hip ratio or body mass index. The National Institutes of Health of the USA and the World Health Organisation suggest that WC and BMI are the most available and reliable means of identifying overweight, establishing the risks related to different phenotypes, and monitoring treatments for weight reduction [36]. A number of different descriptions of the site for WC measurements exist in the literature, the best probably being immediately above the iliac crest. The repeatability of WC is around 1% while its correlation with trunk adiposity (assessed by DEXA), when gender is taken into account, is high with a  $r^2$  ranging from 0.82 for men to 0.92 for women [37].

## ■ Functions associated with body fat deposition

### 1) Energy intake

#### **Biological meaning and relevance in relation to diseases.**

Energy intake is controlled and influenced by many physiological and biochemical factors [38]. Information from different levels of the organism (gastrointestinal tract, concentrations of hormones and metabolites in the blood) is processed to sensations of hunger or satiety. Many promising ways to influence energy intake may therefore be relevant to the factors affecting meal size, or else be acting on hunger and/or satiety. For example, the post-meal feeling of fullness or satiety could be modulated by specific functional foods.

Satiety refers to the effects of a food or meal after eating has ended [39]. It should be distinguished from satiation, which is the process that brings a period of eating to an end [40]. Both these concepts have to do with inhibitory factors that counteract the stimulation influences of factors such as hunger, appetite, palatability and variety of food stimuli [39, 40]. Among the strategies that could be used to deter excessive food intake, some could address factors occurring at meal time, e. g. decreasing the energy density of foods by adding water or "fibre", or facilitating the selection of certain foods (e. g. with low energy density or low glycaemic index), by using appropriate palatability enhancers in nutritionally valuable foods [41], presenting reasonable food portions, avoiding excessive portions of high energy density foods [39].

Other strategies could deal with events occurring after the meal has been terminated, i. e. during the satiety period. The “Satiety cascade”, proposed by Blundell [42], suggests many possible approaches. For example, evidence suggests that nutrient metabolism is directly or indirectly related to post-ingestive satiety. Protein, carbohydrate and fat exert hierarchical effects on satiety in the order protein > carbohydrate > fat [43, 44].

In addition, other factors such as the energy density of food or the Glycaemic Index are considered also to influence satiety and energy. The glycaemic index (GI) was proposed as a system for classifying carbohydrate-containing foods according to the post-prandial glycaemic response [45]. The rate of carbohydrate digestion and absorption has significant effects on post-prandial hormonal and metabolic responses and by these means it may affect hunger and satiety.

Dietary energy density (ED) can be defined as the amount of energy content per unit weight or volume of food (e. g. kJ/g). It has been shown that energy density of food has a robust and significant effect on satiety and satiation independently of palatability and macronutrient composition and has been suggested as an important determinant of energy intake and by this, of energy regulation [39, 46]. In common foods, the two most important determinants of dietary ED are water and fat, because fat is more than twice as energy-dense as either carbohydrates or protein and water has zero energy. In addition to fat and water, other factors, particularly dietary fibre influences the ED of food [46].

There is good evidence that increasing satiety, e. g. by consuming a low ED diet or a low-GI diet, reduces energy intake. The majority of short-term or small-scale studies in humans that have addressed this question showed a reduction in subsequent hunger or increased satiety, or both following consumption of low-GI compared to high-GI foods [47, 48]. In addition, short-term studies have shown that consumption of low-ED diets promotes satiety, reduces hunger, and decreases energy intake with no marked differences between different dietary manipulations used to change energy density [46]. Although energy density exerts profound effects in short-to-medium term studies (from a few days to a few weeks), it might be possible that subjects behave differently in longer-term intervention studies [43]. Whether these short-term changes in satiety persist in the long term and induce or facilitate changes in energy intake that will be eventually translated into weight loss is still controversial; however, this problem was addressed in a review of relevant longer-term studies in which among different approaches to weight reduction the effects of low-ED diets were also evaluated: virtually in all studies a weight loss was achieved [46]. So far, there are no long-term clinical trials examining the effects of dietary glycaemic index on body-weight regulation.

A further possibility to influence energy balance by

lowering energy intake may be to reduce the availability of food energy. If some of the energy ingested is not absorbed completely, this could reduce net energy available to meet metabolic demands and herewith can lead to weight loss [49]. In contrast to a pharmacological approach with a specific lipase inhibitor, so far there are no convincing human studies showing similar effects of foods or food components [50, 51]. However, there is at least one study that shows *in vitro* that a green tea extract also inhibits lipases of the gastrointestinal tract [52].

Besides targeting food energy availability there are other possibilities to reduce the available energy to the body, e. g. by replacing food components with ingredients having lower energy content or reduced absorption rate. Fat replacement, for instance, could be achieved by several polysaccharides, e. g. inulin, modified starches or sucrose polyesters, which act to partly or totally mimic fat in food without the negative aspects of the high caloric value of fat. Replacement of carbohydrates, e. g. sucrose or glucose, in foods can be achieved by using a carbohydrate of similar sweetness and taste but different physiological and energetic properties like polyols (sugar alcohols) [38].

The replacement of food components with lower energy ingredients reduces the available energy and the caloric density of the food item. The effects of low-energy density diets on weight management have not been completely elucidated. Overweight is a consequence of an energy imbalance and weight maintenance requires a balanced caloric intake. Laboratory experiments in animals and clinical studies in humans have repeatedly shown that dietary factors, particularly fat and energy intake are strongly and positively associated with excess body weight. By contrast, the population-based studies of diet and obesity have reported inconsistent results. Such inconsistencies have been attributed to a number of factors, including weakness in the study design and systematic measurement errors in the dietary data [2]. There is robust evidence from cross-sectional and longitudinal studies to support that an energy-dense and a high fat diet, and therefore a high-energy intake is an independent risk factor for weight gain and obesity [53]. Fat intake seems to have the highest impact on energy balance and most population studies have shown a positive association between fat intake and overweight [54]. However, what really matters is total energy intake, and in some cases, particularly in children and adolescents, also excessive consumption of carbohydrate-rich foods (soft drinks) can be predictive of overweight [55].

A review of methods used to assess food and energy intake was presented in the FUFOS document, in the Section devoted to Functional Foods and Behaviours Functions, including hunger and satiety [56]. This review underlined major flaws that are susceptible to alter the validity of results in a large proportion of studies.



**Markers of energy intake (Table 2).** To substantiate claims that are made on reduction of energy intake, markers are necessary that record the energy intake and its reduction. There are accurate tables for converting food eaten into energy consumed that might be helpful to determine energy intake under conditions of a controlled diet. But under free-living conditions the measurement of energy intake seems to be more difficult, because of difficulties in the accurate evaluation of the amount of a food consumed by an individual. There are several methods that are usually applied to estimate energy intake in individuals, they are based on either a retrospective or a prospective evaluation or a combination of the two.

Retrospective methods include dietary recall (e. g. a 24-hour recall of all food eaten during the previous 24 hours), food frequency questionnaires and dietary histories (diet history interview and questionnaire). All of

them rely very much on an individual's memory and motivation to recall his or her food and drink consumption.

The 24-h recall method requires that the respondent report to an interviewer all foods and beverages consumed in the previous 24h period specifying the amount for each of them. The strength of the 24-h recall is its ability to collect detailed qualitative information about foods consumed.

The dietary history method inquires about the frequency with which various foods are consumed and also gathers information about the typical content of meals. One of the strengths of the diet history is its detailed assessment of usual meal pattern in addition to the frequency of consumption.

Prospective methods are dietary records based on weighted portions, household measures or the double portion technique (duplicate foods). In the dietary

**Table 2** Characteristics of markers related to energy intake

Marker/Method	Methodological characteristics	Biological characteristics (relevance to function)	Comments
Dietary recall (24-h recall)	<ul style="list-style-type: none"> <li>retrospective</li> <li>guided interview to recall and describe all foods consumed by a trained interviewer</li> <li>reactivity of the assessment method</li> <li>rely heavily on the individuals memory</li> <li>ability to collect detailed qualitative information</li> <li>respondent burden is low</li> <li>validity is poor/not given</li> </ul>	<ul style="list-style-type: none"> <li>does not provide adequate data</li> <li>not representative: does not reflect the patients usual intake pattern</li> <li>reported may not be actual intake but rather what interviewer wants to hear</li> </ul>	<ul style="list-style-type: none"> <li>problem of underreporting</li> <li>not valuable for intake assessment especially in overweight people</li> <li>suitable for population estimates; low intraindividual reproducibility</li> </ul>
Dietary history	<ul style="list-style-type: none"> <li>retrospective</li> <li>ask for information about usual patterns of intake</li> <li>advantage of covering a rather long period of time</li> <li>not reactive</li> <li>limitations relate to validity</li> <li>incomplete responses may be given</li> </ul>	<ul style="list-style-type: none"> <li>underestimation can occur</li> <li>consistency of intake is assumed</li> <li>unless quantities are elicited absolute energy intake cannot be obtained</li> </ul>	<ul style="list-style-type: none"> <li>susceptibility to the influence of social desirable effects since it reduces awareness of food consumption</li> </ul>
Dietary record	<ul style="list-style-type: none"> <li>prospective/concurrent</li> <li>self-monitoring of food and drink intake over a specified period</li> <li>can be used to determine baseline status and to track intake patterns during and after treatment</li> <li>reliant on compliance</li> <li>respondent burden is great</li> </ul>	<ul style="list-style-type: none"> <li>advantage: permits recording of all foods and drinks in whatever quantities are consumed</li> <li>food intake may be altered</li> <li>underreporting is common</li> </ul>	<ul style="list-style-type: none"> <li>reactive effect (may be often regarded as beneficial)</li> </ul>
Double portion technique	<ul style="list-style-type: none"> <li>prospective</li> <li>a duplicate portion of the foods and beverages is collected</li> <li>accurate</li> <li>permits direct chemical analysis</li> <li>high respondent burden</li> </ul>	<ul style="list-style-type: none"> <li>intakes may be altered</li> <li>differences between duplicate portions and weighed records are large (~7 % for energy)</li> </ul>	<ul style="list-style-type: none"> <li>less affected by forgetting than retrospective methods</li> <li>may be reactive: intake may be altered</li> </ul>
Visual analogue scale (VAS) (satiety)	<ul style="list-style-type: none"> <li>recording of sensations, e. g. hunger, satiety, fullness</li> <li>sensitive under laboratory conditions</li> <li>reliability and validity appears more pronounced under laboratory conditions</li> </ul>	<ul style="list-style-type: none"> <li>reliable for appetite research</li> <li>good evidence that increasing satiety decreases energy intake</li> <li>some evidence that weight is also influenced</li> </ul>	<ul style="list-style-type: none"> <li>best used in within-subject repeated measures design where the effect of different treatments can be compared under similar circumstances</li> </ul>

record the respondent is required to record all foods and beverages and the amount of each consumed on a daily basis. The recording of dietary intake is usually conducted over a period of 3–14 days. One of the strengths of the dietary record method is its ability to capture quantitative information, because all foods and beverages are weighed or measured before consumption. Because of this, the weighed food record has been used as the standard to which other dietary intake methodologies have been compared [57]. Supplementary information on the methods described above can be found in the paper ‘PASSCLAIM – physical performance and fitness’ of Saris et al. [7].

Dietary assessment methods generally differ in the ease with which representative samples can be obtained as well as in their validity and reliability and thus in the uses to which they should be put [1].

Retrospective methodologies are generally thought to be subject to random error due to poor recollection of past diet as well as systematic error due to the underreporting of true intake (this applies particularly to fat intake).

Prospective methodologies are believed to be subject to reporting bias, because the act of recording intake is thought to influence the respondent’s usual food choices and alter their intake during the recording period. Physical and psychological characteristics of study participants play an important role in the observed reporting bias. Subjects might be reporting according to expected instead of real intake [58].

While it is relatively easy to measure meal size, and therefore the effects of factors susceptible to influence it, measuring satiety is somewhat more complex. Claims that a food increases satiety should be supported by demonstrating that post-meal events are indeed modified in a significant way following intake of a specific food product.

Many indices have been proposed to assess satiety effects. A tool frequently used for assessing the intensity and duration of satiety is the “visual analogue scale”, or “VAS”. VAS are horizontal or vertical lines, anchored at the extremities by words describing extreme states of a sensation, e. g. “as hungry as ever”, and “not hungry at all”. Many subjective feelings (hunger, fullness, desire to eat, etc.) can be rated by making a mark along the line corresponding to the intensity of the presently experienced sensation [42].

The VAS is very simple to use, but it merely gives a representation of the subject’s present sensation. Although this is of obvious interest, it is crucial to also obtain objective measures of a food satiating power. One of the most important indices of the satiating power of a food is the impact of that food upon subsequent intake. In the classic “pre-load paradigm”, a test meal is administered at a specified interval after the intake of a fixed “pre-load” food whose satiating power is being tested.

The amount of food spontaneously ingested in the test meal represents an index of how much (or how little) the pre-load inhibited later intake or, in other words, induced satiety. A pre-load food with high satiety power will strongly inhibit later intake.

Another way to measure the satiating power of a food load is derived from classic animal studies in which the very concept of satiety was developed. After a meal of varying dimensions, an animal will remain without eating for a certain period of time before returning to the food source. This inhibition of eating for certain duration is the classic definition of satiety. Therefore in animal or human experiments as well, one way to measure the satiety effect of a food is to measure the duration of the inhibition of eating that follows intake. High satiety power results in a long delay before eating is resumed. Two foods of equal energy value, but different nutrient composition for example, could lead to different satiety duration [59].

The use of a “Satiety Index” computed on the model of the Glycemic Index has been proposed a few years ago [60] but little progress has been achieved in the development, validation or use of such an index.

Commonly, the effect of food or dietary treatment on satiety is determined in preload studies. In these studies a fixed amount of a defined food (a preload) is consumed and after an interval of time, the effect of the preload on subsequent intake (e. g. subsequent meal or the remainder of the day) is measured.

