IFIS core food information

# The potential role of the intestinal gut microbiota in obesity and the metabolic syndrome

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## 1. Introduction

The incidence of obesity has reached alarming levels worldwide, thus increasing the risk of development of metabolic disorders (e.g. type 2 diabetes, coronary heart disease (CHD) and cancer). Among the causes of obesity, diet and lifestyle play a central role. Although the treatment of obesity may appear quite straightforward, by simply re-addressing the balance between energy intake and energy expenditure, practically it has been very challenging. In the search for new therapeutic targets for treatment of obesity and related disorders, the gut microbiota and its activities have been investigated in relation to obesity. The human gut microbiota has already been shown to influence total energy intake and lipid metabolism, particularly through colonic fermentation of undigestible dietary constituents and production of short chain fatty acids (SCFA). Recent studies have highlighted the contribution of the gut microbiota to mammalian metabolism and energy harvested from the diet (Turnbaugh *et al.* 2006; Martin *et al.* 2007). A dietary modulation of the gut microbiota and its metabolic output could positively influence host metabolism and, therefore, constitute a potential coadjutant approach in the management of obesity and weight loss.

# 2. The human colonic microbiota

## 2.1 Composition, development and functions of the human gut microbiota

The human gastrointestinal tract harbours a diverse collection of microorganisms, the majority of which reside in the colon. The adult human gut contains around $10^{14}$ bacterial cells and up to an estimated 1000 different bacterial species, thus constituting the largest microbial community associated with the human body and an organ with relevant metabolic output and capacity (Eckburg *et al.* 2005; Nicholson *et al.* 2005).

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Early studies on the composition of the gut microbiota were limited by the shortcomings of conventional microbiological techniques, which relied upon the ability of bacteria to grow under specific defined environmental conditions. Many bacterial species within the gastrointestinal tract are as yet uncultured and are not represented in studies using traditional, culture-based methodology (Suau *et al.* 1999). Molecular techniques, based around the phylogenetic information encoded by bacterial 16S rRNA genes have made possible direct characterisation of the gut microbiota in a culture-independent manner (Zoetendal *et al.* 2004b; Tuohy and McCartney 2006). Techniques such as fluorescent *in situ* hybridisation (FISH) and quantitative PCR allow direct enumeration of bacterial populations within mucosal biopsies and faecal material at both the phylogenetic genus and species levels (Amann *et al.* 1991; Harmsen *et al.* 2002; Lay *et al.* 2005). Complementary molecular techniques such as PCR-denaturing gradient gel electrophoresis (DGGE) and sequencing of whole community 16S rRNA gene libraries allow the determination of species diversity within the complex gut microbiota as well as monitoring changes within this diversity over time (Suau *et al.* 1999; Muyzer *et al.* 1993; Zoetendal *et al.* 2004a). Recent studies using these approaches have shown that the two most abundant bacterial phyla found in the healthy human large intestine are the Gram negative Bacteroidetes and the Gram positive, low GC% Firmicutes (Eckburg *et al.* 2005; Louis *et al.* 2007). Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia phyla are relatively less abundant (Eckburg *et al.* 2005). The dominant bacterial groups have been identified as the *Clostridium coccoides-Eubacterium rectale* group, *Clostridium leptum* group, *Bacteroides-Prevotella* species, *Bifidobacterium* and *Atopobium* species (Zoetendal *et al.* 2006). Clone sequencing of total 16S rRNA sequences recovered from a single adult faecal sample revealed that only 24% of clones corresponded to previously identified bacterial phylotypes, indicating that the vast majority of bacteria comprising the gut microbiota

(about 70%) correspond to novel bacterial lineages, the majority of which fall within the three dominant groupings (Suau *et al.* 1999). More recent studies have confirmed this molecular view of gut microbiota diversity (Eckburg *et al.* 2005). Human gut microbial composition is characterised by a large inter-individual variation and appears to be stable over extended periods of time (Zoetendal *et al.* 1998; Lay *et al.* 2005). Although genetic factors play an important role in the development of our gut microbiota, environment also drives species acquisition (Zoetendal *et al.* 2001).

Colonisation of the human gastrointestinal tract starts at birth (i.e. vertical transmission of microbes to babies from the vagina and faeces of their mothers; Mandar and Mikelsaar 1996) and selection occurs at the microbial, as well as at the host level. On the one hand, the host selects for microbial divisions that are functionally redundant (i.e. with functionally similar sets of genes). This type of selection confers the characteristic of gut microbial stability and resistance to changes that would otherwise be disruptive for the host organism. The presence of endogenous and exogenous nutrients (i.e. mucins and breast milk, respectively) and adhesion sites on the host intestinal mucosa select for a beneficial or 'symbiotic' microbiota (Mountzouris *et al.* 2002; Isaacs 2005), where bacterial populations beneficial to the host emerge (Nicholson *et al.* 2005). On the other hand, competition between members of the gut microbiota exerts a selective pressure which favours microbial populations with distinct functional and metabolic traits (i.e. specialised genomes; Lay *et al.* 2005).

Interactions between the gut microbiota and the host can play an important role in human health at many levels. Gastrointestinal microbial communities contribute towards several mammalian physiological processes, including fermentation of indigestible complex dietary polysaccharides and proteins (Table 1 - see Appendix, page 29), biotransformation of bile acids, vitamin synthesis, immune system and intestinal mucosal architecture development and defence against pathogens. The main functions of the human gut microbiota and their physiological role are summarised in Table 2 - see Appendix, page 30.

## 2.2 Impact of dietary macronutrients on the gut microbiota

Constituents of the diet that escape digestion in the human upper gastrointestinal tract reach the colon and become available for bacterial fermentation. Among the dietary components, carbohydrate is the major fermentative substrate of the resident gut microbiota, and is therefore the

most actively metabolised. The proximal colon is the main site for bacterial saccharolytic fermentation, which leads to production of SCFA. However, the amount of dietary carbohydrate entering the colon depends on many factors, including the type of carbohydrate, mode of preparation (e.g. cooking method), food form (e.g. particle size), rate of digestion and transit time, amount of fat and proteins present in the food, and presence of digestive enzyme inhibitors (Jenkins *et al.* 1982; Thorne *et al.* 1983; Yoon *et al.* 1983; Cummings and Stephen 2007). The amount of dietary carbohydrate reaching the colon has been observed to be correlated with the amount of dietary fibre present in the food (Jenkins *et al.* 1987; Steinhart *et al.* 1992). Certain types of food, such as legumes and cereals, particularly where the whole-food structure is maintained, provide a physical arrangement that protects carbohydrate from being digested in the small intestine and allows a greater proportion to enter the large bowel. Also, starch is not totally absorbed in the small intestine and a consistent fraction may reach the distal bowel (Englyst *et al.* 1996; Wolever *et al.* 1986). Resistant starch reaching the colon consists of a mixture of amylose ($\alpha$-1,4-linked glucose residues) and amylopectin (amylose chains linked to an amylose scaffolding by $\alpha$-1,6-linkages) and is mainly broken down by amylolytic bacteria, such as *Bacteroides* and *Bifidobacterium* species (Louis *et al.* 2007). Some other plant food constituents reaching the colon are insoluble fibres, such as lignin and cellulose, which can only be incompletely degraded by gut microbes (e.g. *Ruminococcus* species). Despite this, soluble fibres (e.g. hemicelluloses, xylans, pectins, inulin-type fructans) may be released from plant cell wall matrices and become available for fermentation. Some of these fibres, such as inulin-like fructans, display a prebiotic-type fermentation leading to increased numbers of colonic bifidobacteria and lactobacilli.

Dietary proteins reaching the colon also have an impact on microbial metabolism. Bacterial proteolytic fermentation leads to production of branched-chain amino acids, principally in the distal part of the colon. Microbial degradation of certain amino acids causes formation of pro-carcinogen substances, such as phenols, indoles, sulfides, ammonia, amines and *N*-nitroso compounds (Clinton *et al.* 1988; Geypens *et al.* 1997; Roediger *et al.* 1996; Hecht 1997). High protein diets, such as Western-style diets characterised by high red meat consumption and low fibre intake, are now recognised as risk factors of colorectal cancer (Lewin *et al.* 2006; Ward *et al.* 2007).

Dietary fat entering the colon can also affect gut microbial metabolism. Previous investigations have shown that approximately 5% of the total fat from the diet is not digested and absorbed in the small intestine and thus can reach the large intestine (Vulevic *et al.* 2004). The intestinal microbiota has been previously shown to convert dietary choline to trimethylamines (TMA), which are then excreted in the urine (al-Waiz *et al.* 1992). More recently, Dumas *et al.* (2006), using proton-nuclear magnetic resonance ($^1$H-NMR) biofluid metabolite profiling, showed that microbial metabolism of choline into methylamines (TMA, trimethylamine-*N*-oxide – TMAO – and dimethylamine) strongly contributes to non-alcoholic fatty liver disease (NAFLD) and insulin resistance in a mouse model pre-disposed to this condition. In the study, a reduction of plasma phosphatidylcholine bioavailability, due to increased bacterial TMA production, seemed to trigger hepatic steatosis (Dumas *et al.* 2006), which has been previously described in association with choline-deficient diets (Buchman *et al.* 1995). NAFLD is a liver condition associated with insulin resistance and type 2 diabetes (discussed later). Therefore, this study highlights the contribution of the gut microbial metabolism to complex metabolic diseases, such as the metabolic syndrome. The study also shows the utility of high-resolution analytical techniques, such as $^1$H-NMR, to identify differential metabolites in disease states or upon dietary intervention (Lindon *et al.* 2007).

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Evidence suggests that gut bacteria can metabolise dietary phospholipids and produce detrimental substances, such as diacylglycerol (DAG), which is considered a tumour-promoting factor (Friedman *et al.* 1989; Morotomi *et al.* 1990). Through induction of changes in the pH of the colonic environment, as a consequence of bacterial fermentation, and through modulation of enterohepatic circulation, the gut microbiota can create favourable or unfavourable conditions for colonic DAG production (MacDonald *et al.* 1978; Vulevic *et al.* 2004). A modulation of the gut microbiota aimed at increasing the growth and activity of certain beneficial saccharolytic bacterial populations might reduce the amount of DAG produced as a result of a high-fat diet.

## 2.3 Modulation of the gut microbiota

The possibility of modulating the gut microbiota through dietary means has led to the development of functional foods such as probiotics and prebiotics. The FAO/WHO Expert Panel in 2001 defined probiotics as "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host". Probiotic bacteria for human consumption are

usually members of the genus *Lactobacillus* or *Bifidobacterium* and the yeast *Saccharomyces boulardii*. Both the lactobacilli and bifidobacteria have been associated with a number of health-promoting activities. There is convincing evidence for improved intestinal transit, reduced duration of diarrhoea, improved lactose tolerance and alleviation of allergic conditions (e.g. atopic eczema), including inflammatory bowel disease (Isolauri *et al.* 2000; Marteau *et al.* 2001; Sheil *et al.* 2007; Ouwehand 2007). The mode of probiotic action is likely to be multi-factorial and product/strain specific, but will include aspects of microbial physiology (anti-microbial and SCFA production), microbial ecology (competition for nutrients and adhesion sites with pathogens) and host physiological response (immuno-regulation, regulation of mucin or defensin production; Macfarlane and Cummings 2002; Tuohy *et al.* 2003).

A complementary, and, in many ways, more direct approach towards dietary microbiota modulation is the use of prebiotic oligosaccharides. A prebiotic is a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth or the activity, or both, of one or a limited number of bacteria already resident in the colon" (Gibson and Roberfroid 1995). Some prebiotics, predominantly oligosaccharides or short chain polysaccharides, facilitate a modulation of the colonic microbiota whereby relative numbers of bifidobacteria and/or lactobacilli increase, sometimes at the expense of bacterial groups seen as potentially detrimental e.g. *Clostridium perfringens*, *Bacteroides fragilis* and *Escherichia coli* (Gibson and Roberfroid 1995). Thus, prebiotics may be viewed as functional foods which selectively stimulate an individual's own beneficial bacteria within the colonic microbiota. Fructooligosaccharides, inulin (and its derivative oligofructose, OFS), lactulose and galactooligosaccharides are all well-characterised prebiotics and can be reproducibly shown to bring about this modulation of the gut microbiota, typified by increased numbers of bifidobacteria (Tuohy *et al.* 2005). According to the European Consensus on 'Scientific Concepts of Functional Foods', a functional food is, by definition, a food that has been "demonstrated to affect beneficially one or more target functions in the body, thus resulting in improved stage of health and well-being and/or reduction of risk of disease" (Diplock 1999). Prebiotics have been associated with a number of specific health-promoting effects, including improved bowel habits, mineral absorption, protection against colon carcinogenesis and inflammatory bowel diseases, immunomodulation and resistance to infections, production of

gastrointestinal endocrine peptides and appetite regulation, and lipid homeostasis (Roberfroid 2007).

### 2.3.1 Effect of prebiotics on obesity-associated metabolic disorders

Investigation of the role of prebiotics, especially inulin-type fructans, in regulating satiety, glycaemia and lipogenesis has increased in the last decade, probably in association with the increased incidence of metabolic diseases such as the metabolic syndrome and type 2 diabetes. Both animal and human studies support the efficacy of OFS supplementation in modulating food intake through a mechanism that involves secretion of intestinal endocrine peptides. Cani *et al.* (2005) showed that in Wistar rats, addition of 10% (wt/wt) OFS to a high-fat diet reduced energy intake, body weight gain, fat mass development and blood triacylglycerol (TAG), while increasing caecal and colonic proglucagon mRNA levels, glucagon-like petide 1 (GLP-1) and glucagon-like petide 2 (GLP-2) colonic contents.

In a further study, GLP-1 was proven necessary to exert the OFS-induced anti-diabetic effect (Cani *et al.* 2006b). In particular, the study showed that in high-fat fed diabetic mice with a functional GLP-1 receptor, supplementation with OFS (10:90, wt OFS:wt diet) improved glucose tolerance, glucose-induced insulin secretion, hepatic insulin sensitivity and reduced body weight. On the contrary, GLP-1 receptor knockout mice were totally insensitive to the effects of OFS on glucose homeostasis (Cani *et al.* 2006b). The same effects of OFS on satiety (i.e. reduced hunger after a meal) and energy intake (i.e. reduced food consumption) were observed by the same group in a pilot study with ten healthy men and women fed OFS or placebo maltodextrin (16 g/day) for 2 weeks (Cani *et al.* 2006a).

Convincing evidence from animal and human studies supports the effect of inulin-type fructans on lipid metabolism and homeostasis. Several studies in rats fed with different types of diets (i.e. high-carbohydrate diet, high sucrose/fructose diet, fibre-free diet, high-fat diet) showed that fructan supplementation consistently decreased fasted and postprandial

triglyceridaemia, while cholesterolaemia was less affected (Kok *et al.* 1998; Fiordaliso *et al.* 1995). In particular, a decrease in very low-density lipoproteins (VLDL, described later) plasma levels has been observed in animals after treatment with fructans, although VLDL lipid composition remained unaltered (Fiordaliso *et al.* 1995). Mechanisms underlying the observed hypolipidaemic effects include a reduction of hepatic lipogenesis, but not of cholesterol synthesis (Letexier *et al.* 2003). Increased bacterial colonic fermentation increases the concentration of SCFA reaching the liver after absorption via the portal vein. A sufficiently high hepatic concentration of propionate, such as that induced by fructan supplementation, has been proven efficacious in inhibiting colonic acetate uptake and decreasing the mRNA levels of fatty acid synthase (Fas; Agheli *et al.* 1998; Daubioul *et al.* 2002). Therefore, by reducing the availability of substrates (i.e. acetate) and enzymes (i.e. Fas) for lipid synthesis, propionate might reduce hepatic lipogenesis. In addition, Delzenne and Kok (1999) showed that the expression and activity of other hepatic lipogenic enzymes, such as acetyl-CoA carboxylase, malic enzyme and adenosine triphosphate (ATP) citrate lyase, might be reduced after OFS administration. Insulin and glucose play a major role in regulating hepatic lipogenesis, and their involvement in fructan-associated hypolipidaemic effects has also been investigated. OFS supplementation in rats was shown to induce a moderate decrease in postprandial and basal glucose and insulin levels, while the response to an oral glucose load was not modified (Agheli *et al.* 1998). Some contradictory results were obtained in studies with diabetic rats, where basal glucose and insulin levels and the response to a glucose load were not modified by OFS administration (Daubioul *et al.* 2000). Therefore, the effect of fructan supplementation on glycaemia, insulinaemia and glucose tolerance, also in relation to the effect on hepatic lipogenesis, still remains unclear.