## 2) Energy expenditure (Table 3)

Energy expenditure (EE) includes several components: resting metabolic rate (RMR), the thermogenic effect of exercise, the thermogenic effect of food and facultative thermogenesis. RMR is usually the greatest contribution (60–75 %) to total daily energy expenditure and is due to EE for maintaining normal body functions and the homeostasis plus a component due to activation of the sympathetic nervous system.

The role of energy expenditure in energy regulation remains a subject of continuing controversy. It has been frequently hypothesised that low energy expenditure causes obesity, although direct evidence of this hypothesis has been difficult to obtain in humans [61]. Cross-sectional studies in children and adults have shown that energy expenditure, including physical activity-related energy expenditure, are similar in lean versus obese subjects, especially after controlling for differences in body composition [62]. On the other hand, new data have emerged from studies conducted over the last decade, demonstrating that energy expenditure is a critical factor contributing to successful energy regulation in normal individuals, as well as to the dysregulation of energy balance that characterises obesity. Reduced energy expenditure appears to facilitate weight gain in individu-

**Table 3** Characteristics of markers related to energy expenditure (EE)/fat oxidation\*

Marker/Method	Methodological characteristics	Biological characteristics (relevance to function)	Comments
Indirect calorimetry*	<ul style="list-style-type: none"> <li>indirect calculation of the heat released by chemical processes from the rate of oxygen consumption</li> <li>non-invasive</li> <li>good validity</li> <li>indirect calculation of fat oxidation (respiratory quotient)</li> </ul>	<ul style="list-style-type: none"> <li>highly accurate for determining resting energy expenditure</li> <li>limitations by physiological effects (e. g. hyperventilation) and the influence of metabolic processes such as ketogenesis and lipogenesis</li> <li>predicts body weight gain and long-term success of weight reducing regimens</li> </ul>	<ul style="list-style-type: none"> <li>requires separate analysis of protein oxidation (urinary nitrogen) to obtain non-protein respiratory quotient</li> <li>inexpensive and reproducible</li> <li>troublesome and time consuming</li> </ul>
Doubly labelled water (DLW)	<ul style="list-style-type: none"> <li>administration and measuring of elimination rates of stable isotopes</li> <li>simple and convenient technique</li> <li>gives an overall mean value of EE for a period of 2–3 weeks</li> <li>good validity</li> <li>not possible to measure 24h EE and its components, and the day to day variability</li> </ul>	<ul style="list-style-type: none"> <li>highly accurate for measuring total EE</li> <li>some evidence that underestimation of EE in heavier subjects</li> </ul>	<ul style="list-style-type: none"> <li>regarded as the gold standard for determining energy expenditure</li> </ul>

als susceptible to obesity and also appears to reduce the extent of body energy loss during undereating in both lean and obese individuals [63]. Moreover, it was shown recently that changes in nonexercise activity thermogenesis (NEAT) mediate resistance to weight gain with overfeeding in sedentary adults and that the thermogenic potential of fidgeting-like and low-grade activities may contribute to energy balance [64, 65].

Several foods or food components are supposed to influence energy expenditure, e. g. caffeine, capsaicin, gingerols and shogaols (the pungent principle of ginger and spices) or green tea extract. Therefore, including such ingredients could be a viable approach to stimulate energy expenditure and to influence body weight [4, 39, 66].

Claims on increasing energy expenditure should have to be supported by measuring it with reliable methods. There are several methods available that can be used to determine energy expenditure in humans.

#### – Indirect calorimetry

The energy expended by an individual especially under resting conditions can be assessed by indirect calorimetry. Indirect calorimetry based on respiratory exchange measurements has been successfully used from the beginning of the 20<sup>th</sup> century to obtain an estimate of heat production (energy expenditure) in human subjects and animals. Indirect calorimetry is based on the fact that the heat released by chemical processes within the body can be indirectly calculated from the rate of oxygen consumption (VO<sub>2</sub>). In addition to ventilated hood systems, the energy expenditure can be accurately determined by using respiration chambers (described in detail in [7]) although, due to several disadvantages

(e. g. interfering with everyday life and the normal activity pattern), they seem to be less useful in practice. Indirect calorimetry is used to measure energy expenditure under resting conditions. The errors inherent to this classical technique can stem from various sources, e. g. model of calculation and assumptions, calorimetric factors used, technical factors and human factors. The physiological and biochemical factors influencing the interpretation of calorimetric data include hyperventilation and the accumulation or loss (via breath, urine or sweat) of intermediary metabolites (gluconeogenesis, ketogenesis) [67, 68].

#### – Doubly labelled water

In addition to calorimetric methods the most commonly used method to assess total energy expenditure in humans under natural conditions of life is the doubly labelled water (DLW) measurement [1]. This technique involves the administration of a bolus of water containing enriched quantities of the stable isotopes deuterium (<sup>2</sup>H) and oxygen-18 (<sup>18</sup>O). The oxygen-18 is eliminated from the body in the form of carbon dioxide (C<sup>18</sup>O<sub>2</sub>) and water (H<sub>2</sub><sup>18</sup>O) and the deuterium is eliminated in water (<sup>2</sup>H<sub>2</sub>O). The difference in elimination rate between these two isotopes is a measure of carbon dioxide production, which can then be used to calculate energy expenditure by the standard equations employed for indirect calorimetry. The DLW technique is used to measure total energy expenditure in free-living subjects. It was originally established for use in animals and later validated for use in humans. The DLW technique has been shown to be accurate to 1 % with a coefficient of variation of 2 to 12 % [69]. The technique is quite robust with regard to accuracy but is subject to error if some of the

dose water is lost, if the subject switches to a new source of water that has different background isotope abundance, or if the specimens are contaminated with ambient water or mislabelled with regard to time and date. DLW also requires calculating energy expenditure from CO<sub>2</sub> production, and thus an error in the assumed macronutrition composition of the diet can introduce a bias. The precision of the technique is potentially quite variable between centres, because a small random error in measuring the isotope enrichments of the physiological specimens can introduce random errors in the calculated energy expenditure of 3 to 15%. Because of this it is important to determine the repeatability of the analyses in any given laboratory [57, 69].

The doubly labelled water method tends to underestimate energy expenditure especially in heavier and fatter subjects. This underestimation in heavier subjects is possibly related to larger sequestration of deuterium during fat synthesis [70].

#### – Heart rate monitoring

In addition, other methods exist, e. g. heart rate monitoring [71]. For a detailed description of the method, see Saris, W. H. M. et al. [7]. Because of a variety of confounding factors, e. g. variations in stroke volume, eating meals, variation in posture, heart rate seems to be less useful for an estimation of energy expenditure [1].

### 3) Fat storage and oxidation

Subjects prone to obesity show a reduced ability to oxidise dietary and endogenous fat, and, in the presence of excess energy, store fat in adipose tissue to an extent that exceeds the body's ability to oxidise it, leading to an expansion of body fat stores and weight gain [72]. Conversely, weight-stable obese individuals show high rates of fat oxidation, which is interpreted as an adaptation mechanism to prevent further, unlimited weight gain [73–76]. This mechanism of weight control also applies to obese subjects undergoing a weight loss treatment, since a low rate of lipid oxidation at the end of the weight-reduction period is able to predict weight regain in the following years [77]. Therefore, the measurement of the rate of lipid oxidation and storage *in vivo* (both in absolute values and as percent of lipid intake) as well as the identification of exogenous and endogenous factors able to modulate human fat metabolism is of major importance in obesity research.

The oxidation of nutrients can be assessed, in addition to the evaluation of energy expenditure, by indirect calorimetry (IC), a technique based on the measurement of oxygen consumption and carbon dioxide production. Assuming that all the oxygen is used to oxidise degradable fuels, that all carbon dioxide produced in those reactions is recovered, and knowing the stoichiometry of

oxidative reactions *in vivo*, the rates of oxidation of macronutrients can be computed. An indicator of the relative contribution of protein, carbohydrate and fat to total fuel oxidation (or energy production) at a given time is the ratio between carbon dioxide production (VCO<sub>2</sub>) and oxygen consumption (VO<sub>2</sub>), which is named respiratory quotient (RQ); this ratio is quite different for the three macronutrients (mean RQ = 1 for carbohydrates, 0.809 for animal protein and 0.707 for fat). Special metabolic conditions which often take place in obese and insulin resistant individuals during the post-absorptive state are associated with RQs above or below this range (*de novo* lipogenesis, RQ > 1; ketogenesis, RQ < 0.707), but these biosynthetic processes generally occur along with oxidation and are not “seen” by IC [78].

Coupling IC with the infusion of labelled lipids offers the possibility of assessing not only fat oxidation, but also short-term fat storage. However, fat deposition and mobilisation can also be followed macroscopically on a long-term basis, by measuring body fat and body fat distribution with different techniques (see above).

### ■ Mechanisms involved in the regulation of body fat deposition

#### Hormones and other messengers

There are no doubts that the complex relationships between energy intake, energy expenditure and energy deposition and the pathogenesis of obesity involve regulation mechanisms mainly related to intra- and extracellular signalling molecules and receptors encoded by a large number of important genetic determinants in the control of body weight.

Hormones, hormone receptors and different protein and protein-derived molecules are involved in the two aspects of energy balance, i. e. control of food intake and energy expenditure/deposition [79–81].

#### – Thyroid hormones

The thyroid gland is part of the hypothalamic-pituitary-thyroid axis, and control of thyroid hormone secretion is exerted by classical negative feedback. Thyroid-releasing hormone (TRH) from the hypothalamus stimulates TSH from the pituitary, which stimulates thyroid hormone release. Increased thyroid hormone levels activate fat mobilisation, leading to increased concentrations of fatty acids in plasma. They also enhance oxidation of fatty acids in many tissues. Finally, plasma concentrations of cholesterol and triacylglycerols are inversely correlated with thyroid hormone levels. Thyroid hormones also stimulate almost all aspects of carbohydrate metabolism, including enhancement of insulin-



dependent entry of glucose into cells and increased gluconeogenesis and glycogenolysis to generate free glucose.

#### – Glucocorticoids

Most glucocorticoid activity in most mammals derives from cortisol, also known as hydrocortisone. The name glucocorticoid is related to their action in glucose metabolism. In the fasted state, cortisol stimulates several processes that collectively increase and maintain normal concentrations of glucose in blood. These effects include stimulation of gluconeogenesis from non-hexose substrates such as amino acids and glycerol. Enhancing the expression of enzymes involved in gluconeogenesis is probably the best-known metabolic function of glucocorticoids. Another effect is mobilisation of amino acids from extrahepatic tissues to be used as substrates for gluconeogenesis. Glucocorticoids inhibit glucose uptake in muscle and adipose tissue and stimulate triglyceride breakdown in adipose tissue, which liberates fatty acids used as energy substrate in tissues like muscle, whereas the released glycerol provides a substrate for gluconeogenesis.

#### – Growth hormone

Growth hormone (GH) is secreted from the anterior pituitary gland at a rate, which reaches the maximum during puberty when it starts to decline. The progressive reduction in GH levels during adulthood and ageing involves a number of changes in the GH axis, including decreased secretion of growth hormone-releasing hormone from the hypothalamus and decreased serum levels of insulin-like growth factor-1 (IGF-1). Normal ageing is accompanied by a number of catabolic effects on body compartments, including a decrease in lean mass and bone density and an increase in fat mass. A model to study the effect of GH on body composition is GH replacement therapy in adults with severe GH deficiency. These patients are at increased risk of death from cardiovascular disease, and, relative to age-matched controls, show increased fat mass, reduced muscle mass and strength, lower bone density, and higher serum lipid concentrations [82]. GH replacement therapy for a few months leads to increased lean mass and decreased fat mass, especially in the visceral area.

#### – Leptin and leptin receptors

The name leptin derives from the Greek leptos (thin) and is applied to a protein hormone with important effects in regulating body weight, metabolism and reproductive function. Leptin is encoded by the obese (*ob*) gene and expressed predominantly in the adipocytes. It appears that the increase in size of adipocytes, due to ac-

cumulation of triglycerides, stimulates leptin synthesis. Once synthesised, leptin is secreted through a constitutive pathway and not stored in the cell. At this time, the mechanisms responsible for regulating leptin expression in adipocytes are unknown. It is likely that a number of hormones modulate *ob* gene expression, including glucocorticoids and insulin. Leptin's effects on body weight are mainly mediated through effects on hypothalamic centres that control hunger, possibly due to inhibition of synthesis of neuropeptide Y, a very potent stimulator of feeding behaviour. Additional effects are on energy expenditure, measured as increased oxygen consumption, higher body temperature and loss of adipose tissue mass. The mechanisms by which leptin exerts its effects on metabolism are largely unknown and are likely quite complex. In contrast to dieting, which results in loss of both fat and lean mass, treatment with leptin promotes lipolysis in adipose tissue, but has no apparent effect on lean tissues. Mutations in *ob* or *db* genes appear to be a very rare cause of morbid obesity in humans, but both have been described. Recent studies with obese and non-obese humans demonstrated a strong positive correlation of serum leptin concentrations with percentage of body fat, and higher concentrations of *ob* mRNA in fat from obese compared to thin subjects. This suggests that obese people are in some way insensitive to leptin, rather than suffering from leptin deficiency.