Experimental data showing the effect of dietary supplementation with fructans on cholesterolaemia are also uncertain. Fiordaliso *et al.* (1995) reported that OFS administration (10% wt/wt of the diet) in rats lowered esterified- and VLDL-cholesterol levels. However, several other authors reported no decrease in plasma cholesterol concentrations in rats fed with OFS in their diet (Agheli *et al.* 1998; Delzenne *et al.* 2002). Different from OFS, inulin was reported to induce a significant hypocholesterolaemic effect in rats and in hamsters (Levrat *et al.* 1994; Trautwein *et al.* 1998). Human studies

partly confirmed the findings from animal studies, in that a reduction of plasma TAG concentration was observed after dietary supplementation with inulin, although these effects were much more evident in hyperlipidaemic individuals than in normolipidaemic people. Moreover, inulin appeared to be more efficacious than OFS in lowering hypertriacylglycerolaemia in humans. Studies in normolipidaemic subjects did not show any effects of OFS on TAG or cholesterol levels (van Dokkum *et al.* 1999; Luo *et al.* 2000). However, the results from dietary intervention studies with inulin in normolipidaemic subjects are inconsistent. Brighenti *et al.* (1999) showed a decrease in TAG and cholesterol following inulin supplementation (9 g/day), while other studies did not report any effect of inulin on plasma lipid levels (Pedersen *et al.* 1997; van Dokkum *et al.* 1999). More promising results on the hypolipidaemic effect of inulin-type fructans were obtained from studies with hyperlipidaemic individuals. OFS supplementation was associated with a significant decrease in plasma cholesterol (Hidaka *et al.* 1991), while inulin supplementation was shown to decrease plasma cholesterol (Davidson *et al.* 1998) and TAG concentrations (Jackson *et al.* 1999a; Causey *et al.* 2000). Jackson *et al.* (1999a) also showed a significant decrease in basal insulin concentrations, which might have influenced the decrease in plasma TAG levels. Interestingly, Balcazar-Munoz *et al.* (2003) showed that inulin significantly reduced both cholesterol and TAG concentrations, compared with placebo, when supplemented (7 g/day) in the diet of obese hyperlipidaemic subjects. This study suggests that inulin supplementation might improve some obesity-associated risk factors for atherosclerosis and cardiovascular disease.

The results of studies in mice performed by Cani *et al.* (2007b) support the strengthening effect of prebiotics on the intestinal mucosal barrier and also show some evidence for a systemic effect of prebiotics on inflammatory markers and risk factors of the metabolic syndrome. In particular, decreased plasma levels of inflammatory bacterial lipopolysaccharide (LPS) and of pro-inflammatory cytokines were observed in OFS-fed mice compared with cellulose-fed control mice, thus suggesting an improvement in gut epithelial cell integrity and a reduced translocation of bacteria and bacterial LPS through the intestinal epithelium into systemic circulation (Cani *et al.* 2007b). The reduced endotoxaemia and inflammatory tone were found concomitantly with improved glucose tolerance and insulin sensitivity and reduced body fat

percentage. Importantly, the observed effects were found to be positively and significantly correlated with increased caecal levels of *Bifidobacterium* species.

## 3. Obesity and metabolic disorders

### 3.1 Incidence, causes and consequences of obesity

Obesity and overweight are, by definition, described as excessive or abnormal fat accumulations that may impair health. Body mass index (BMI) is generally used to classify obesity and overweight. BMI represents the weight in kilograms divided by the square of the height in $m^2$. Individuals with a BMI equal to or greater than 25 $kg/m^2$ are considered overweight, while individuals with a BMI equal or greater than 30 $kg/m^2$ are defined as obese (World Health Organisation (WHO) 2000).

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According to the latest WHO global estimate, approximately 1600 million adults are overweight and at least 400 million are obese (WHO 2006). By 2015, the WHO calculates that about 2300 million adults will be overweight and about 700 million will be obese. The last National Health Service (NHS) statistics revealed that in England, 38% of adults were classified as overweight and 24% as obese (The Information Centre, IC 2008). These data become more alarming when the NHS prediction for 2050 of 60% of men and 50% of women in England being classed as obese is considered (Foresight 2007). Moreover, the prevalence of obesity is dramatically increasing among children, with about 20 million obese children under the age of five worldwide and 16% obese children aged 2-15 years in England (WHO 2006; The Information Centre, IC 2008). The obesity epidemic does not only affect Western countries, but its incidence is dangerously increasing in low/middle-income developing countries, especially in urban settings, where people are adopting Western lifestyles (Food and Agriculture Organisation of the United Nations (FAO) 2002).

The main cause of obesity is an imbalance between energy intake and energy expenditure, predominantly caused by a dietary shift towards high calorie foods (i.e. foods high in fat and sugars but low in fibres, micronutrients, vitamins and minerals) and decreased physical activity, due, in part, to sedentary lifestyles. Recent studies over the last decade have also revealed

an important genetic contribution to obesity. Quantitative genetic analysis has shown that the chances of developing obesity are increased from 30 to 70% in people with obese relatives (Bell *et al.* 2005; Farooqi 2005). According to the last update of the human obesity gene map, more than 600 genes, markers and chromosomal regions are involved in body weight regulation and obese phenotypes (Rankinen *et al.* 2006). The genetics of obesity include gene encoding factors involved in regulation of food intake (e.g. leptin, neuropeptide Y and gastrointestinal peptides, such as ghrelin, cholecystokinin, GLP-1, peptide YY3–36), as well as factors implicated in energy expenditure (e.g. $\alpha$-adrenoreceptors, which regulate thermogenesis and lipolysis, and uncoupling proteins, which modulate heat-generating uncoupled mitochondrial respiration) and transcription factors regulating adipogenesis and adipocyte differentiation (e.g. PPAR$\gamma$; Martínez-Hernaíndez 2007). Although genetics predisposes a subpopulation to obesity, obesity does not usually occur in the absence of an obesogenic environment (e.g. energy dense diet, high in fat and refined carbohydrate and low in fibre, in accordance with low physical activity). The interaction between genotype and environment may play a substantial role in the development of obesity; since the individual's genetic background drives the response to certain environmental factors (e.g. overfeeding; Marti *et al.* 2008). Although we may not be able to alter an individual's chances of becoming obese through changing their genes, we can intervene on the obesogenic environment (e.g. by modifying the type of diet) to try and reduce risk.

The principal health consequences of obesity and overweight include increased risk of cardiovascular disease (mainly heart disease and

stroke), diabetes, musculoskeletal disorders (e.g. osteoarthritis), some cancers (e.g. endometrial, breast and colon), NAFLD, sleep apnoea and gallbladder disease (Haslam and James 2005).

Current approaches for the treatment of obesity principally target a reduction of calorie intake coming from fats (especially saturated fats) and sugars (in particular simple refined sugars) and an increased consumption of fruit, vegetables, whole grains, legumes and nuts. In addition, an increase in energy expenditure through regular physical exercise is also recommended for body weight loss and maintenance.