#### – Neuropeptide Y, melanocortin and melanocortin-4 receptors

Neuropeptide Y (NPY) is the most abundant neuropeptide in the brain. NPY is known to be an extremely potent stimulator of feeding behaviour. Feeding behaviour in rodents is blocked by injection of antibodies or antisense RNAs against NPY. More importantly, leptin appears to act, at least in part, by inhibiting NPY synthesis and release in the hypothalamus. In addition, mutations that interfere with signalling via the hypothalamic melanocortin-4 receptor lead to obesity that is at least partially explained by perturbations of NPY expression. Melanocortins are a group of pituitary peptide hormones that include adrenocorticotropin (ACTH) and the alpha, beta and gamma-melanocyte-stimulating hormones. Five melanocortin receptors have been identified to date, all of which are G-protein coupled receptors. Melanocortin stimulation of certain hypothalamic neurons, via the melanocortin-4 receptor, inhibits feeding behaviour by inhibiting NPY. This has been studied in the "yellow obese" mouse, another mutant strain that develops adult-onset obesity, hyperinsulinaemia, hyperglycaemia, hyperphagia, and has a striking yellow colour due to a mutation in the *agouti* gene.

### – Mitochondrial uncoupling proteins

Mitochondrial uncoupling proteins are 34 kDa mitochondrial membrane proteins first discovered in brown fat (UCP1) and subsequently identified in white fat (UCP2) and muscle cells (UCP3). They allow mitochondria within those cells to uncouple oxidative phosphorylation, which “short circuits” the proton gradient across the inner membrane, leading to diminished production of ATP, but generating heat. Some research suggests that they may play an important role in energy expenditure and thus body weight in man. Studies in Pima Indians, a population particularly prone to obesity and diabetes, have found a negative correlation between skeletal muscle UCP3 expression and BMI and a positive correlation between UCP3 mRNA and resting metabolic rate [83].

### – Beta 3-adrenergic receptors

These receptors are highly expressed in brown fat and to a lesser extent in white fat, where also beta 1- and beta 2-receptors are present. Binding of norepinephrine to this receptor on fat cells leads to increased transcription of the mitochondrial uncoupling protein, allowing increased heat production via hydrolysis of fatty acids. A possible role for beta 3-adrenergic receptors in the pathogenesis of human obesity is suggested by many studies showing an association between a polymorphism of the beta 3-AR gene with a tryptophan to arginine substitution at codon 64. This polymorphism was also shown to be associated to earlier age of onset of diabetes, insulin resistance and capacity to gain weight [79]. However, there are also studies showing lack of association between this variant and obesity, leaving open the question if this is a major mechanism in human obesity.

### – Serotonin (5HT),

This neurotransmitter, synthesised in the brain from the amino acid tryptophan, has been studied in humans for its action in inhibiting feeding behaviour. Dietary factors influencing blood tryptophan concentrations, or its uptake across the blood-brain barrier may influence 5HT synthesis. Such factors include other amino acids (AA), such as branched-chain AA, which compete for transport, or carbohydrates through their effect on AA uptake mediated by insulin. Some data indicate that the hypophagic action of 5HT acts preabsorptively, possibly through interactions with CCK and enterostatin. Changes in serotonin metabolism have been reported in eating disorders, such as anorexia and bulimia, in obesity and type 2 diabetes.

### – Endogenous opioids

They include beta-endorphin, dynorphin and enkephalines and are believed to induce stress-related hyperphagia, by acting on several sites in the hypothalamus. Some studies suggest that these compounds may influence meal size and macronutrient preference (for fat rather than carbohydrate) in humans.

### – PPAR $\gamma$

See previous section on “Leptin and leptin receptors”.

### Control of appetite by gastro-intestinal peptides

The finding that some peptides (Ghrelin, GLP-1 CCK...) or systems (cannabinoids...) expressed or present in the gastro-intestinal tract exert an effect on food intake implies strongly that there is a regulatory axis or a dialogue between the gastro-intestinal system and the hypothalamic pituitary unit. Nutrients could constitute an interesting relay and important modulators of such systems to control food intake and obesity-associated metabolic disorders.

### – Glucagon-like peptide 1

The glucagon-like peptide 1 is a proglucagon-derived peptide, secreted from gut endocrine cells in response to nutrient ingestion [84]. It links to specific G protein-coupled receptors, expressed in several central and peripheral tissues. In pancreatic b-cells, it promotes insulin secretion and b-cell proliferation and differentiation; those effects contribute to its blood glucose lowering effect, together with its influence on gastric emptying. Experimental findings in animals have shown that intra-cerebroventricular or peripheral administration of GLP-1 or analogues reduces food intake, suggesting both direct and indirect effects on central nervous system satiety centers through neuronal relay mechanisms [85]. GLP-1 was also shown to enhance satiety and reduce energy intake in human volunteers [86]. All those actions represent ideal properties for an agent designed for the management of type 2 diabetes. However, the short lifetime, and the peptidic nature of GLP-1, do not favour its development per se as a therapeutic agent. Inhibitors of dipeptidyl-peptidase IV, the enzyme responsible for the cleavage of GLP-1 in biological samples, are now proposed as an interesting mean to promote satiating action of GLP-1 in obesity. The elucidation of nutrients able to promote endogenous GLP-1 production and/or activity would provide a new way to control food/energy intake.

### – Cholecystokinin

Cholecystokinin (CCK) is a polypeptide hormone released by the upper small intestine in response to the presence of food in the lumen, particularly fat. CCK has both peripheral and central receptors. Peripherally, it is known to regulate gallbladder contraction and thus bile secretion, gastric emptying and pancreatic hormone secretion. In addition, CCK relays signals to the brain, inhibiting eating behaviour in humans and causing meal termination [41]. Antagonists to CCK have been shown to increase food intake in rats, whereas CCK stimulants have been shown to decrease food intake both in rats and in humans [87].

### – Ghrelin

Ghrelin, a 28 amino acid peptide hormone of mostly expressed in stomach endocrine cells, has been recently identified as the natural ligand for the growth hormone secretagogue receptor, which is widely distributed in the body [88]. Ghrelin is considered to have a significant role in the integration of glucose metabolism and insulin secretion inhibition during fasting [89]. Endogenous ghrelin levels and expression are increased in fasted rats, and decreased with refeeding [90]. Moreover, ghrelin can stimulate food intake and weight gain when given either systemically or into the brain in rodents; it also reduces fat utilisation in rats [91, 92]. The appetite activity of centrally administered ghrelin can be blocked by co-administration of a NPY-Y1 receptor antagonists, thus suggesting interplay of NPY, agouti related protein, and ghrelin in the central nervous system.

### – Endogenous cannabinoid system.

Epidemiological reports describing the appetite-stimulating properties of cannabinoids, and the recent insight into the molecular mechanisms underlying cannabinoid action have proposed a central role of the cannabinoid system in obesity [93]. The endogenous cannabinoid system comprises 1) specific receptors – the G-protein coupled-cannabinoid receptor (CB1) – being largely expressed in the central and enteric nervous system, 2) endogenous ligands – which are mainly ethanolamide or glyceride derivatives of mono- or long chain fatty acids – and 3) degradation enzymes for the ligands, such as fatty acid amide hydrolase [94]. Cannabinoid system activation may induce food overconsumption by amplifying the palatability or orosensory reward of food. Interactions of cannabinoid system with dopaminergic, serotonergic, and opioid pathways have been shown in several models.

The cannabinoid system is thus a potential and promising molecular target in the control of food intake: cannabinoids antagonists are now in phase III of devel-

opment in the therapeutical control of obesity [95]. Since cannabinoids analogues may be present in the diet as such, or at a higher amount as fatty acids precursors, a promising future will come from a better knowledge of nutrients/cannabinoid system interactions.

### ■ Future research needs and conclusions

For all aspects of overweight, either for the endpoint itself or the functions that influence body fat deposition, energy intake and energy expenditure, markers exist and are adequate to substantiate claims.

Regarding the evaluation of energy intake, under-reporting of absolute energy intake is common, particularly among obese patients. On the other hand, simple self-administered questionnaires based on food groups, designed for dietary assessment in epidemiological studies, show an acceptable agreement with more complex tools and are sufficiently accurate for clinical work and research [96]. Nevertheless, findings on misreporting of food intake, especially in obese subjects [97], underline that there is not yet a fully reliable method for the accurate determination of dietary intake.

For satiety, a function associated with energy intake, subjectively perceived hunger is translated into an objective marker by means of the visual analogue rating scale [40]. Measurement of satiety by this instrument seems to predict well the effect of the eaten food on the energy intake in the next meal. There is good evidence that lowering energy intake either by reducing the appetite or by lowering the energy density of the eaten foods is able to facilitate body weight reduction. However more controlled intervention trials are needed to assess whether the effects on body weight are sustained also in the long term.

Regarding energy expenditure, the doubly labelled water (DLW) method is considered a suitable and accurate method to measure energy expenditure in free-living conditions but might provide a slightly underestimated figure in overweight subjects. The DLW technique was shown to be highly accurate and is currently considered as the gold standard for the measurement of total energy expenditure in humans [57].

For most claims related to overweight and energy balance the reliability and validity of the markers seems to be good. The relevance of the markers to function seems to be satisfactory for a substantiation of the corresponding claims. Nevertheless, properly controlled intervention studies in humans are needed to evaluate in the long term the effects of modifications of the existing markers of the target function on the end point overweight.

## The metabolic syndrome

### ■ Background

Reduced insulin sensitivity is an important causative factor of type 2 diabetes. Not only is it present in most cases of type 2 diabetes mellitus, but it is also an independent risk factor for the development of the disease [98]. Moreover, impaired insulin sensitivity is associated with an increased prevalence of atherosclerotic vascular disease and is probably an independent risk factor for cardiovascular disease [99].

While the mechanisms linking impaired insulin sensitivity with type 2 diabetes have been extensively elucidated in recent years (they will be briefly illustrated in this section and in the next on Diabetes), the reasons of the association between an impairment of insulin action and cardiovascular disease are not completely clear.

As a matter of fact, impaired insulin sensitivity is often associated with other metabolic disturbances and cardiovascular risk factors; this condition has been defined as the insulin resistance syndrome or metabolic syndrome [100]. There is no internationally agreed definition of the metabolic syndrome, which is generally considered as an association of impaired glucose regulation (Impaired Glucose Tolerance, IGT or Impaired Fasting Glucose, IFG) or type 2 diabetes, hypertension, hypertriglyceridaemia, low HDL, central obesity [101, 102]; a recent statement from the National Cholesterol Education Panel (NCEP) of the USA attempts to define diagnostic criteria for the metabolic syndrome based exclusively on these clinical parameters (Table 4) [103]. On the basis of this definition the prevalence of the metabolic syndrome is around 25% of the general population and is not different in males and females, but it may vary with genetic background [104]. Other abnormalities often associated with the metabolic syndrome are microalbuminuria, hyperuricaemia, non-alcoholic liver steatosis, and coagulation disorders. There is growing evidence pointing to insulin resistance as the common aetiological factor of this condition, and its as-

**Table 4** Clinical Diagnosis of the metabolic syndrome: Three or more of the following abnormalities

Component	Trait	Defining Value
Abdominal obesity	Waist circumference	W: > 88 cm M: > 102 cm
Atherogenic dyslipidaemia	Triglycerides HDL – Cholesterol	> 150 mg/dl W: < 50 mg/dl M: < 40 mg/dl
Elevated BP	Ambulatory BP	SBP ≥ 130 mm Hg DBP ≥ 85 mm Hg
Elevated FPG	FPG	≥ 110 mg/dl

W women; M men; BP Blood Pressure; FPG Fasting Plasma Glucose

sociated increased risk of cardiovascular disease [105, 106].

Improving insulin sensitivity by (functional) foods or changes of food composition could possibly constitute the basis for health claims – as reduced risk claims or improved function claims. Insulin sensitivity can be regarded as a critical function; however, its impairment can also represent a marker of increased risk for the development of the metabolic syndrome/diabetes mellitus and possibly coronary heart disease or, even, an intermediate endpoint, at least for type 2 diabetes (Fig. 2).

### ■ Potential claims for health effects

Claims based on modifications of the target function:

- Improves insulin sensitivity
- Increases insulin-mediated glucose disposal
- Helps to reduce the risk for the metabolic syndrome

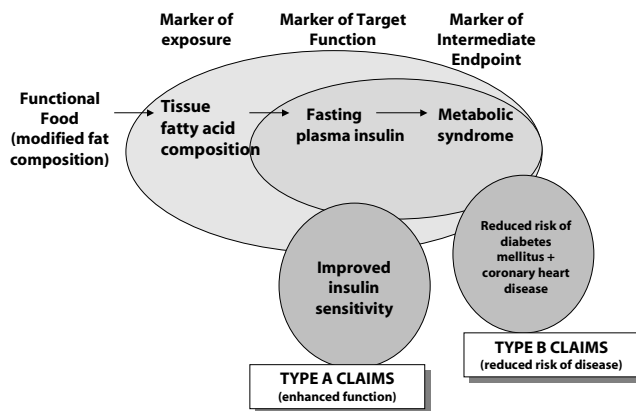
Claims based on modifications of other relevant associated functions:

- Improves fat composition in the body
- Reduces saturated fat in the body
- Lowers plasma NEFA
- Ameliorates serum oxidant-antioxidant balance
- Improves physiological vaso-dilation
- Reduces inflammation associated with impaired insulin sensitivity and/or hyperglycaemia
- Improves vascular function in individuals with impaired insulin sensitivity and/or hyperglycaemia

### ■ Target function: insulin sensitivity

#### Biological meaning

Plasma insulin levels play a relevant role in the regulation of the intermediary metabolism: not only do they



**Fig. 2** Example of claims for a functional food influencing insulin sensitivity



control plasma glucose levels by inhibiting liver glucose production and stimulating glucose uptake primarily in muscle tissues, but also induce a number of other biological effects. As a matter of fact, plasma insulin promotes the storage of substrates in fat, liver and muscles by stimulating lipogenesis as well as protein and glycogen synthesis, and by inhibiting lipolysis, glycogenolysis and protein breakdown. In addition, insulin regulates water and electrolyte balance and stimulates cell growth and differentiation. This multifaceted hormonal activity involves a complex network of signalling pathways, activated by the binding of the hormone with its receptor. Different proteins act as insulin receptor substrates at the cellular level for the various biological effects of insulin, which explain why selective impairments of insulin activity have been described [107].

Both genetic and environmental factors can interfere with the transmission of the insulin signal thus impairing one or more of the activities regulated by insulin in one or more tissues; this impairment, called insulin resistance or impaired insulin sensitivity, does not lead, with the exception of few rare cases, to a completely absent insulin activity, but only to a reduced effect which can be overcome by increased insulin levels.

### Relevance in relation to diseases

The degree of insulin resistance is directly related to the metabolic disorders associated with the metabolic syndrome and shows a graded and independent relationship with the incidence of diabetes [108].

With impaired insulin sensitivity, plasma insulin levels are increased, due to the closed loop between plasma insulin and glucosed levels: when the activity of the hormone is reduced, peripheral glucose utilisation is impaired which leads to a slight increase in plasma glucose levels; in turn, this stimulates insulin secretion and thus restores a normal glucose utilisation but at the cost of increased plasma concentrations of insulin [108]. This occurs in all individuals – with or without diabetes – having insulin resistance: they represent as much as one quarter of the general population; however, in some of them impaired insulin secretion is also present and, due to the concomitance of both defects, stable hyperglycaemia develops [106].

Since insulin resistance is selective, in the presence of this condition high plasma insulin levels are associated with abnormal metabolic effects (leading to hyperglycaemia, hypertriglyceridaemia, low HDL, high blood pressure, impaired fibrinolysis and all other conditions clustering in the metabolic syndrome) but also with an excessive stimulation of cell growth which may facilitate the development of arteriosclerosis and, possibly, neoplasia. This occurs because the insulin effects on cell growth and proliferation are mediated by an intracellular signalling pathway different from that employed for

the metabolic effects. In addition, hyperinsulinaemia predisposes to overweight and may aggravate insulin resistance as a consequence of a down regulation of the transduction system of the insulin receptor signal [107].

### Available evidence on possible markers of the function and their links with the disease process (Table 5)

The “gold standard” for measurement of insulin sensitivity is the hyperinsulinaemic euglycaemic clamp technique [109, 110], which directly reflects peripheral insulin sensitivity. This method is technically demanding but has a relatively good precision and reproducibility (coefficient of variation for repeated measurements less than 20%). A less cumbersome alternative to the clamp technique is the frequently sampled intravenous glucose tolerance test (FSIGT) [111], which gives a computer – derived indirect measure of insulin sensitivity. The insulin sensitivity estimated from the frequent sampling intravenous glucose tolerance test (FSIGT), or the “minimal model” [112], is rather closely correlated with the insulin sensitivity index derived from the euglycaemic clamp, but the day to day variation is larger (CV 20–27%).

A number of other indirect estimates of insulin sensitivity have been used, e. g. fasting insulin concentrations, the HOMA model and oral glucose tolerance test. Although significant correlations to the insulin sensitivity measured by the euglycaemic clamp are demonstrated, these methods provide only limited quantitative information concerning insulin action [113, 114].

There are two types of procedures to assess glucose utilisation in the whole-body using the technique known as ‘glucose clamping’. In the hyperglycaemic clamp, glucose is infused intravenously in a manner calculated to raise the plasma glucose concentration to some pre-chosen value, e. g. 10 mmol/l. Insulin secretion is stimulated, endogenous glucose production is suppressed and glucose utilisation is stimulated. The rate of glucose infusion is varied every 5 or 10 minutes according to the blood glucose concentration measured at the bed-side, to keep the blood glucose concentration constant (i. e. ‘clamped’) [109]. Under those conditions, the rate at which glucose enters the plasma must be the same as the rate of glucose utilisation. If endogenous glucose production is assumed to be suppressed, then the rate of glucose entry is the rate of intravenous infusion, which is known. Hence the rate of glucose utilisation can be assessed *in vivo*. More accurate measurement requires that a tracer technique be added to measure endogenous glucose production. In the ‘euglycaemic hyperinsulinaemic clamp’, insulin is also infused, to raise the plasma insulin concentration, usually to a high but physiological level. Glucose is also infused at a variable rate as above, to maintain euglycaemia (typically 5 mmol/l). This enables the comparison of glucose utilisation rates in different

**Table 5** Characteristics of markers related to insulin sensitivity

Biomarker	Methodological characteristics	Biological characteristics (relevance to function)	Comments
'Glucose clamp' technique	Invasive laboratory-based procedure; not suitable for screening large numbers. Coefficient of variation (day-to-day within individual) ~ 10 %	Often taken as the 'gold standard' for measuring the sensitivity of glucose metabolism to insulin.	
Fasting glucose and insulin concentrations; homeostatic model assessment ('HOMA')	Correlation coefficient with insulin sensitivity measured by 'glucose clamp' is approximately 0.64 [281]	Fasting plasma insulin alone is a useful index of insulin resistance in epidemiological studies (fasting insulin increases with insulin resistance). Calculation of HOMA (using also fasting glucose concentration) probably improves this measurement	HOMA combines insulin sensitivity of liver and peripheral tissues
Glucose and insulin concentrations after oral glucose challenge (oral glucose tolerance test) with application of simple model	Easy to perform; large numbers of subjects can be screened although it needs specialised laboratory for measurement of insulin. Coefficient of variation (day-to-day within individual) ~ 10–20 %. Correlation coefficient with insulin sensitivity measured by 'glucose clamp' varies from 0.4 to 0.9 for different models [282]	Probably measures effect of insulin on hepatic glucose production as well as muscle glucose uptake. Since this is a 'dynamic' test it should reflect insulin sensitivity in normal daily life	There are many possible models: reviewed in [282]
Short insulin tolerance test	Easy to perform (measure fall in plasma glucose after intravenous insulin injection) although requires medical supervision. Insulin measurement not needed. Correlation with 'clamp' is high (some claim 0.90) [281]	Probably measures effect of insulin on hepatic glucose production as well as muscle glucose uptake	
Frequently-sampled intravenous glucose tolerance test (FSivGTT) with 'minimal model'	A rather invasive procedure suitable for a clinical setting; multiple samples are taken and insulin and glucose measurements are needed. But often considered simpler to perform than a glucose clamp. However, values cannot be computed for all subjects and the correlation with the clamp is not strong (e. g. 0.5) [281]	Using the computer-based 'minimal model' insulin sensitivity and a number of other parameters can be calculated	

groups, or between different treatments under fixed conditions of glucose and insulin concentrations.

Alternatively, a constant infusion of a glucose tracer, labelled with either a radioactive or a stable isotope, can be used to measure glucose turnover and to distinguish glucose production and glucose utilisation, even in relatively non-steady-state conditions [115]. Again this is a whole-body assessment. Glucose utilisation by specific tissues, such as skeletal muscle and adipose tissue, can be studied *in vivo* either by arterio-venous difference measurements [116] or by imaging techniques such as positron emission tomography [117, 118].

In prospective studies of these markers, high fasting insulin (a surrogate marker for insulin resistance) and impaired glucose tolerances predict future development of type 2 diabetes and the metabolic syndrome [119].

Impairment of insulin-stimulated glucose utilisation, measured using the euglycaemic-hyperinsulinaemic clamp technique or with the FSIGT, is also associated with increased risk of deterioration of glucose tolerance and development of type 2 diabetes as well as the metabolic syndrome [120].

In conclusion, insulin sensitivity can be measured accurately using validated methods (insulin levels, HOMA, clamp, FSIGT). Insulin sensitivity is an independent risk marker for the development of diabetes and is closely related to the cluster of metabolic aberrations associated with the metabolic syndrome. If a specific (functional) food can be shown to significantly improve insulin sensitivity, as measured by validated methods, this could be a ground for specific health claims.

## ■ Functions associated with insulin sensitivity

### 1) Lipotoxicity

The impairment of glucose utilisation by high circulating concentrations of non-esterified fatty acids (NEFA, also called free fatty acids, FFA) has long been recognised. The mechanisms whereby elevated fatty acid availability impairs insulin-stimulated glucose utilisation were put forward by Randle et al. 40 years ago [121]. This effect has been clearly demonstrated in humans in vivo by artificial elevation of plasma NEFA concentrations by infusion of a triacylglycerol (TG) emulsion with heparin [122, 123]. A consistent body of evidence points to this as an important mechanism for impairment of glucose utilisation in insulin resistance. The ability of insulin to suppress NEFA concentrations is impaired in many insulin resistant conditions (summarised in [124]) and in type 2 diabetes; for instance, plasma NEFA concentrations are consistently elevated during the 24-hour cycle compared with non-diabetic subjects [125]. More recently, attention has shifted away from circulating NEFA concentrations towards accumulation of TG within other tissues as a marker of insulin resistance. Accumulation of skeletal muscle TG is closely associated with insulin resistance [126, 127]. TG in the liver is also associated with insulin resistance [128–130]. The amounts of hepatic TG associated with insulin resistance are less than in typical ‘fatty liver’ disease and this condition has become known as non-alcoholic steatohepatitis (NASH). Fat can also accumulate in the pancreas. In obesity, the pancreas often contains many adipocytes. More specifically, it seems that accumulation of TG within the insulin-secreting islet  $\beta$ -cells is associated (in the long term) with impairment of insulin secretion, and therefore may contribute to risk of development of type 2 diabetes.

It is difficult to be certain whether increased tissue TG content causes insulin resistance, or vice versa, but in animal models in which the TG content of tissues is manipulated (e. g. by adenoviral expression of leptin) there is a strong correlation between insulin resistance and the TG content of muscle, liver and pancreas over a wide range of tissue TG concentrations [131]. Muscle TG is also related to circulating NEFA concentrations, and they both increase in parallel with insulin resistance [132]. Therefore nutritional or pharmaceutical treatments that reduce lipid availability to skeletal muscle and other tissues may have some benefit in improving glucose utilisation.

Nicotinic acid is one component of the B-vitamin niacin. It has been used for decades as a drug treatment for hyperlipidaemia. One of its specific actions is to suppress NEFA release from adipose tissue. Some studies using a nicotinic acid analogue, acipimox, show improved glycaemic control in type 2 diabetes [133]. Niacin

is widely available but its effects on glucose utilisation in typical dietary amounts have not been assessed.

Other dietary components that reduce NEFA concentrations, apart from promoting insulin secretion, have not been elucidated. Some dietary components may have adverse effects by raising plasma NEFA concentrations. Diets with unphysiologically large amounts of sucrose may lead to elevated NEFA concentrations, particularly in the post-prandial period when glucose utilisation should be stimulated [134]. Feeding rats a high-fructose diet induces insulin resistance but evidence that this occurs in humans with typical dietary amounts is not strong.

### 2) Body fat composition

**Relevance in relation to diseases.** Experimental studies in animal models and epidemiological and observational studies in humans indicate that the amount and type of dietary fat may be of importance for the development of insulin resistance and diabetes mellitus [135, 136]. An important part of this relationship is probably mediated by the strong association between (abdominal) obesity and insulin resistance/diabetes [137, 138]. A high proportion of dietary fat, and possibly also a low proportion of some polyunsaturated fatty acids, seem to contribute to a higher incidence of obesity. However, also after adjustment for body weight or body mass index there remains in certain, but not in all, studies a significant and in some cases independent relationship between dietary fat composition on the one hand and prevalence/incidence of insulin resistance or diabetes mellitus on the other [136]. The most consistent finding from epidemiological studies seems to be a protective effect of a high proportion of polyunsaturated (vegetable) fatty acids in the diet but also indications of a direct association between high proportions of saturated and trans fatty acids in the diet and development of insulin resistance and type 2 diabetes mellitus [139, 140].

**Relationships between diet, dietary markers (fatty acid composition in body tissues) and function/disease endpoints (insulin sensitivity/type 2 diabetes).** In experimental studies in animals insulin sensitivity has been shown to be related to the fatty acid composition of the skeletal muscle membranes, which is dependent on the fatty acid composition of the diet [141].

In humans it has been repeatedly shown that the fatty acid composition of body tissues (serum lipids, phospholipids in erythrocyte membranes, triglycerides in adipose tissue, phospholipids in skeletal muscle membranes) at least partly reflects the dietary fat composition, and changes of the fatty acid composition reflect changes of dietary fat composition [142–145]. The fatty acid pattern mirrors the average composition of the dietary fat during the preceding weeks (serum lipids, ery-

throcytes), months (possibly skeletal muscle) and many months to years (adipose tissue). The strength of the relationships between the proportion of a specific fatty acid in the diet and that in the body tissues varies much between different fatty acids and for different tissues [141]. The proportions of many fatty acids in the body are mainly determined by the rate of endogenous synthesis and/or metabolism. The essential fatty acids cannot be synthesised in the body. There are strong relationships between long chain essential fatty acids in the diet, as well as between some of their metabolites (e. g. the so called fish fatty acids eicosapentaenoic acid and docosahexaenoic acid) and the corresponding fatty acids in the tissues, respectively. The relationships between amounts of saturated fatty acids in the habitual diet and in body tissues are weaker and for some fatty acids virtually absent (e. g. stearic acid) while somewhat stronger relationships are seen for myristic and palmitic acid. The relationship between the proportion of oleic acid in the diet and in body tissue is very weak or absent. This means that certain, but not all, fatty acids in the body tissues potentially can be used as markers for intake of dietary fat. Two saturated fatty acids with an uneven number of carbon atoms (pentadecaenoic acid and heptadecaenoic acid) can be used as specific markers for intake of dairy fat [146].

There are significant, and rather strong, relationships between the proportions of some fatty acids in the body tissues and insulin sensitivity. Insulin resistance, and insulin resistant states, are associated with a fatty acid pattern in plasma characterised by an increased proportion of palmitic acid and a low proportion of linoleic acid with a distribution of other fatty acids indicating an increased activity of delta-9 and delta-6 desaturase. These changes are probably to a large extent related to the type of fat in the diet [147]. Also, significant relationships between the fatty acid composition of adipose tissue triglycerides and skeletal muscle phospholipids, respectively, and insulin sensitivity have been demonstrated.