## 3.2 The metabolic syndrome

Metabolic syndrome (MS) or syndrome X is a constellation of heterogeneous characteristics linked with abdominal obesity and insulin resistance as the central components. Although about 50% of obese people develop MS (Shaw *et al.* 2005), excess body visceral fat, as determined by waist circumference, is more indicative of MS than obesity itself, as defined by BMI (Pouliot *et al.* 1994). MS worldwide is increasing rapidly, with about a quarter of the world's population having been diagnosed with MS. These statistics are of concern, particularly when considering that MS confers substantial risk of type 2 diabetes and cardiovascular disease development (Isomaa *et al.* 2001; Dunstan *et al.* 2002; Shaw *et al.* 2005). MS involves multiple metabolic pathways which culminate in dyslipidaemia (elevated plasma TAG, low plasma high-density lipoprotein (HDL) cholesterol and elevated low-density lipoprotein 3 (LDL ) cholesterol concentrations), abdominal obesity, microalbuminurea and hypertension (Reaven 1988; Stern 1996). There are several definitions of the MS, and all recognised definitions include a measure of obesity or central obesity, hypertension, dyslipidaemia and a measure of insulin resistance (Balkau and Charles 1999; Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, National Cholesterol Education Program Adult Treatment Panel III, NCEP ATP III 2001; Alberti *et al.* 2006). However, these features are often accompanied by other related abnormalities of vascular function and lipoprotein metabolism. A summary of the requisites for MS diagnosis and a list of additional parameters associated with MS, according to the International Diabetes Federation (IDF 2005), are shown in Tables 3 and 4 - see Appendix, page 31. There are also underlying genetic factors affecting important aspects of the MS, such as insulin resistance (Saad *et al.* 1991). The incidence of type 2 diabetes is more elevated in people with close family relations with type 2 diabetes (Turner *et al.* 1995). Moreover, the prevalence of obesity and insulin resistance is higher

among some ethnic groups, such as those from the Indian subcontinent, Asians, Hispanics, Native Americans and African Americans (Fernandez *et al.* 2004; Cossrow and Falkner 2004). Therefore, ethnic group-specific cut-points were introduced to optimally define the MS in these subpopulations (e.g. lower waist circumference; Tan *et al.* 2004; Misra *et al.* 2005).

## 3.3 Risk factors associated with obesity and MS

### 3.3.1 Lipid risk factors associated with MS

The combined dyslipidaemia of elevated TAG, low HDL and a high proportion of $LDL_3$, often referred to as 'atherogenic lipoprotein phenotype' (ALP), is commonly observed in diabetics and those with MS, and closely linked to the development of CHD (Griffin and Packard 1994). Many epidemiological studies have shown a positive correlation between fasting total cholesterol concentrations, especially LDL cholesterol levels, and CHD mortality (Verschuren *et al.* 1995). Accumulation of LDL in the plasma leads to a deposition of cholesterol in the arterial wall, a process which involves oxidative modification of the LDL particles. The oxidised-LDL binds to scavenger receptors on macrophages in an uncontrolled manner. When these macrophages become lipid loaded, they convert to foam cells which form the basis of the early atherosclerotic plaque. Progression of the narrowing of the arteries eventually leads to angina pectoris, myocardial infarction and death. It has been estimated that every 1% increase in LDL cholesterol level leads to a 2-3% increase in CHD risk (Gensini *et al.* 1998). Much of the public health dietary recommendations have been based on the 'cholesterol lowering' strategy. The value of this was unequivocally supported by the findings of the West Scotland Heart Study (Shepherd *et al.* 1995) where lipid-lowering treatment was advised for middle-aged men with moderately raised blood cholesterol, but with no CHD. A 33% reduction in cardiovascular mortality and a 26% reduction in LDL cholesterol were reported in men receiving the active drug (prevastatin) compared with a placebo. Low HDL cholesterol levels have also been found in association with MS and represent an independent risk factor for CHD, with a strong inverse relationship between fasting plasma HDL levels and the risk of development of CHD (Gensini *et al.* 1998; Assmann *et al.* 1998). Elevated levels of circulating TAG constitute another lipid abnormality associated with MS (Alberti *et al.* 2005).

**Many epidemiological studies have shown a positive correlation between fasting total cholesterol concentrations, especially LDL cholesterol levels, and CHD mortality**

Raised plasma TAG concentrations, both in the fasted and fed (postprandial) states, are also a recognised independent risk factor for CHD (Patsch *et al.* 1992; Steinberg *et al.* 1996). In addition to TAG-rich lipoprotein directly

sequestering lipid into the developing atherosclerotic plaque (Rapp *et al.* 1994) and negatively affecting endothelial and platelet function, high levels of TAG impact on the atherogenicity of other lipoproteins, resulting in a reduction of HDL-cholesterol levels and an increase in the proportion of LDL, in particular the atherogenic $LDL_3$ particle. Many studies have demonstrated that those with CHD have a preponderance of small dense $LDL_3$ particles (Austin *et al.* 1994; Austin 1998). The increase in atherogenic potential of small dense $LDL_3$ particles, compared with less dense $LDL_1$ and $LDL_2$ particles, is believed to be due to a number of factors, including increased residency in the circulation as a consequence of poor receptor binding, resulting in reduced removal; increased susceptibility to oxidation and reduced antioxidant association; and their ability to infiltrate the endothelium and be endocytosed by the scavenger receptor of foam cells (Chapman *et al.* 1998).

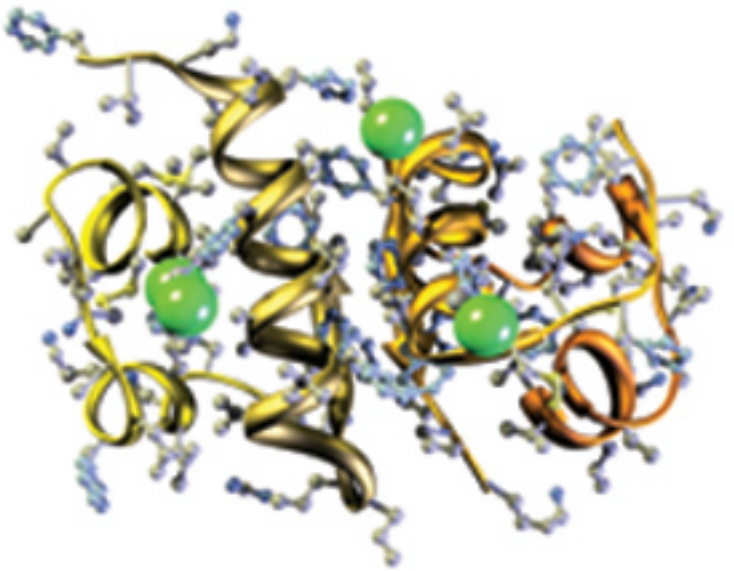
### 3.3.2. Hepatic fat metabolism

The liver plays an important role in human lipid metabolism, since it is the site for both lipid oxidation and synthesis. A schematic representation of fatty acid metabolism and *de novo* lipogenesis in the liver is shown in Figure 1 - see Appendix, page 32. Hepatically synthesised TAG may be excreted into the circulation in the form of VLDL or stored locally in the liver. In pathological conditions, such as alcoholism and NAFLD, the hepatic fat deposits may increase to an extent that impairs normal liver functions. As mentioned earlier, SCFA produced in the colon upon microbial fermentation of non-digested dietary carbohydrate reach the liver through the portal vein and may play a relevant role in *de novo* lipogenesis. Acetate is transformed in acetylCoA and utilised for fatty acid and cholesterol synthesis. Propionate is an important regulator of *de novo* lipid synthesis, since it may inhibit utilisation of acetate by the hepatocytes for fatty acid synthesis (Demigné *et al.* 1995) and it has also been suggested to reduce cholesterol synthesis by inhibiting the activity of HMGCoA reductase, a rate limiting enzyme for the synthesis of cholesterol (Chen *et al.* 1984). Thus, the ratio of acetate:propionate entering the liver from colonic fermentation plays an important regulatory role in the types and quantities of fats produced in the liver and released into circulation, where they can play a role in the development of cardiovascular disease (Wolever *et al.* 1991, 1996).

### 3.3.3. Insulin and human metabolism

Insulin is a peptide hormone synthesised in the pancreatic β-cells, where it is stored in secretory vesicles until it is needed in the circulation. When the blood glucose concentration rises above 5 mmol/L, the β-cells act as a 'glucose

sensor' and release insulin from secretory granules. Insulin secretion is also stimulated by amino acids and fatty acids. An increase in plasma fatty acids (e.g. after a meal) potentiates the insulin response to glucose. Conversely, if fatty acid levels remain high for several hours after a meal, insulin secretion is down-regulated through a mechanism that is not fully understood (Frayn 2003b).



Binding of insulin to its receptor triggers several cellular responses and regulates the expression of a large quantity of genes, mainly involved in induction of glucose utilisation and lipogenesis (through increased expression of sterol-regulatory element binding protein-1c – SREBP-1c – and of carbohydrate responsiveness element binding protein – ChREBP) and suppression of fat oxidation (through inactivation of hormone-sensitive lipase – HSL).