Changes in dietary fatty acids, and consequently of the fatty acid composition of body tissues, may influence insulin sensitivity through many mechanisms, e. g. by affecting membrane lipid composition, metabolism, signal-transduction pathways, and by direct control of gene expression. It has to be remembered, however, that the fatty acid composition may also be influenced by other factors such as genetic predisposition and early pre- or perinatal environmental influence.

Epidemiological and observational studies indicate that the dietary fat quality may influence insulin sensitivity and the risk to develop type 2 diabetes [148, 149]. An association between dietary fat quality and insulin sensitivity is also supported by the significant relationships between insulin sensitivity and the fatty acid composition of body tissues, which are dependent on the fatty acid composition of the diet. To prove a causal re-

lationship between dietary fat composition and insulin sensitivity it has to be demonstrated that a change of dietary fat quality only can affect insulin sensitivity, as earlier shown in animal models. Recent studies seem to supply these data [148–150] indicating that substitution of unsaturated for saturated fatty acids in the diet improves insulin sensitivity also in humans.

**Fatty acid composition as a biomarker for insulin sensitivity (function) or diabetes (risk) after dietary intervention.** The only lipid fraction where fatty acids could be studied and used as biomarkers is probably the lipids in plasma or serum (or in erythrocyte membranes). Skeletal muscle biopsies are not possible to use as a routine tool (technically demanding and potentially painful) and the very long turn over time of fatty acids in adipose tissue makes it unsuitable to study effects of dietary changes during most controlled trials.

The most consistent data with regard to a relationship between fatty acid composition in body tissues and insulin sensitivity, and incidence of diabetes, are available for serum or plasma lipid fatty acid composition [150]. The proportions of fatty acids are relatively easy to measure with good precision and rather low variability. In some studies the fatty acid composition was significantly related to insulin sensitivity or to diabetes independent of other factors. There are plausible mechanisms behind the relationships and evidence for causality between diet and insulin sensitivity from intervention studies. A problem is, however, what to measure. Most of the fatty acids are closely interrelated. Insulin sensitivity is characterised by a fatty acid “pattern” rather than by the proportion of one or a few fatty acids. The relationships are not very strong and not always consistent. The evidence that a change of fatty acid pattern is a direct marker of a change of insulin sensitivity is lacking (see below).

During intervention studies, where changes of dietary fat quality have caused changes of insulin sensitivity, there have concomitantly been changes of the plasma lipid fatty acid composition mirroring the change of the dietary fatty acid pattern [148–150]. The changes of plasma lipid fatty acid composition have been in a direction which earlier, in observational studies, has been associated with a better insulin sensitivity. Up to now, however, there are no trial data showing that changes of the proportions of specific fatty acids in the plasma lipids of the participants were significantly related to changes of insulin sensitivity. This obviously limits the usefulness of measurement of plasma lipid fatty acids as markers for insulin sensitivity.

**Grounds for possible health claims.** From experimental and observational studies in humans it is reasonable to conclude that changes of dietary fat composition could affect insulin sensitivity or influence the risk to develop



type 2 diabetes [139, 140]. In the perspective of health claims for (functional) foods or food components it is conceivable that dietary induced changes of insulin sensitivity could form the basis for claims – both enhanced function claims if insulin sensitivity is improved, and reduced risk claims if insulin resistance is defined as an intermediate end-point for the development of the metabolic syndrome/diabetes mellitus and, possibly, coronary heart disease (Fig. 2). The fatty acid composition of body tissues, which at least partly reflects the dietary fat composition and is related to insulin sensitivity (see below), might potentially be regarded as a marker of a function relevant for the development of insulin resistance and possibly also for the risks of the endpoints diabetes mellitus and coronary heart disease.

### 3) Oxidative stress

Insulin resistance may cause elevated plasma free radical concentrations, which, in turn, might be responsible for a deterioration of insulin action, with hyperglycaemia being a contributory factor [151, 152]. In adipocytes cultured *in vitro*, insulin increases the production of hydrogen peroxide, which may mimic insulin action, thus creating a vicious circle between hyperinsulinaemia and free radicals production. Prospective epidemiological studies demonstrate that high serum vitamin E levels, a marker of antioxidant activity, are associated with decreased risk of type 2 diabetes [153]. In the same line is the observation that plasma concentrations of lipid hydroperoxides are higher in healthy, insulin resistant volunteers as compared to insulin-sensitive ones, while plasma concentrations of vitamin E are significantly lower [151].

Increased blood glucose levels, by stimulating the

production of reactive oxygen species, and by depleting the customary antioxidant defences may further contribute to enhance oxidative stress [154]. Increased intracellular production of reactive oxygen species through hyperglycaemia may result from protein kinase C activation of nicotinamide adenine dinucleotide phosphate oxidase. On the other hand, the increased production of sorbitol following hyperglycaemia causes an intra-cellular depletion of reduced coenzyme NADPH, thereby causing impaired regeneration of reduced glutathione [155].

Oxidative stress might also be involved, in turn, in glycaemic regulation, even if this relationship is still a matter of debate [156].

Antioxidants such as vitamin E and vitamin C have been shown to improve insulin action in healthy, elderly, and non insulin-dependent diabetic subjects [156, 157]. Parenteral administration of anti-oxidant alpha-lipoic acid significantly enhances insulin-stimulated glucose transport system and both oxidative and non-oxidative pathways of glucose metabolism in insulin-resistant rat skeletal muscle [158]. However, some studies, such as the one reported in the Insulin Resistance and Atherosclerosis Study, do not support the hypothesis of improved insulin sensitivity through antioxidant vitamins supplementation in humans [98].

**Markers of oxidative stress related to insulin resistance (Table 6).** Damage caused by oxidation, or the risk thereof, can be assessed by evaluating the overall antioxidant defence or by measuring oxidation products of lipids, proteins, and DNA. Available markers of oxidative stress have been reviewed by an EU concerted action (EUROFEDA) which has produced a consensus paper which provides detailed evaluation of the validity of the

**Table 6** Characteristics of markers related to oxidative stress, inflammation and vascular function associated with impaired insulin sensitivity

Marker	Methodological characteristics	Biological characteristics (relevance to function)	Comments
<b>Pro-antioxidant status assessment</b>			
Cu-Zn superoxide dismutase	spectrophotometric detection		increased in diabetes
S/E TBARS	spectrophotometric detection	non-specific marker of peroxidation	increased in diabetes
S/U 8-iso-PGF F2 isoprostane	HPLC	oxidative stress marker with vaso-active properties	increased in diabetes
U 8-oxo-7,8-dihydroguanine	spectrophotometric/HPLC	DNA damage marker	
<b>Inflammation</b>			
P C-reactive protein	RIA method	early and sensitive criteria	increased in diabetes
TNF- $\alpha$ /IL-6	ELISA	major origin: activated immune cells, also adipose tissue	increased in diabetes
<b>Vascular function</b>			
P cGMP	RIA	endothelium vasodilatation markers	decreased in diabetes
Endothelium dependent vasodilation	forearm blood flow measurement ultrasound measurement of brachial artery diameter	Specifically due to NO activity endothelium dependent dilation	invasive non-invasive
Adipose tissue blood flow	<sup>133</sup> Xe washout ethanol escape/urea discovery	microdialysis	

different methods employed for their measurement [159]. This subject has been also covered in PASSCLAIM by ITG A in relation with the risk of cardiovascular diseases [160].

Several markers of oxidative stress have been employed in human studies; among them, some have been found specifically associated with type 2 diabetes and insulin resistance. Some evidence is also available that in diabetic patients they are correlated with the degree of blood glucose control and that treatment with hypoglycaemic drugs is able to normalise them. The markers of oxidative stress more often utilised in individuals with diabetes or other conditions associated with insulin resistance are peroxidation products appearing in the serum (such as thiobarbituric reactive substances TBARs), or in the urine (such as dicarboxylic acids); however many concerns on their specificity and validity have been raised. More reliable are the measurements of plasma lipid peroxides and urinary isoprostanes. In particular, F<sub>2</sub>-isoprostane (15-F<sub>2t</sub>-IsoP) is proposed as a marker of oxidative stress associated with stimulation of smooth muscle cells, and thus with vaso-active properties [161]. A paradoxical increase in detoxification enzymes (such as Cu-Zn SOD activity) may also be observed in connection with deranged glucose metabolism [162]. DNA damage may be assessed by measuring 8-oxo-7,8-dihydroguanine in lymphocytes or urine. All these markers are also proposed to assess the putative involvement of nutrients on oxidative stress and possible consequences on lipoprotein oxidation and atherogenesis (see ITG-A paper) [160].

In order to support claims on the ability of a functional food to protect against oxidative damage associated with hyperglycaemia and/or impaired insulin activity, several validated and biologically relevant markers should be used.

#### 4) Inflammation

The association between the presence of markers of inflammation, on the one hand, and abnormalities of lipid and carbohydrate metabolism, obesity and atherosclerosis, on the other hand, has been shown in many studies. Inflammation probably causes impaired insulin sensitivity, hyperlipidaemia, and atherosclerosis both directly, and through induced hyperglycaemia [163].

One of the possible links between impaired insulin sensitivity and inflammation is represented by Tumour Necrosis Factor alpha (TNF  $\alpha$ ), as illustrated in both animal and human studies. High serum TNF  $\alpha$  concentrations – generated through TNF injection in animals, or through lipopolysaccharides injection – reduce insulin sensitivity by stimulating stress hormone production, and by induction of suppressor of cytokine signalling protein 3 [164]. Despite the fact that TNF- $\alpha$  on its own promotes insulin resistance in isolated cell systems, its

role as a modulator of insulin sensitivity *in vivo* remains uncertain (its neutralisation by antibodies is unable to increase insulin sensitivity in humans) [164]. However, a decrease in TNF- $\alpha$  ameliorates vascular function in type 2 diabetic patients.

Positive correlations between glycosylated haemoglobin and markers of oxidative stress in polymorphonuclear cells have been shown in population studies from Japan in which similar associations between C reactive protein and blood mononuclear cell oxidative stress and between blood pressure and polymorphonuclear oxidative stress were shown [165]. A link exists between inflammation and oxidative stress on the one hand and vascular function on the other hand [166].

**Markers of inflammation (Table 6).** Population studies have shown that markers of inflammation correlate with indices of insulin resistance, and the metabolic syndrome. For example: plasma C-reactive protein (CRP), interleukin 6 (IL-6), TNF- $\alpha$ , and TNF-R are elevated in the obese and type 2 diabetic patients; similarly increased in these conditions is plasminogen activator inhibitor (PAI-1), regulated through the key proinflammatory product NF- $\kappa$ B [163, 167, 168]. A link was also established between an increase in CRP and insulin resistance (not with insulin secretion) in 396 subjects at high risk for type 2 diabetes [169]. Serum levels of inflammatory markers, in particular highly sensitive CRP, have been found to be strong predictors of increased risk for type 2 diabetes and cardiovascular disease, independent of other traditional risk factors [170].

#### 5) Vascular function

The initiation and progression of atherosclerosis in the presence of insulin-resistance may be linked with impaired endothelial function that can be detected at the earliest stages of obesity and insulin resistance [171]. On the other hand, impairments of vascular function also participate to the development of insulin resistance in several organs.

Endothelial cell integrity, and responsiveness to hormones or reactive substances, leads the regulation of blood flow in all organs, and may have consequences on oxygen and substrates – namely glucose – cellular uptake. An increase of muscle blood flow can independently induce a higher glucose uptake during infusion of insulin; blood flow has been proposed to be rate limiting for insulin-stimulated glucose uptake, namely in healthy subjects [172]. There is accumulating evidence to suggest that insulin resistance of muscle *in vivo* in term of glucose uptake could be partly due to impaired insulin-mediated capillary recruitment [173]. In human adipose tissue, there is a close relationship between insulin sensitivity and regulation of post-prandial blood flow, independent of adiposity; impaired regulation of adipose

tissue blood flow could thus be part of the insulin resistance syndrome [174].

In addition, vascular damage, by promoting atherosclerosis, is a major cause of morbidity and mortality in diabetes mellitus [175]. Changes in endothelial cellular structure, function and regulation of transcription factors is clearly linked to oxidative stress.

Excess production of superoxide may lead to inappropriate activation of intra-cellular proteins and enzymes, which contribute to the pathogenesis of atherosclerosis. Possible mechanisms include the activation of protein kinases by reactive oxygen species, and stimulation of collagen accumulation by superoxide, which, additionally, may trigger the release of platelet derived growth factor and platelet aggregation.

It has also been shown that matrix metallo-proteinases are activated by hyperglycaemia, in particular increased oxidant activity increases the transcription of MMP-9 in vascular endothelial cells. This enzyme is considered to have a role in plaque rupture.

#### **NO and serum lipids as mediators of vascular alterations.**

Inactivation of nitric oxide impairs endothelial dependent vascular relaxation.

Endothelial derived nitric oxide (NO) has an important dual role. On one hand this free radical has a positive function, by mediating vasorelaxation through the activation of soluble guanylate cyclase thereby stimulating the production of cGMP. However, it has a potential harmful effect by triggering inflammation. Interaction of superoxide and other ROS with NO depletes NO activity and impairs vasodilatation responses [176].

ROS react with NO to produce the peroxynitrite anion, which induces tissue injury by causing protein nitration on aromatic amino acid residue, and the production of highly reactive hydroxyl anions. On the other hand, higher levels of circulating nitrite/nitrate – metabolites of monocytes and macrophages derived – NO can be found in serum of diabetic patients; increased NO production may in turn induce immune responses and inflammatory reactions that cause cell damage.

The abnormal increase in serum non esterified fatty acids (NEFA) in insulin resistant subjects, has a modulating (decrease) effect on NO-synthase activity – through a PI3 kinase mediated mechanism – and thus participates to endothelial dysfunction [177, 178]. Some data suggest that NEFA-induced vascular oxidative stress (increase in ROS generation in endothelial and vascular smooth muscle cell) could contribute to endothelial dysfunction in insulin-resistant patients [178, 179].

An independent and cumulative effect of post-prandial hypertriglyceridaemia and hyperglycaemia on endothelial function was shown in diabetic patients, suggesting oxidative stress as a common mediator of such an effect [180].

In an intervention study, vitamin C infusion improved the vasodilatory response to acetylcholine in obese subjects exhibiting insulin resistance, a phenomenon which might be related to its anti-oxidant effect [181]. A similar treatment also decreased steady-state plasma glucose in 22 patients with coronary spastic angina [182]. The beneficial effects of vitamin C on vascular function could be mediated through an increased intracellular concentration of tetrahydrobiopterin, an essential co-factor required for enzymatic activity of NO synthase [183]. A prospective study in non-diabetic individuals, provided evidence that vitamin E supplementation prevents the induction of protein kinase C activity in the aorta exposed to hyperglycaemia, preserves endothelial function, and reduces vascular oxidative stress. This corroborates experiments in rats showing that supplementation with vitamin E and Se preserves aortic relaxation in response to acetylcholine and calcium [184]. Such examples clearly illustrate the narrow relationship between oxidative stress and vascular function in humans.

**Markers of vascular function (Table 6).** The most currently used method to measure vascular reactivity is to measure dilation of the brachial artery, after a period of forearm ischaemia. Endothelium dependent vasodilation may also be assessed by measuring the increase of forearm blood flow after infusion with acetylcholine; it is negatively correlated with BMI, waist-to-hip ratio, fasting insulin, and insulin resistance estimated by the homeostasis model assessment (HOMA-IR) [181]. A study with 44 patients with type 2 diabetes lead to the proposition that (plasma) cGMP might be a marker of endothelium-dependent vasodilatation in diabetic patients, which is related to hyperglycaemia, and is thus controlled by sulfonylurea, biguanides and/or acarbose [152].

Adipose tissue blood flow may also be estimated by <sup>133</sup>Xe washout techniques, which seems to be more responsive to physiological changes than ethanol escape or urea recovery by microdialysis [174].

#### **■ Mechanisms involved in the regulation of insulin sensitivity**

##### **Hormones and other messengers**

Several endocrine factors have been shown to interfere with insulin action or glucose metabolism, and, therefore, have been suggested to play a role in the pathogenesis of the metabolic syndrome. As the effects of hormones on obesity and fat distribution have been covered in the section on obesity, this section focuses on the effects on insulin action and glucose metabolism.

### – Glucocorticoids

Glucocorticoids exert acute insulin-antagonistic effects on glucose metabolism, independent of their effects on body weight and body composition. These include both impairment of insulin-mediated glucose disposal and stimulation of hepatic glucose production [185, 186]. This observation, together with the insulin resistance of Cushing disease, has led to the hypothesis that cortisol is also involved in insulin resistance in other pathophysiological conditions, e. g. central obesity and metabolic syndrome [187–189]. As these conditions of insulin resistance are not unavoidably associated with elevated circulating cortisol levels [187], recent studies focus on the role of locally produced cortisol in their pathogenesis; glucocorticoids can also be produced locally from inactive 11-keto forms (e. g. cortisone) through the enzyme 11 beta-hydroxysteroid dehydrogenase type 1 (11 $\beta$ -HSD1).

Indeed, the metabolic syndrome seems to be associated with tissue specificity in 11 $\beta$ HSD1 activity, since this enzyme shows decreased activity in liver and enhanced activity in adipose tissue [189, 190]. Thus, locally increased levels of cortisol in adipose tissue may be involved in the pathogenesis of the metabolic syndrome. This view is further supported by the development of a transgenic mouse model, showing that selective overexpression of 11 $\alpha$ -HSD1 in adipose tissue results in visceral obesity, marked insulin resistance and hyperlipidaemia [191].

### – Sex steroids

Although sex steroids have been associated with insulin resistance and the metabolic syndrome [192, 193], it is at present unclear to what extent this association is mediated by the effects of these hormones on adiposity.

In (both pre- and postmenopausal) women, increased androgenicity, as assessed by increased testosterone and decreased sex hormones binding globulin (SHBG), is strongly associated with insulin resistance, but also with an unfavourable body fat distribution [194]. In men, on the other hand, longitudinal epidemiological data demonstrate that relatively low testosterone levels are associated with insulin resistance. Again, this relationship is highly dependent on the magnitude of adiposity [195].

### – Growth hormone

The relationship between growth hormone (GH) and glucose metabolism is complex. In general, growth hormone counteracts the effects of insulin on glucose utilisation by peripheral tissues as well as on hepatic glucose production [195]. On the other hand, GH is a potent stimulator of the release of insulin-like growth factor I

(IGF-I), which exerts potent insulin-like activities on glucose metabolism [196]. Intervention studies on the effects of GH treatment on glucose metabolism in GH deficient patients have yielded controversial results. Thus, both an improvement [197, 198] and a deterioration of insulin sensitivity and glucose metabolism have been observed [199].

The metabolic syndrome is associated with low levels of growth hormone (GH). In moderately obese, middle-aged men, nine months of GH treatment improved insulin sensitivity [197]. This was associated with reduced total body fat and resulted in a specific and marked decrease in both abdominal subcutaneous and visceral adipose tissue. Therefore, it cannot be excluded that the effects of the treatment on glucose homeostasis were mediated through alterations in the degree and localisation of adiposity.

### – Glucagon

Glucagon exerts its main insulin-antagonistic effect on the liver by increasing glycogenolysis and gluconeogenesis, with less significant effects on peripheral glucose utilisation [200, 201]. Glucagon levels may be increased in patients with type 1 and type 2 diabetes, but otherwise hyperglucagonaemia is hardly found [202]. Therefore, it is unlikely that glucagon plays a significant causal role in the insulin resistance of the metabolic syndrome.

### – Catecholamines

The catecholamines epinephrine and norepinephrine are also classified as counterregulatory hormones, i. e. they possess hyperglycaemic properties. Both epinephrine and norepinephrine have been shown to directly stimulate hepatic glucose production [201]. Also, epinephrine has been shown to limit glucose utilisation by muscle and adipose tissue [185].

In the metabolic syndrome, a positive relationship between sympathetic nervous system activity (an important source of circulating norepinephrine) and plasma insulin levels has been observed [203]. Another abnormality often associated with the metabolic syndrome is the reduction of plasma epinephrine concentrations, both at rest and in response to a stimulus such as physical activity [204]. Still, the patho-physiological role of catecholamines in modulating the insulin resistance associated with the metabolic syndrome needs to be established.

### – Adipocyte hormones

White adipose tissue secretes a number of peptide hormones, which are thought to be involved in energy metabolism. *Leptin* has been shown to improve insulin action in laboratory animals and in human muscle cells



[205]. Plasma leptin levels are elevated in human obesity and in the presence of the metabolic syndrome, but the correlation between leptin levels and insulin resistance – independent of fat mass – is weak [206]. Therefore, the likelihood that leptin is involved in the pathogenesis of the metabolic syndrome is rather low. The recently discovered *resistin* has been indicated as a potential link between obesity and diabetes [207]. Initial studies in rodents suggested that resistin is upregulated in obesity and may be involved in the development of insulin resistance. Also in human adipose tissue resistin is detectable, albeit at a very low level [208]. Studies on the expression of resistin in normal, insulin-resistant or type 2 diabetic humans have given inconsistent results [209]. Plasma concentrations of *adiponectin* are reduced in obese animals and humans, and in patients with type 2 diabetes mellitus [208]. In vitro and animals studies show that adiponectin stimulates fatty acid oxidation, decreases plasma triglycerides, and improves glucose metabolism by increasing insulin sensitivity [210]. The role of resistin as well as adiponectin in the regulation of insulin action in humans remains to be established.

#### – Peroxisome proliferator activated receptors (PPARs)

PPARs are a class of nuclear receptor/transcription factors. There are three isoforms: PPAR $\alpha$ , expressed mainly in liver and muscle, PPAR $\beta$  (also called PPAR $\delta$ ), expressed ubiquitously, and PPAR $\gamma$ , expressed mainly in adipose tissue [211]. The name PPAR comes from their role in the response to exogenous toxins and drugs that cause peroxisome proliferation, but their probable physiological role is to sense excess fatty acids and respond to them. It is believed that the endogenous ligands are fatty acids or, more probably, fatty acid derivatives such as prostaglandins. Activation of PPAR $\alpha$  upregulates fatty acid oxidation and also affects the synthesis of apolipoproteins in a favourable way (increasing HDL-cholesterol concentrations). By this mechanism fibrates, a class of hypolipidaemic drugs, act to improve dyslipidaemia, which is characteristically observed in association with insulin resistance. Activation of PPAR $\gamma$  induces differentiation of new fat cells and stimulates the pathways of fat storage. The thiazolidinedione (or glitazone) drugs activate PPAR $\gamma$  to improve insulin sensitivity, possibly by removing excess fatty acids from the circulation. Thus, both PPAR $\alpha$  and PPAR $\gamma$  could be seen as increasing disposal of excess fatty acids. PPAR $\delta$  is less well understood but recent evidence with specific pharmacological agonists also suggests beneficial effects on HDL-cholesterol [212].

A common polymorphism in the gene encoding for PPAR $\gamma$  (Pro12Ala) has been observed in humans. In meta-analyses the rarer allele (Ala) is associated with decreased risk of developing diabetes but increased risk of obesity, particularly at young ages [213, 214]. Al-

though apparently paradoxical, this could be understood by hypothesising that the presence of the rare allele caused improved sequestration of fatty acids in adipose tissue, thus leading to overweight; however, by removing them from the circulation, less free fatty acids were available for oxidation in muscles and liver, thus promoting glucose utilisation. Because the natural ligands are fatty acids or their derivatives, it is possible that specific dietary components could be found to affect the PPAR system, although none has been identified as yet.

#### Trace elements and minerals

##### – Chromium

Chromium is considered by many as essential micronutrient for humans. The best evidence for this has been the resolution of impaired glucose tolerance in response to chromium supplementation in patients on long-term parenteral nutrition [215, 216]. Similar results have been obtained in laboratory animals: rats fed a chromium deficient diet develop insulin resistance and glucose intolerance, which can be reversed by subsequent chromium supplementation [217].

It is argued that chromium is an “insulin-sensitising” agent in that it facilitates the association of insulin with its receptor. The postulated mechanism involves an oligopeptide, chromodulin, which binds chromic ions in response to an insulin stimulated chromic ion flux, and which can then bind to an insulin receptor thereby activating tyrosine kinase [218].

Studies on glucose metabolism and insulin resistance in humans both with and without diabetes have been inconsistent in demonstrating the benefits of chromium (either provided as a chloride, picolinate, nicotinate, or as chromium-enriched yeast). Support for the use of chromium supplements derives from a study in 155 diabetic subjects in China, showing lowered plasma glucose and insulin concentrations as well as reduced HbA1c after supplementation with chromium picolinate [219]. In contrast, a recent meta-analysis of 15 clinical trials performed in Western countries on the effect of chromium on glucose and insulin responses did not reveal any effect in healthy subjects, while the effects in diabetics were inconclusive [220].

Moreover, the safety of chromium complexes has become a matter of discussion, since several *in vitro* studies report mutagenic and pro-oxidative activities of chromium complexes (in particular chromium picolinate) [221, 222]. It has to be underlined that there is no evidence of a relationship between chromium intake with the habitual diet and insulin sensitivity at the population level.

### – Vanadium

Many *in vitro* and *in vivo* studies have shown that vanadium has insulin-like effects in liver, skeletal muscle and adipose tissue [223]. In rodent models of obesity and type 2 diabetes mellitus, vanadium improves glucose metabolism by restoring both hepatic and skeletal muscle insulin sensitivity [223, 224]. A few clinical trials using vanadyl sulfate as a source (vanadyl appears to be the active intracellular form of vanadium) have shown beneficial effects of the trace element on insulin sensitivity in type 2 diabetics [224, 225]. However, long-term safety of vanadium supplementation at the effective dosages remains to be assessed. Moreover, also for this trace element there is no evidence of a relationship between its intake with the habitual diet and glucose/insulin metabolism at the population level.

### – Magnesium

Magnesium deficiency has been shown to occur in 25–38% of patients with diabetes mellitus [226]. Moreover, plasma magnesium levels seem to be inversely correlated to insulin sensitivity. Accordingly, epidemiological studies show that high daily magnesium intake is predictive of a lower incidence of type 2 diabetes.

Magnesium is a cofactor in various enzyme pathways involved in glucose metabolism, and it is suggested to improve insulin action through restoration of the impaired tyrosine-kinase activity at the insulin receptor level. This has been confirmed in human trials, showing that chronic magnesium administration improves insulin action in type 2 diabetic patients [227, 228]. In another trial, magnesium supplementation reduced blood concentrations of fructosamine, a short-term marker of glycaemic control [226]. Still, since other studies did not report improvement of glycaemic control after magnesium supplementation [229, 230], the evidence for its efficacy in diabetes remains inconclusive. Even more unproven are its effects in the non-diabetic population

### – Zinc

Zinc is suggested to be an essential trace element for adequate glucose homeostasis. Thus, rats fed a zinc-depleted diet showed impaired glucose metabolism and insulin resistance when compared to rats fed a zinc adequate diet [231]. The mechanisms by which zinc influences glucose metabolism are not clear. The element is involved in the presecretory storage of insulin hexamers in the pancreatic beta cells; it may be involved in the association between insulin and its receptor (i. e. the zinc sandwich); and *in vitro* studies indicate that zinc facilitates tyrosine kinase phosphorylation in insulin signal transduction [232].