Insulin plays a central role in the regulation of the interaction between carbohydrate and fat metabolism. Insulin up-regulates both fatty acids and cholesterol synthesis from acetyl-CoA, respectively, by increasing the activity and expression of the enzymes Fas and acetyl-CoA carboxylase, and by acutely activating the enzyme 3-hydroxy-3-methylglutaryl-CoA (HMGCoA) reductase. Despite this, net lipogenesis (i.e. when the rate of lipogenesis exceeds the rate of fat oxidation) does not occur in normal healthy subjects after eating a carbohydrate-rich meal. In contrast, when healthy people consume high-carbohydrate diets or consume more calories than their normal energy requirements for several days, net lipogenesis will arise (Acheson *et al.* 1984; Aarsland *et al.* 1997).

Another important metabolic interaction between glucose and fat metabolism is the 'glucose-fatty acid cycle'. When plasma glucose levels rise, insulin response leads to suppression of fat mobilisation and, therefore, inhibition of the release of fatty acids from adipose tissue into the circulation. Consequently, glucose becomes the preferred source of energy for the muscle. When plasma glucose concentration falls (i.e. in-between meals), insulin secretion decreases and plasma non-esterified fatty acid (NEFA) concentration rises. The rate of free fatty acid oxidation increases in the muscle, while muscular glucose uptake and glycolysis are inhibited. When plasma glucose

levels are low, this mechanism allows circulating glucose to be utilised by the brain, which cannot extract energy from fatty acids. The glucose-fatty acid cycle can be disrupted in abnormal situations, such as stress or MS. In unusual conditions, such as when both glucose and NEFA levels are high, the ability of muscle to take up and metabolise both glucose and NEFA is reduced in relation to plasma concentration. This state of reduced sensitivity to the insulin action is also known as 'insulin resistance' (Frayn 2003c).

### 3.3.4. Insulin resistance

Insulin resistance is the abnormally low response of organs and tissues to the action of insulin, culminating in hyperinsulinaemia and eventually in hyperglycaemia. When the elevated insulin levels fail to control plasma glucose concentrations, frank diabetes results, which is progressively associated with a reduction in plasma insulin concentrations due to exhaustion of pancreatic β-cells. It is not clear whether insulin resistance is the primary cause of the abnormalities associated with the MS or the consequence of obesity. However, it is believed that insulin resistance is a predominant factor in the abnormalities in both lipid and carbohydrate metabolism associated with MS. There is a close link between obesity, especially visceral or central adiposity (VAT) and insulin resistance (Pouliot *et al.* 1994; Wahrenberg *et al.* 2005). As adipose tissue (especially visceral adipose tissue) expands, the concentrations of circulating NEFA increases, due to the lack of normal insulin-stimulated suppression of HSL-mediated release of NEFA from the adipocyte (Frayn *et al.* 1997). Continuous delivery of NEFA to the liver is linked to several negative effects including enhanced gluconeogenesis, increasing hepatic glucose output and hence hyperglycaemia (Lam *et al.* 2003). Impaired binding and hepatic extraction of insulin from the circulation has also been previously reported among individuals presenting with elevated NEFA concentrations, further exacerbating hyperinsulinaemia (Wajchenberg 2000). Finally, hepatic reesterification of excess NEFA to TAG and subsequent incorporation into liver-derived VLDL, increases circulating plasma TAG and apo B100. Elevated plasma TAG concentrations enable enhanced neutral lipid exchange between TAG-rich lipoproteins and the cholesterol containing lipoproteins HDL and LDL, via cholesteryl ester transfer protein (CETP), leading to the remodelling of these lipoproteins, a preponderance of $LDL_3$ and reduced HDL concentrations (Karpe 1999). In addition, impaired NEFA suppression results in elevated plasminogen activator inhibitor 1 (PAI-1), interleukins and tumour necrosis factor alpha (TNFα ), that promote atherogenesis. This, combined with insulin resistance and dyslipidaemia, significantly elevates the risk of atherogenesis

due to endothelial dysfunction, increased collagen, impaired fibrinolysis and thin, unstable plaques (Despres *et al.* 2000).

### 3.3.5. Type 2 diabetes

Diabetes globally affects around 200 million people and constitutes a leading cause of death in the Western world. Type 2 diabetes accounts for 90% of all diabetes and is five times more likely to develop in obese or MS individuals (Stern *et al.* 2005). Type 2 diabetes is characterised by two major factors, a peripheral insulin resistance and an insulin secretory cell deficit (Yki-Jarvinen 1995; Cerasi 1995). Diabetes is associated with a significant increase in the development of cardiovascular disease in addition to other micro- and macro-vascular conditions (Candido and Zanetti 2005). Endothelial dysfunction is one of the consequences of insulin resistance and it contributes to the accelerated atherosclerosis associated with type 2 diabetes (Hink *et al.* 2003). Among the principal causes of type 2 diabetes are obesity, energy dense diets (e.g. high-glycaemic index, high-saturated fat diets), increased urbanisation and a sedentary lifestyle, and an ageing population (WHO 2003). All of these causes are converging in Western European countries, making type 2 diabetes probably the greatest health challenge facing the European population in the near future.

### 3.3.6. The role of leptin

Leptin, originally termed the 'anti-obesity hormone', is an adipocyte-secreted hormone acting on the brain to regulate energy homeostasis (Ahima and Flier 2000). Leptin is mainly produced by the white adipose tissue in direct proportion with the amount of energy stored as fats (Shuldiner *et al.* 2001). The effect of leptin on the brain is to reduce food intake by affecting appetite (Williams *et al.* 2000). The hormone also mediates lipid depletion from the liver and peripheral tissues (Hynes and Jones 2001).

**Leptin, originally termed the 'anti-obesity hormone', is an adipocyte-secreted hormone acting on the brain to regulate energy homeostasis**

Leptin-deficient humans and mice show TAG accumulation in the adipose tissue and also in the liver, muscle and other peripheral tissues (Unger 2002). However, only 5–10% of obese individuals have insufficient leptin production. Contrary to strong evidence of the association between leptin and energy regulation, elevated leptin levels are more commonly associated with human obesity, with obese individuals presenting with elevated circulating leptin concentrations, which correlate with the percentage of body fat. This apparently disparate finding is due to the fact that 90–95% of obese and overweight individuals are leptin-resistant and insensitive to endogenous

leptin production. Hyperleptinaemia is correlated with hyperphagia, insulin resistance and other conditions of MS (Ren 2004).

High leptin levels constitute a marker of CHD risk. Being a metabolic hormone like insulin, leptin has vascular effects, regulating the sympathetic tone and arterial blood pressure (Haynes *et al.* 1997). Leptin also regulates vascular relaxation through a mechanism mediated by nitric oxide (Lembo *et al.* 2000; Vecchione *et al.* 2002). Finally, high leptin levels affect the heart rate, causing tachycardia in moderately obese and hypertensive individuals. This effect might lead to cardiac hypertrophia and heart failure (Blüher and Mantzoros 2004; Ren 2004; Swoap and Overton 2004; Cohen and Friedman 2004).

Little is known about the regulation of leptin production in adipocytes, but a recent study in mice has confirmed *in vitro* studies with cultured human adipocytes that the SCFA propionate can increase leptin production. Xiong *et al.* (2004) showed that acute administration of propionate to mice increased circulating leptin levels in a process mediated through the orphan G protein-coupled receptor 41 (GRP41). This increase in circulating leptin occurred at propionate concentrations within the physiological range and suggests that propionate produced from carbohydrate fermentation in the colon plays a role in leptin regulation, and thus the leptin mediated physiological processes including feeding behaviour, metabolic rate, sympathetic nerve activity, reproduction and immune response.

## 3.4. Reducing risk factors of MS through dietary intervention

Diet can play an important role in the development of obesity, MS, diabetes and CHD, and dietary change can modify the risk of these diseases. There is abundant and convincing evidence that high dietary intake of saturated fatty acids (SFA) contributes to the development of ALP and is closely linked to CHD risk (Laaksonen *et al.* 2001). A reduction of SFA intake has also been shown to positively impact on insulin sensitivity (Vessby *et al.* 2001; Perez-Jimenez *et al.* 2001). There are a number of strategies which can be exploited to reduce dietary SFA intake, such as changing the quantity or quality of dietary fat. A reduction of dietary SFA can be achieved through low-fat, high-carbohydrate diets or through moderately high-fat diets, in which SFA are substituted by monounsaturated fatty acids (MUFA). Reducing the percentage of energy derived from dietary fats is the current strategy recommended by the UK government for reducing CHD (Department of Health 1994). This specifically

addresses reducing elevated plasma cholesterol levels. However, although low-fat diets have been associated with successfully lowering total and LDL cholesterol, studies report that such diets have been linked to elevation of plasma TAG and suppression of beneficial HDL (Garg 1998; Parks *et al.* 1999; Mensink *et al.* 2003). This response would be prudent to avoid particularly in those individuals presenting with MS, characterised by hypertriglyceridaemia. Replacement of SFA with MUFA has been demonstrated as efficacious in improving postprandial lipaemia and insulin sensitivity in individuals with impaired glucose tolerance (Roche *et al.* 1998; Zampelas *et al.* 1998; Louheranta *et al.* 2002). The efficacy of modifying the type of dietary fats in reducing lipid risk factors for CHD has been extensively reviewed (Williams *et al.* 2004). A meta-analysis of human studies demonstrated that the replacement of dietary SFA with polyunsaturated fatty acids (PUFA) and MUFA lowered fasting plasma LDL cholesterol levels (Gardner and Kraemer 1995). A limited number of studies have investigated the effects of fat type on postprandial TAG levels with some (Weintraub *et al.* 1988; Jackson *et al.* 1999b; Zampelas *et al.* 1994), but not all (Bergeron and Havel 1995) showing that acute or chronic *n*-6 PUFA or MUFA ingestion, in comparison with SFA ingestion, enhanced the clearance of TAG-rich lipoproteins and chylomicron remnants (CMR), and lowered concentration of VLDL. Moreover, it has for a long time been recognised that high dietary intake of long chain *n*-3 PUFA (LC *n*-3 PUFA) is associated with lower risk of CHD (Dyerberg and Bang 1982). Increased consumption of foods high in LC *n*-3 PUFA (i.e. predominantly oily fish) is also a UK government recommendation to tackle CHD. The recent report entitled *Advice on fish consumption: benefits and risks* recommends a daily intake of 0.45 g of LC *n*-3 PUFA (Food Standards Agency 2004). The benefit of dietary intervention with LC *n*-3 PUFA on secondary prevention of a cardiac event has been investigated in several randomised, controlled trials and reported to significantly reduce CHD (Burr *et al.* 1989; GISSI-Prevenzione Investigators 1999). Therefore, evidence shows that there are established mechanisms for MUFA and PUFA in protecting against metabolic syndrome. However, we know very little about the impact of high-fat diets or diets with different types of fat on the microbial ecology of the gut, and, considering the importance of the gut microbiota in generation of metabolically important SCFA and regulation of intestinal and/or systemical inflammation, it is an area of research which deserves closer scrutiny.

A dietary intervention study in young healthy subjects showed that both a low-fat, high-carbohydrate diet and a high-MUFA diet were equally efficacious in improving glucose tolerance (Perez-Jimenez *et al.* 2001). Despite this, the high glycaemic load (GL) of low-fat, high-carbohydrate diets, may negatively

impact on insulin sensitivity, by increasing the demand for insulin secretion (Coulston *et al.* 1983; Knopp *et al.* 2000). Such adverse effects of high-GL diets may exacerbate insulin resistance in MS individuals. Recent human nutritional intervention studies have shown that the quality or type of dietary carbohydrate may also influence insulin sensitivity (Frost *et al.* 1998; Brynes *et al.* 2003). In particular, low-glycaemic index (GI, discussed later) diets have been shown to attenuate the negative effects of a high-GL on insulin sensitivity (Frost *et al.* 1998; Brynes *et al.* 2003). The GI of a food is an indexing of the glycaemic response to a fixed amount of available carbohydrates from a test food and the same amount of available carbohydrates from a standard food (e.g. glucose, white bread), consumed by the same subject (Jenkins *et al.* 2002a; Monro 2003). Dietary intervention studies have shown that the GI of a food may improve insulin sensitivity and increase HDL cholesterol concentrations (Frost *et al.* 1999), decrease LDL cholesterol and TAG in hyperlipidaemic individuals (Jenkins *et al.* 1987) and decrease C-reactive protein levels (Liu *et al.* 2002). Moreover, other studies showed that the low-GI of a food reduced the risk of developing type 2 diabetes (Salmeron *et al.* 1997; Meyer *et al.* 2000). Low-GI foods may be considered dietary constituents, which slowly release carbohydrate, thus resulting in a slow rate of glucose absorption and a low rise in blood glucose levels (Wong and Jenkins 2007). Several factors influence the GI of a food, including gastrointestinal transit, absorption and digestion, the nature of the carbohydrate in the food, the cooking method, the presence of fibre, fat and proteins (Jenkins *et al.* 2002b). A low-GI food is characterised by an attenuated glucose response and consequently lower postprandial rise in gut hormones and insulin (Brand-Miller *et al.* 2002). While high-GL diets may worsen insulin resistance in obese subjects, low-GI diets may address this metabolic imbalance by improving glycaemic control (Ludwig 2002). A food classified as low-GI is more slowly digested and absorbed in the small intestine, thus increasing the likelihood of undigested carbohydrates reaching the colon. As discussed earlier in this section, dietary carbohydrate that escapes digestion in the upper gastrointestinal tract constitute available substrate for colonic bacterial fermentation. Increased saccharolytic activity in the colon is seen as beneficial, driving a well-balanced ecosystem through the energy released by fermentation and providing important nutrients for the host, not least the SCFA acetate, butyrate and propionate. Although much work has been conducted on the fermentation of non-digestible carbohydrates, fibre and prebiotic oligosaccharides by the gut microbiota, there is little or no information on the response of the gut microbiota to low-GI foodstuffs. It is likely that such foods will act in a manner similar to prebiotics and certain dietary fibres, and may have similar health-promoting

**Low-GI foods may be considered dietary constituents, which slowly release carbohydrate, thus resulting in a slow rate of glucose absorption and a low rise in blood glucose levels**

effects. It is important to emphasise that the fibre content of a low-GI food is also valuable. Increased dietary fibre content associated with a low-GI food can have an additive effect to the merits of low-GI foods and bring about a further improvement of glucose and lipid metabolism in hyperlipidaemic individuals, and in individuals with type 2 diabetes or with high risk of developing type 2 diabetes (Bjorck and Elmstahl 2003). This may be due to an increased fermentation of these indigestible carbohydrates by the gastrointestinal microbiota, thus modifying the relative proportions of colonic and systemic propionate and acetate. There is a need for well-designed intervention studies in human volunteers to examine the impact of diets of varying GI on the gut microbiota and biomarkers of chronic disease such as MS.

## 4. Obesity, inflammation and gut microbiota

Obesity appears to be associated with a 'low grade' or 'chronic' inflammation, characterised by increased production of cytokines and acute-phase reactants in white adipose depots, which eventually lead to impaired insulin sensitivity and glucose tolerance (Wellen and Hotamisligil 2005). The white adipose tissue (WAT) and the liver play an important role in the pathophysiology of obesity-associated inflammation, since they constitute the anatomical site for both metabolic cells (i.e. adipocytes and hepatocytes) and immune cells (i.e. Kupffer cells and macrophages; Hotamisligil 2006). The WAT of obese people is infiltrated with macrophages, which are responsible for the production of high levels of IL-6, TNF-$\alpha$ and IL-1. Conversely, weight loss has been shown to be associated with a decrease in macrophage infiltration and a concomitant reduction of inflammatory molecule gene expression (Clement *et al.* 2004). Pro-inflammatory molecules produced in the WAT might have systemic effects and contribute to the pathogenesis of insulin resistance and CHD. Such cytokines mainly act through activation of NF-$\kappa$B and c-jun $NH_2$-terminal kinase (JNK) cellular pathways, which interact with insulin signalling via inhibitory serine or threonine phosphorylation of insulin receptor substrate (IRS; Hotamisligil *et al.* 1996a, 1996b; Aguirre *et al.* 2000). In particular, TNF-$\alpha$ over-expressed in WAT of obese humans, has been shown to inactivate insulin receptor and to induce insulin resistance (Hotamisligil *et al.* 1996b; Hotamisligil 2006). IL-6, on the other hand, has been linked to increased hepatic production of C-reactive protein (CRP) and, therefore, to increased-CRP plasma levels in obese subjects, which can lead to cardiovascular complications (Ford 2003).