Diabetes is associated with a 20–40% decrease in

serum zinc levels, which may be the consequence of hyperzincuria and/or decreased gastrointestinal absorption [233] or they could reflect the customary hypozincemic response to stress. It appears the hyperzincuria, at least, is a result more of hyperglycaemia than of any specific effect of endogenous or exogenous insulin on the renal tubule. Still, the resulting zinc deficiency may be involved in a further deterioration of glucose metabolism, caused either by a further decline in insulin action, or by provoking adequate insulin secretion [233] or they could reflect tissue catabolism. However, conclusive human data on the effects of zinc supplementation on glucose homeostasis is lacking.

### – Calcium

Although the mechanism of action is poorly understood, high levels of cytosolic free calcium may impair insulin action in target cells (i. e. skeletal muscle and adipose tissue) [234, 235]. Elevated cytosolic free calcium levels have been observed in patients with obesity and non-insulin-dependent diabetes mellitus and in some patients with hypertension. One month of therapy with the calcium-channel blocker, nitrendipine, reduced plasma insulin concentrations and restored adipocyte insulin-mediated 2-deoxyglucose uptake in obese hypertensive subjects [234].

Recently, attention has been focused on the apparent association between calcium and adiposity [236, 237]. Epidemiological data revealed an inverse relationship between calcium intake on the one hand and body weight or the prevalence of obesity on the other. Various human and animal trials suggest that standard to high calcium diets, with dairy products being the preferred source of calcium, may be helpful in reducing body fat mass and, consequently, the incidence of obesity, when compared to low calcium diets [236, 237]. It is suggested that dietary calcium lowers 1.25-dihydroxyvitamin D, thereby inhibiting calcium influx in adipocytes. The resulting decrease in intracellular calcium levels facilitates lipolysis and concomitantly suppresses lipogenesis, hence, counteracts adiposity [237].

However, the evidence available on the inverse relationship between calcium intake and insulin sensitivity in humans is far from being conclusive.

### – Other elements

Several other trace elements have been associated with blood glucose regulation and insulin resistance. In rats, very low dietary *copper* results in hyperglycaemia and low concentrations of circulating insulin, as well as pancreatic atrophy [238]; insulin release from the pancreas is inhibited, which can also be induced by high dietary *iron* (low dietary copper causes high hepatic and pancreatic iron) [238]. *Lithium* stimulates glycogen syn-

these activity in rat skeletal muscle [239]; however, human studies on the effects of lithium on glucose homeostasis have provided conflicting results. Tungstate has shown to improve glycaemic control in streptozotocin diabetic rats as well as in diabetic Zucker rats; this is at least partially due to a stimulation of hepatic glycogen synthesis [240, 241]. However, the possible anti-diabetic effect of tungstate has not yet been verified in humans. *Selenium* has been shown to mediate insulin-like actions, including stimulation of glucose uptake [242]. In streptozotocin-diabetic rats, selenium administration significantly lowered blood glucose levels, but so far, human data are lacking.

### ■ Future research needs and conclusions

The link between impaired insulin sensitivity, evaluated by validated methodologies, and the incidence of type 2 diabetes and the metabolic syndrome is clearly established. Therefore functional foods able to improve validated markers of insulin sensitivity may possibly apply for a type A health claim. Among the other functions associated with insulin sensitivity plasma NEFA levels have, so far, the strongest evidence; however more intervention trials of sufficiently long duration are needed before a clear cause-effect relationship is established.

According to the available evidence, probably it is not yet possible to use for health claims changes of fatty acid composition in human body tissues (serum lipids) induced by (functional) foods. Dietary intervention studies are needed to show direct and significant relationships between individual changes of function/disease risk (insulin sensitivity) and markers of dietary intake (fatty acid composition, preferably the proportion of a single fatty acid or the ratio between some of them), thus allowing an accurate prediction of the magnitude of the improvement of insulin sensitivity on the basis of the observed change of the body fatty acid profile induced by dietary manipulations. Studies on a molecular level to increase our knowledge about the mechanisms underlying the effects of dietary fat on insulin sensitivity would be very valuable. Also, documentation of changes of incidence of endpoints (diabetes, coronary heart disease) related to specific changes of insulin sensitivity is needed.

All the markers of oxidative stress – some of them have been assessed by ITG-A group – need to be validated further in the context of glucose homeostasis disturbances. Validation of the markers as clear indicators of risk of development of cardiovascular disease and/or inflammatory disease has not been performed so far. Some of these cytokines may come from sources other than macrophages, since TNF- $\alpha$  is over-expressed in adipose tissue of obese insulin-resistant humans. A

question remains open: is the source of cytokines (such as TNF- $\alpha$ , coming from either adipose tissue or immune cells) an important factor to consider when assessing the involvement in conditions associated with impaired insulin sensitivity?

As for previous sections, there is a need to evaluate the accuracy of measurements of vascular function and their sensitivity as predictors of disease risk.

Since oxidative stress, inflammation, and vascular reactivity seem inter-related, most nutrients apt to act on these targets exhibit anti-oxidant properties. Many data published until now refer to “relationship” or “correlation” between oxidative status/inflammation and/or vascular function, on one hand, and hyperglycaemia/insulin resistance and/or diabetes risk on the other hand. Fundamental research is needed in order to approach the biochemical mechanisms underlying these relations, in order to point out the key metabolic targets (and then to assess the more relevant marker(s) of the function).

However, also in relation to markers of inflammation, oxidative stress and vascular function diet based intervention studies are needed in order to establish a causative link between these functions and insulin sensitivity.

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## Diabetes mellitus

### ■ Background

Diabetes mellitus is a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia associated with impaired carbohydrate, fat and protein metabolism. These abnormalities are the consequences of either inadequate insulin secretion or impaired insulin action, or both. Diabetes can be diagnosed in three ways, and – in the absence of specific symptoms of the disease – each must be confirmed on a subsequent occasion: casual plasma glucose concentration  $\geq 200$  mg/dl (11.1 mmol/l), or fasting plasma glucose  $\geq 126$  mg/dl (7.0 mmol), or 2-hour plasma glucose  $\geq 200$  mg/dl (11.1 mmol/l) during an OGTT with 75 g of glucose.

Diabetes has been recently reclassified into four distinct types: type 1, type 2, gestational diabetes mellitus, other specific types [100, 243]. Type 1 diabetes is characterised by a cell-mediated autoimmune destruction of pancreatic  $\beta$ -cells that results in a partial or total inability to secrete insulin and in life-long need for insulin administration. Type 2 diabetes, until recently referred to as non-insulin-dependent diabetes, is characterised by disorders of insulin action and secretion, either feature being the predominant impairment. The specific aetiologies of this form of diabetes are yet to be found, but it is known that most of these patients are obese or have increased body fat predominantly in the abdominal re-

gion. Type 2 diabetes mellitus accounts for almost 85–95% of the cases of diabetes. Its estimated prevalence in the caucasian population is 4–6%: half of these are diagnosed, while a similar number remains unrecognised. The prevalence is known to be much higher in older people and in some ethnic communities (up to 40% of Pima Indians). WHO has predicted that the global prevalence of Type 2 diabetes will more than double, from 135 million in 1995 to 300 million in the next few years [244, 245].

Other specific types of diabetes are due to less common aetiologies – where the underlying defect may be genetic – and include diabetes secondary to pancreatic disease, endocrine disorders, infections, drug or chemical toxins. Gestational diabetes is defined as any degree of glucose intolerance with onset or first recognition during pregnancy [100, 243].

Long-term complications of diabetes include retinopathy (with potential loss of vision), nephropathy (leading to renal failure), peripheral neuropathy (with risk of foot ulcers), autonomic neuropathy (which contributes to erectile dysfunction and cardiac arrhythmia). However most of the morbidity and mortality associated with diabetes is attributable to macrovascular complications such as myocardial infarction, heart failure and acute stroke. Diabetes is associated with an age-adjusted cardiovascular mortality that is between 2 and 4 times that of the non-diabetic population, while life expectancy is reduced by 5 to 10 years in middle-aged patients with diabetes [246–249].

Two other categories of impaired glucose metabolism are Impaired Glucose Tolerance (IGT), diagnosed by the 2-h plasma glucose after OGTT > 140 and < 200 mg/dl (> 7.8 and < 11.1 mmol/l) with fasting value < 126 mg/dl (7.0 mmol/l), and Impaired Fasting Glucose (IFG), defined by fasting plasma glucose > 110 and < 126 mg/dl (> 6.1 and < 7.0 mmol/l). These two categories can be considered a metabolic state halfway between normal glucose homeostasis and diabetes and represent risk factors for the development of diabetes and/or cardiovascular disease [100, 243, 250, 251].

Most of the negative effects of diabetes on health are mediated by hyperglycaemia; as a matter of fact not only the symptoms but also the long-term complications of the disease are consequences of increased blood glucose levels [252, 253]. Therefore, the regulation of blood glucose levels represents a key function of the human body, which has to be preserved/restored in order to reduce the risk of complications and to avoid the deterioration of the quality of life. Blood glucose concentrations, in turn, are regulated by two associated functions: 1) glucose delivery into the bloodstream; 2) glucose utilisation by tissues [254].

The regulation of blood glucose levels has also a fundamental role in the prevention of diabetes since the development of this disease is always preceded by eleva-

tions of blood glucose levels either in the fasting state (IFG) or post-prandially (IGT) or both which, although below the diagnostic cut-off for diabetes, are still above the normal levels and clearly identify an increased risk for the development of this disease [251, 255]. Indeed all pharmacological and non-pharmacological interventions able to reduce glycaemia with whatever mechanism, tested so far in people with prediabetes, have proven effective in the prevention of type 2 diabetes [15, 16, 256].

Therefore the regulation of blood glucose levels is a function of paramount importance in the natural history of diabetes and its complications; a strong evidence, based not only on epidemiological studies but also on clinical intervention trials, identifies markers of this function as intermediate end-points both for diabetes (in people with a pre-diabetic condition) and for its long-term complications (in people with diabetes) [257, 258].

### ■ Potential claims for health effects on foods

Claims based on modifications of the target function:

- Improves blood glucose control
- Reduces plasma glucose levels
- Limits the post-prandial glucose rise
- Improves glucose tolerance
- Reduces the risk of type 2 diabetes
- Helps to reduce the risk of long-term complications of hyperglycaemia

Claims based on modifications of other relevant associated functions:

- Lowers glucose delivery in the bloodstream
- Increases glucose disposal
- Increases the first phase of insulin secretion

### ■ Target function: regulation of blood glucose levels

#### Biological meaning

Blood glucose levels are very stringently regulated to provide a steady fuel supply to the brain, which relies almost exclusively on glucose for its energy needs. In the brain, like in all non-insulin dependent tissues, glucose uptake is regulated by glucose concentrations in the arterial blood and, since the ability of neural cells to store glucose is minimal, optimal brain function depends on stable glucose levels, with a very narrow range of fluctuations in the post-prandial period as well as in the fasting state. A complex hormonal system, which involves insulin and other hormones with anti-insulin actions, is able to regulate glucose production and disposal keeping blood glucose concentrations between 4 and



8 mmol/L almost all day long, irrespective of the size and composition of the meals or the duration of the fasting period [254].

In the presence of insulin resistance and/or impaired insulin secretion (glucose stimulated early insulin response) blood glucose levels tend to rise. This is the effect of both inappropriately high liver glucose output and impaired glucose utilisation. In this situation both the amount of carbohydrate in the meal and the rate of digestion of carbohydrate foods play a relevant role in the regulation of the post-prandial blood glucose rise [38].

### Relevance in relation to diseases

An elevation of blood glucose concentrations, as seen in impaired fasting glucose, impaired glucose tolerance or diabetes, could in principle be caused by either an increase of glucose production or an impairment of glucose utilisation [254].

Hyperglycaemia is not only the cause of most symptoms affecting the quality of life of diabetic patients but represents also the cause of the long-term specific complications of diabetes (retinopathy, nephropathy, neuropathy) and also contributes to a large extent to the excess risk of cardiovascular disease associated with this condition. High plasma glucose concentrations – but below the diagnostic cut-off level for diabetes – may also predispose to ischaemic cardiovascular disease in the non diabetic population but, since this condition is often asymptomatic, it may go unrecognised and untreated for years while its ill effects on the arteries persist [259].

Various mechanisms have been proposed to link high plasma glucose concentrations with the occurrence of angiopathy at the level of small or large arteries. Glycosylation of proteins in the arterial wall is most probably one of the key mechanisms, since this process is able to modify many of the physico-chemical properties of these molecules, thus facilitating the occurrence of ischaemic processes: the arteries progressively lose their elasticity and increase the thickness of their wall which, eventually, leads to significant reduction of blood flow [260]. Other mechanisms involve accelerated arterial cell proliferation, alterations of lipid metabolism and haemostasis, endothelial dysfunction and many other abnormalities triggered by hyperglycaemia. Oxidative stress is an acknowledged pathogenetic mechanism in diabetic micro- and macrovascular complications. Increased uptake of glucose in the arterial wall stimulates protein kinase C activity, which activates peroxidase and the cyclo-oxygenase pathway to overproduce reactive oxygen species (ROS). ROS are also produced by circulating mononuclear cells of type 2 diabetic patients [154, 156, 167]. In turn, ROS may increase endothelial permeability, macrophage migration, and the secretion of endothelin, those events being involved in the develop-

ment of atherosclerosis [151]. Advanced glycation end-products created via hyperglycaemia bind to specific receptors (RAGE) and induce oxidative stress, increase endothelial adhesiveness for monocytes, and increase expression of vascular cell adhesion molecule type 1 [152]. Advanced glycation endproducts mediate their toxic effects by causing structural and functional changes in proteins, and by interacting with receptors, which increase the formation of intracellular oxidant signalling, and eventual activation of nuclear factor kappa beta [261]. Polymorphisms in RAGE might indicate the susceptibility of individuals to oxidant damage [262].

ROS are also associated with microvascular complication (kidney and nerve being well-known targets), but also lead to alteration of pancreatic  $\beta$ -cell and endothelial cell function (see also the previous section on markers of inflammation, oxidative stress and vascular function associated with impaired insulin sensitivity).

Chronically elevated plasma glucose levels impair both glucose utilisation and insulin secretion and therefore may contribute to self-perpetuation or, even, worsening of the metabolic abnormalities responsible for impaired glucose homeostasis. Elevations of blood glucose levels (above the normal range but below the diagnostic level for diabetes) either in the fasting state (IFG) or post-prandially (IGT) or both always precede the development of diabetes and may be considered as intermediate end-points for this condition [251, 255].

### Available evidence on markers and their links with diseases (Table 7)

The evidence that high plasma glucose levels induce microangiopathy and represent a risk factor for macroangiopathy is strong and consistent. Many epidemiological studies have shown that the degree of hyperglycaemia and its duration are strong predictors of future development of microangiopathy, in both type 1 and type 2 diabetic patients. Furthermore, in vitro and animal studies are also consistent in supporting high blood glucose as a pathogenetic factor for long-term diabetic complications. Microangiopathy represents a specific complication of diabetes, since it is never observed in individuals with slightly elevated blood glucose concentrations (IGT or IFG).

In earlier studies the only marker of blood glucose control in diabetic patients was fasting blood glucose concentration, while in subsequent researches more sophisticated parameters were employed. As a matter of fact the ability to predict the development of small vessel complications has been substantially improved by the measurement of glycated haemoglobin which represents an integrated marker of blood glucose control over the past 2–3 months; moreover the possibility to measure blood glucose levels by strips read on a reflectome-

**Table 7** Characteristics of markers related to blood glucose control

Biomarker	Methodological characteristics	Biological characteristics (relevance to function)	Comments
Fasting blood glucose	Low analytical error, good within-subject reproducibility	Good marker in non-diabetic individuals; it is not highly predictive of 24-h blood glucose control in diabetic patients, particularly type 1	Cheap, particularly appropriate for epidemiological studies
Post-prandial blood glucose	Difficult to standardize, large day-to-day variability in relation to meal composition	Seems more relevant for cardiovascular disease risk than the fasting value	Appropriate to evaluate post-prandial effects of foods
Daily blood glucose profile	Performed by strips and meters on repeated occasions, including fasting and post-prandial measurements; acceptable reproducibility	Particularly indicated for diabetic patients; good marker of diabetic complications (if performed on a regular basis) and risk of hypoglycemia	
Glycated haemoglobin	Various methods available; HPLC is the most reliable one – low analytical error within laboratory, poor comparability between laboratories	Gold standard; very reliable quantitative estimate of the risk of microvascular complications in diabetes; as marker of cardiovascular diseases it performs slightly worse, particularly in non-diabetics	Expensive
Fructosamine	Easy to measure; good reproducibility within and between laboratories	Covers a time span of only a few weeks; not particularly reliable as marker of long-term blood glucose control	
Oral Glucose Tolerance Test	Slightly invasive and time consuming; post OGTT blood glucose is less reproducible than fasting glucose within the same subject	Good marker of risk for diabetes and cardiovascular disease in non-diabetic individuals	Not indicated in diabetic patients

ter has allowed the evaluation of blood glucose concentrations directly by patients who can perform this measurement on several occasions in their usual daily life (blood glucose profile). Glycated haemoglobin and the daily blood glucose profile have been found elevated in association with an increased risk of microangiopathy in diabetic patients both cross-sectionally and prospectively [253]. Intervention studies have clearly shown that by improving blood glucose control with a more intensive therapeutic approach, the incidence and progression of all long-term specific complications of diabetes are substantially reduced; this applies to both types of diabetic patients [257, 258].

All markers of blood glucose control are also predictive of cardiovascular disease in type 1 and type 2 diabetic patients; however, the intervention studies so far undertaken for this complication have been unable to elucidate whether the incidence of cardiovascular diseases can substantially be reduced by the optimisation of blood glucose control [258].

Plasma glucose levels are predictive of cardiovascular events also in non-diabetic people; for these individuals the evidence available indicates blood glucose concentrations, measured at fasting and after a standard oral glucose load (OGTT), as good predictors of cardiovascular risk. These measurements are interpreted as markers of blood glucose metabolism respectively in the post-absorptive and in the post-prandial condition. The

possibility that post-prandial plasma glucose levels are particularly relevant in relation to the risk of cardiovascular diseases is supported by many epidemiological studies and is further indicated by evidence linking the glycaemic load, obtained multiplying the carbohydrate content of each food by its glycaemic index (a marker, albeit indirect, of the post-prandial blood glucose response), with the incidence of cardiovascular events [259, 263, 264].

Reduction of the glycemic load may be achieved by either lowering the intake of carbohydrates or selecting carbohydrate-rich foods with a low glycemic index. The latter manoeuvre is particularly relevant since a reduction in dietary carbohydrates often implies an increase in fat intake, which does not seem particularly wise in populations – as in western countries – consuming diets already rich in fat (particularly saturated ones). Most low glycemic index foods are fibre-rich, but food technology is able to influence carbohydrate digestion of carbohydrate rich-foods with a particularly low fibre content, thus reducing their glycemic index [265, 266].

Intervention studies show that drugs as well as non-pharmacological measures able to reduce blood glucose levels, particularly in the post-prandial period, are able to improve the overall cardiovascular risk factor profile and, possibly, also to reduce the incidence of events. However, since these interventions act usually at multiple levels, it is not possible to dissect the beneficial ef-

fects induced by lowering plasma glucose from those secondary to other metabolic improvements (i. e. insulin sensitivity, hyperinsulinaemia etc.) [15, 256].

High plasma glucose levels, at fasting or after an OGTT, are consistently indicated as risk factors for the development of type 2 diabetes in epidemiological studies and, if reduced by appropriate treatments, are paralleled by a lower diabetes incidence [15, 255, 256]. Also the diet's glycaemic load, which is an indirect marker of post-prandial glucose rise, has been shown to predict the development of type 2 diabetes in non-diabetic people [262, 267].

### Validity

The assay of plasma glucose concentrations is easy and reproducible; self-monitoring with strips and meters has also good reproducibility, although it is not as accurate as conventional laboratory methods [268].

Day to day variation of plasma glucose levels in the same individual is rather small in the fasting state while it is higher in the post-prandial period, depending, among other things, on the size and composition of the meal. Plasma glucose concentrations after an oral glucose load (OGTT) are usually employed in non-diabetic individuals as a surrogate marker of post-prandial glucose metabolism; however, although this measurement is more reproducible and easier to standardise than post-prandial glucose measurements, it does not take into account the impact of diet on glucose metabolism, which is not at all negligible. Multiple daily measurements of plasma glucose levels performed by strips and meters are now generally accepted as a reliable marker of 24-hours glucose metabolism [268].

Glycated haemoglobin is probably the best marker of blood glucose control in diabetic patients since it takes into account glucose metabolism both at fasting and post-prandially over the time span of several weeks; it is highly reproducible in the same laboratory while the variability between laboratories is high, depending on the method employed and on other factors not easy to control; in multi-centre studies it is appropriate to centralise this measurement. National and international programs for standardisation of this method are under way. Available data seem to indicate this marker as far less reliable in non-diabetic individuals [268].

Plasma fructosamine levels are also an integrated marker of blood glucose control over time; however it reflects only a time span limited to 2–3 weeks and therefore has never been utilised on a large scale as a marker of a long-term condition [268].

## ■ Functions associated with the regulation of blood glucose levels

### 1) Glucose delivery into the bloodstream

The concentration of glucose in the blood is the net result of two processes: glucose delivery into the bloodstream, and glucose utilisation by tissues. In the overnight-fasted (postabsorptive) state, glucose production is typically around 2 mg/min per kg body weight. After a meal, insulin, secreted from the pancreas in response to a rise in blood glucose concentration, suppresses the release of glucose from hepatocytes, by suppressing glycogen breakdown and gluconeogenesis, and the increase in glucose concentration in the portal vein stimulates net glucose uptake by hepatocytes. By these means, the elevation in blood glucose concentration after a meal is minimised.

In the postabsorptive state, measurements of endogenous glucose production (EGP) in type 2 diabetes show that this is increased only in more severe cases [269, 270].

An accurate measurement requires that a tracer technique be utilised to evaluate endogenous glucose production: a constant infusion of a glucose tracer, labelled with either a radioactive or a stable isotope, can be used to measure glucose turnover and to distinguish glucose production and glucose utilisation, even in relatively non-steady-state conditions [115].

### 2) Glucose utilisation

In the post-prandial state, impairment of glucose utilisation (both hepatic and peripheral) is in large part a very prominent effect of insulin resistance [270–273]. This has been studied most extensively by intravenous infusions of glucose and insulin to mimic the post-prandial state (glucose clamp). In that experimental situation, glucose utilisation in the whole body, or more specifically in skeletal muscle (e. g. the forearm or the leg), is characteristically impaired in people with diabetes compared with those with normal glucose tolerance [108, 274, 275]. A similar impairment of the insulin mediated glucose utilisation has been demonstrated in people with less severe forms of derangements of glucose metabolism (IFG and IGT) as well as in individuals with hypertension, dyslipidaemia, overweight and, even more, is present in individuals with a cluster of these abnormalities (metabolic syndrome). Under those experimental conditions, resembling the post-prandial metabolic state, around 70% of the glucose infused is disposed of by deposition as glycogen in skeletal muscle [276]. Therefore considerable attention has been paid to the pathway of glycogen synthesis in skeletal muscle as a possible primary site of impaired glucose disposal in diabetes [276]. However, nothing has emerged to clearly

pinpoint this pathway, and other evidence suggests that the impairment is at the level of glucose entry into the muscle cell [117, 275, 277].

### ■ Mechanisms involved in the regulation of blood glucose levels

#### Insulin secretion

Plasma insulin levels play a relevant role not only in controlling plasma glucose levels, but also in the overall regulation of the intermediary metabolism: they promote the storage of substrates in fat, liver and muscles, regulate water and electrolyte balance and stimulate cell growth and differentiation. This complex activity involves a network of signalling pathways, activated by the binding of the hormone with its receptor. Different proteins act as insulin receptor substrates at the cellular level for the various biological effects of insulin, which explain why selective impairments of insulin activity have been described [107].

Plasma insulin levels are mainly regulated by plasma glucose concentrations, although many other factors, including nutrients, hormones and the activity of the autonomic nervous system, also play a relevant role [278].

In relation to glucose metabolism the early phase of the glucose stimulated insulin response is particularly important; as a matter of fact it plays a crucial role in the regulation of post-prandial metabolism since it inhibits liver glucose production and stimulates splanchnic glucose disposal, which accounts for as much as one third of the glucose utilised by the total body after a meal [259].

Plasma insulin levels are low in type 1 diabetic patients, in whom autoimmune destruction of the beta cells is the key pathogenetic mechanism of the disease [279]. In type 2 diabetic patients plasma concentrations of insulin are usually normal or high, although they are inappropriate for the elevated ambient glucose and the presence of insulin resistance [254]. However, when stable hyperglycaemia develops impaired insulin secretion is also present; this impairment is particularly evident at the level of the early phase of insulin release after a carbohydrate load which is markedly reduced in type 2 diabetic patients, even if plasma insulin levels are overall normal or high. Reduced early insulin response is not only associated with type 2 diabetes but is also predictive of this condition in at risk individuals [280].

Fasting plasma insulin values are not necessarily a good marker either of plasma insulin concentrations throughout the day or of the insulin secretion kinetics; for this reason the evaluation of the early phase of insulin response has to be performed either after intravenous glucose (given as a load or at a constant infusion and the insulin is measured during the first ten minutes

after glucose injection) or during an OGTT (insulin is measured at thirty minutes) and shows, usually, acceptable reproducibility in the same subject [278].

#### Insulin sensitivity

This aspect has been already considered in the section on the metabolic syndrome.

### ■ Future research needs and conclusions

The link between blood glucose levels and the incidence of long-term diabetic complications, particularly the microvascular complications, is clearly established. Therefore functional foods able to influence markers of blood glucose control in a beneficial way are certainly welcome and may possibly apply for a type A health claim. Such foods should not be regarded as specific for diabetic patients, since blood glucose levels are markers of disease risk (diabetes, cardiovascular disease) also in non-diabetic individuals and, therefore, keeping them under control should be considered beneficial for the population at large.

The role of post-prandial glucose levels in relation to the development of cardiovascular diseases deserves further evaluation, particularly by intervention studies employing a non-pharmacological approach; along this line, large-scale intervention studies utilising a low glycaemic index/load diet might be particularly appropriate.

Obviously, insulin secretion and insulin sensitivity play an important role in the regulation of blood glucose levels; therefore these functions are also relevant in relation to diabetes and may represent possible targets for functional foods.

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### General conclusions

In this ITG we have tried to identify relevant functions contributing to overweight, the metabolic syndrome and diabetes; moreover for each function, and for some other associated functions which also play a role in the pathogenesis of these conditions, we have evaluated possible markers in relation to their methodological validity and to their links with the disease process.

Our task was to review the literature and, from that, try to distillate pieces of information and judgements on the basis of the expert knowledge of the participants in the ITG. Obviously the literature is rather discontinuous and the evidence available not equally strong to support the role of each function and the relevance of each marker. However, our general impression is that in this field, more than in many others, the link between nutrition, biological responses and diseases is clearly estab-



lished and, therefore, there is a strong potential to set up a functional food science.

The major gap in the evidence is always the lack of diet based intervention trials of sufficient duration to be relevant for the natural history of diseases. When such

studies are available, as in relation to the influence of moderate weight reduction and increased physical activity on diabetes prevention in at risk individuals, stimulation of scientific interest is high and the impact on public health strategies is large [15–17].

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