It has been recently shown that dietary fatty acids can directly activate

Toll-like receptors (TLRs) expressed on the surface of adipocytes and macrophages and consequently trigger inflammatory pathways. In particular, TLR4 has been demonstrated to induce inflammatory signalling in mice fed a high-fat diet (Shi *et al.* 2006). As discussed in this section, TLR4 is the receptor for bacterial LPS, the membrane constituent of Gram negative bacteria. Cani *et al.* (2007a) reported that bacterial LPS may be a triggering factor for both inflammation and insulin resistance, following a high-fat feeding in mice. The same authors showed that the 'metabolic endotoxaemia', observed after high-fat intake in the diet or induced by chronic LPS rectal infusion, was associated with adipose tissue macrophage infiltration, hepatic insulin resistance, fasting hyperinsulinaemia and hyperglycaemia, obesity and steatosis. High plasma LPS levels in high-fat fed mice were also associated with a modified caecal microbiota. The levels of dominant microbial populations of the murine gut microbiota (i.e. *E. rectale-C. coccoides* group, *Bifidobacterium* species and Mouse Intestinal Bacteria group) were lower in caecal contents of mice fed a high-fat diet, compared with control mice which were fed a standard chow diet. As bifidobacteria were previously shown to improve intestinal permeability and reinforce mucosal barrier function, the same authors subsequently tested if a restoration of bifidobacteria levels could constitute a remedy for high-fat diet-induced insulin resistance in mice.

OFS supplementation of high-fat fed mice selectively increased bifidobacteria levels, lowered plasma LPS and significantly improved glucose tolerance and inflammatory tone (Cani *et al.* 2007b). These results support the role of the gut microbiota in the inflammatory processes associated with obesity and insulin resistance.

## 4.1. The gut microbiota and energy balance

Evidence from a number of different sources is supporting the possible involvement of the gut microbiota in mammalian energy metabolism. Studies in

mice have highlighted some key aspects of the mammalian host-microbe relationship, suggesting that the gut microbiota plays an important role in retrieving energy for the host from recalcitrant dietary components. Backhed *et al.* (2004) recently compared the body composition of germ-free (GF) C57BL/6 mice with the body composition of conventionally raised (CONV-R) C57BL/6 mice. Young CONV-R mice were shown to have 40% higher body fat content and 47% higher epididymal fat content compared with GF mice, despite consuming a lower amount of the same standard rodent chow diet. In the same study adult GF C57BL/6 mice were colonised with the gut microbiota derived from CONV-R C57BL/6 mice (Backhed *et al.* 2004). The result was a 60% increase in body fat and epididymal fat accompanied by an insulin resistance state within 14 days despite less food intake. Other parameters monitored in the study also suggest an effect of the gut microbiota on the animals' energy homeostasis and hepatic lipogenesis. Fasting glucose and insulin levels increased significantly following colonisation. Leptin levels were also measured. In conventionalised mice (i.e. mice colonised with the gut microbiota of CONV-R mice), blood leptin was significantly higher, reflecting higher lipid content in the adipose tissue. Moreover the colonisation with the gut microbiota of CONV-R mice induced a higher expression of acetyl-CoA carboxylase 1 (Acc1) and Fas, two key enzymes involved in *de novo* lipogenesis. In addition, the expression levels of SREBP-1 and ChREBP, which are transcription factors targeting Acc1 and Fas, were also higher in the liver of CONV-R mice compared to GF. These observations indicate a higher hepatic response to glucose and insulin and a move towards lipogenesis in conventionalised animals compared to their GF counterparts. Interestingly, the conventionalisation also brought about a general increase in the activity of the enzyme LPL, responsible for the release of fatty acids and TAG from circulating lipoproteins in muscle, heart and adipose tissue. The mechanism proposed for such an increase was a suppression of fasting-induced adipose factor (*Fiaf*) in the gut epithelium. *Fiaf* is an inhibitor of LPL; therefore, *Fiaf* suppression in CONV-R mice resulted in an increase of TAG accumulation in the adipose tissue. The same study demonstrated that the rate of absorption of monosaccharides from the large intestine increased after colonisation of GF mice with the gut microbiota from CONV-R animals. Previous studies have also demonstrated that the colonisation of GF animals with the gut microbiota of conventionally raised animals is responsible for an angiogenic effect on the gastrointestinal mucosa (Stappenbeck *et al.* 2002). By increasing the density of the capillaries in the gut mucosa, the microbiota improves efficiency of absorption of monosaccharides from the gut to the portal circulation. The observed increase in body fat in conventionalised animals was interpreted as a consequence of increased uptake of monosaccharides from the gut lumen. The gut microbiota

increases energy that the host can retrieve from the diet by increasing the amount of absorbable monosaccharides and SCFA derived from complex non-digestible polysaccharides. Gut microbial populations are responsible for the breaking down of complex polysaccharides into more simple monosaccharides which can therefore be taken up by the host luminal transporters to the liver.

*Bacteroides* species are highly efficient at processing complex dietary polysaccharides. *Bacteroides thetaiotaomicron*, a prevalent member of the human gut microbiota, has a complex pool of saccharolytic enzymes (i.e. glycosylhydrolases) for the cleavage of plant polysaccharides into oligosaccharides and monosaccharides (Kuwahara *et al.* 2004). Animal studies demonstrated that intestinal colonisation of GF mice only with the species B. *thetaiotaomicron* induced the activity of the host monosaccharide transporters in the gut (Hooper *et al.* 2001). Conventionalisation with *B. thetaiotaomicron* was performed by Backhed *et al.* (2004) to test if just one bacterial species can influence host metabolism and fat storage. The study showed that colonisation of GF mice with *B. thetaiotaomicron* was followed by a 23% increase in body fat. Previous studies demonstrated a promotion of angiogenesis and fortification of the gut mucosal barrier in adult GF mice undergoing gut colonisation with *B. thetaiotaomicron* (Stappenbeck *et al.* 2002; Hooper *et al.* 2003). However, the effect of colonisation of GF mice with polysaccharide-degrading commensal species other than *B. thetaiotaomicron* on the host metabolism was not tested.

*Bacteroides* **species are highly efficient at processing complex dietary polysaccharides**

In conventional mice, the gut microbiota is dominated by the Bacteroidetes and the Firmicutes. Recent studies looking at the composition of the mouse gut microbiota and how it changes with diet have shown that genetically obese *ob/ob* mice had 50% lower Bacteroidetes prevalence and a significantly higher prevalence of Firmicutes compared to lean wild-type mice (Ley *et al.* 2005). Therefore, the increased Firmicutes to Bacteroidetes ratio seemed to promote adiposity in *ob/ob* mice compared to lean *ob/+* and *+/+* mice. These results may appear contradictory in relation to the findings from the experiments on GF mice colonised with *B. thetaiotaomicron*. However, very different animal models were employed in these studies. GF mice have no history of contact with commensal bacteria and their gut physiology and energy metabolism have evolved in complete absence of bacteria and the energy derived from microbial polysaccharide fermentation. Conversely, conventional animals, including *ob/ob*, *ob/+* and *+/+* mice, have evolved alongside a commensal microbiota, which can contribute at least 10% of the daily energy intake through polysaccharide fermentation in the large bowel. Therefore, the host's

interaction with particular bacterial species may not be comparable between these two very different models.

These studies suggest that obesity may affect the composition of the gut microbiota. Further, metagenomic studies in the same groups of animals investigated the metabolic attributes that go along with the 'obese microbiota phenotype' (Turnbaugh *et al.* 2006). Sequencing of bacterial DNA isolated from the caecum of *ob/ob*, *ob/+* and +/+ mice revealed that the gut microbiome of obese mice was enriched with sequences encoding for glycoside hydrolases involved in the breakdown of indigestible dietary polysaccharides (i.e. starch/sucrose, galactose, butanoate), ABC transporters (ATP binding cassette transporters) to import the end-products of polysaccharide processing, and enzymes involved in the formation of the major fermentation end-products. Moreover, higher concentrations of acetate and butyrate were also observed in the caecum of genetically obese mice compared to lean mice. The same study also demonstrated that the observed metabolic characteristics associated with the 'obese microbiota phenotype' (i.e. increased adiposity) were transmissible through transplantation of the gut microbiota from *ob/ob* mice to GF mice. After being colonised with an 'obese microbiota', adult C57BL/6J mice had significantly higher body fat percentage and significantly higher caecal levels of Firmicutes compared to mice that had been colonised with the gut microbiota harvested from lean +/+ donors (Turnbaugh *et al.* 2006).

More recent studies have confirmed this link between body fat and the gut microbiota in humans. Ley *et al.* (2006) monitored the changes in the gut microbiota of obese people over one year of dietary intervention with either a fat-restricted or a carbohydrate-restricted low-calorie diet. Before changing their diet, obese people had significantly higher abundance of Firmicutes and significantly lower predominance of Bacteroidetes compared to lean control individuals. After dietary intervention this profile changed towards a more lean-type profile, with significantly increased levels of Bacteroidetes and significantly decreased levels of Firmicutes. Moreover these changes in gut microbiota were shown to be positively and significantly correlated with body weight loss (Ley *et al.* 2006). However, the studies are based on the analysis of very few subjects and should thus be treated with caution.

The impact of diet on obesity development has also been shown to differ between GF animals and those possessing a gut microbiota. Backhed *et al.* (2007) showed that GF mice were protected from the obesogenic effect of a high-fat, high-sugar Western-style diet. Two possible mechanisms might explain the differential response to diet in GF mice when compared with

microbiota-associated mice. On one side, the LPL inhibitor *Fiaf* was shown to be necessary to protect GF mice from diet-induced obesity, since GF knockout mice lacking *Fiaf* developed obesity in consequence of a Western style diet. Conversely, AMP-activated protein kinase (AMPK) levels in the muscle of GF mice were significantly higher than in conventionalised animals, thus suggesting that GF mice have higher fatty acid oxidation in peripheral tissues, which involves a higher rate of energy burning (Backhed *et al.* 2007).

## 5. Conclusion

Despite recent studies suggesting a different gut microbiota between obese and lean mice and humans, the role of the gut microbiota in the development of obesity is still unclear. The description of the 'obese gut microbiota', mainly by one research group, needs confirmation by others using complementary molecular microbiological techniques. Similarly, very little is known about how the Western-style diet affects the gut microbial composition and how this might relate to important risk factors for cardiovascular and metabolic diseases.

To identify the gut microbiota as a biomarker of obesity, further investigations are needed to test the relevance of the gut microbiota in the aetiology and/or maintenance of obesity. The impact of both genetic and obesogenic environmental factors (e.g. diet) on the gut microbiota of obese people needs closer examination. Further studies should investigate whether the composition and activity of the gut microbiota differ between obese and lean individuals, and should measure the effects of the diet on the gut microbiota in association with recognised risk factors of MS (e.g. modulation of insulin resistance, obese-associated inflammation, enterohepatic recirculation of bile acids). Finally, particular attention should be paid to investigate the role of bifidogenesis in obesity-associated disorders through human dietary intervention studies using prebiotics in obese volunteers on high-fat, high-carbohydrate obesogenic diets.

# 6. Appendix

## Table 1: Relative abundance of dominant human gut bacterial groups and corresponding main acidic fermentation end-products (adapted from Louis *et al.* 2007)*

| Bacterial group | | Abundance (typical % of total bacteria | Fermentation end-products |
|---|---|---|---|
| Firmicutes | Clostridial clusters XIV a + b | 10.8-29 | |
| | *Roseburial/Eubacterium rectale* group | 2.3-8.8 | Butyrate, formate, lactate |
| | *Eubacterium hallii* | 0.6-3.8 | Butyrate, formate, acetate |
| | *Ruminococcus obeum* | 2.5 | Acetate |
| | *Lachnospira* spp. | 3.6 | Formate, acetate, lactate, succinate |
| | Clostridal cluster IV | 25.2 | |
| | *Faecalibacterium prausnitzii* | 3.8-15.4 | Butyrate, formate, lactate |
| | *Ruminococcus bromii, Ruminococcus flavefaciens* | 1.7-10.3 | Acetate, formate, lactate, succinate |
| | Clostridial cluster IX (*Megasphaera* spp., *Veillonella* spp.) | 7.1 | Propionate, various minor acids |
| | Clostridial cluster XVI | | |
| | *Eubacterium cylindroides* | 0.4-1.4 | Butyrate, acetate, lactate, succinate, formate |
| | *Lactobacillus/Enterococcus* | 0.01-1.8 | Lactate, acetate |
| Actinobacteria | *Bifidobacterium* spp. | 2.5-4.9 | Lactate, acetate, formate |
| | *Atopobium* spp. | 2.1-11.9 | Acetate, formate, lactate |
| Bacteroidetes | *Bacteroides-Prevotella* group | 8.5-27.7 | Acetate, propionate, succinate |
| Proteobacteria | *Escherichia coli, Salmonella, Klebsiella, Desulfovibrio* | 0.1-0.2 | Lactate, acetate, succinate, formate |

*The abundance is expressed as mean values of the percentage of total bacteria, based on FISH data from several publications, as reported by Flint (2006). The reported fermentation end-products are indicative of cultured representatives

## Table 2: Main functions of the human gut microbiota and their physiological role in the host organism

| Function | Physiological effects | References |
|---|---|---|
| Fermentation and production of short chain fatty acids (SCFA) | Source of energy (acetate, propionate → brain, muscle, heart; butyrate → colonocytes) | Hooper *et al.* 2002; Fleming and Floch 1986 |
| | Anticancer properties (butyrate → inhibition of proliferation and induction of apoptosis) | Siavoshian *et al.* 2000; Bornet *et al.* 2002; Pryde *et al.* 2002; Grunstein 1997 |
| | Lipid metabolism (acetate substrate for *de novo* lipogenesis; propionate → inhibitor of HMG-CoA synthase and reductase) | Wong *et al.* 2006; Wolever *et al.* 1991; Jenkins *et al.* 1991; Bush and Milligan 1971; Rodwell *et al.* 1976 |
| | Decrease of pH of the gut environment (↑solubility of bile acids,↑minerals absorption, ; ammonia absorption and inhibition of the growth of potentially pathogenic clostridia) | Cummings *et al.* 1987; Coudray *et al.* 2003; Vince *et al.* 1978; Roberfroid 2005 |
| Deconjugation of bile acids and enterohepatic circulation | Production of secondary bile acids (potential procarcinogens) | Heuman *et al.* 1989; Tannock 2004; De Smet *et al.* 1998; Marteau *et al.* 1995; Jackson and Lovegrove 2002; Watkins 1985; Ridlon *et al.* 2006 |
| | ↓fat emulsification and absorption | |
| | ↓absorption of bile acids into the liver | |
| | ↑bile acids excretion | |
| | ↑hepatic bile acids synthesis from cholesterol | |
| Immunomodulation | Barrier effect to invading pathogens (production of antimicrobial compounds and bacteriocins; induction of IgA production; competition for nutrients and mucosal attachment sites; lowering of intestinal pH; induction of mucin production) | Bernet *et al.* 1994; Cotter *et al.* 2005; Gibson *et al.* 1997; Mack *et al.* 2003; Maqueda *et al.* 2008; Macpherson *et al.* 2005 |
| | Maintenance of mucosal barrier integrity (activation of toll-like receptor (TLR) signalling pathways) | Rakoff-Nahoum *et al.* 2004; Cario *et al.* 2007 |
| | Intestinal anti-inflammatory effect (suppression of nuclear factor (NF)-kB pathways;↓expression of pro-inflammatory cytokines, chemokines, and pro-inflammatory enzymes, i.e. nitric oxide synthase (NOS) and cyclooxygenase-2 (COX-2)) | Schiffrin and Blum 2002; Cui *et al.* 2004; Riedel *et al.* 2006; Lammers *et al.* 2003; Watson and McKay 2006 |

## Table 3: Definition of the metabolic syndrome (MS) according to the International Diabetes Federation (IDF; adapted from Alberti *et al.* 2006)*

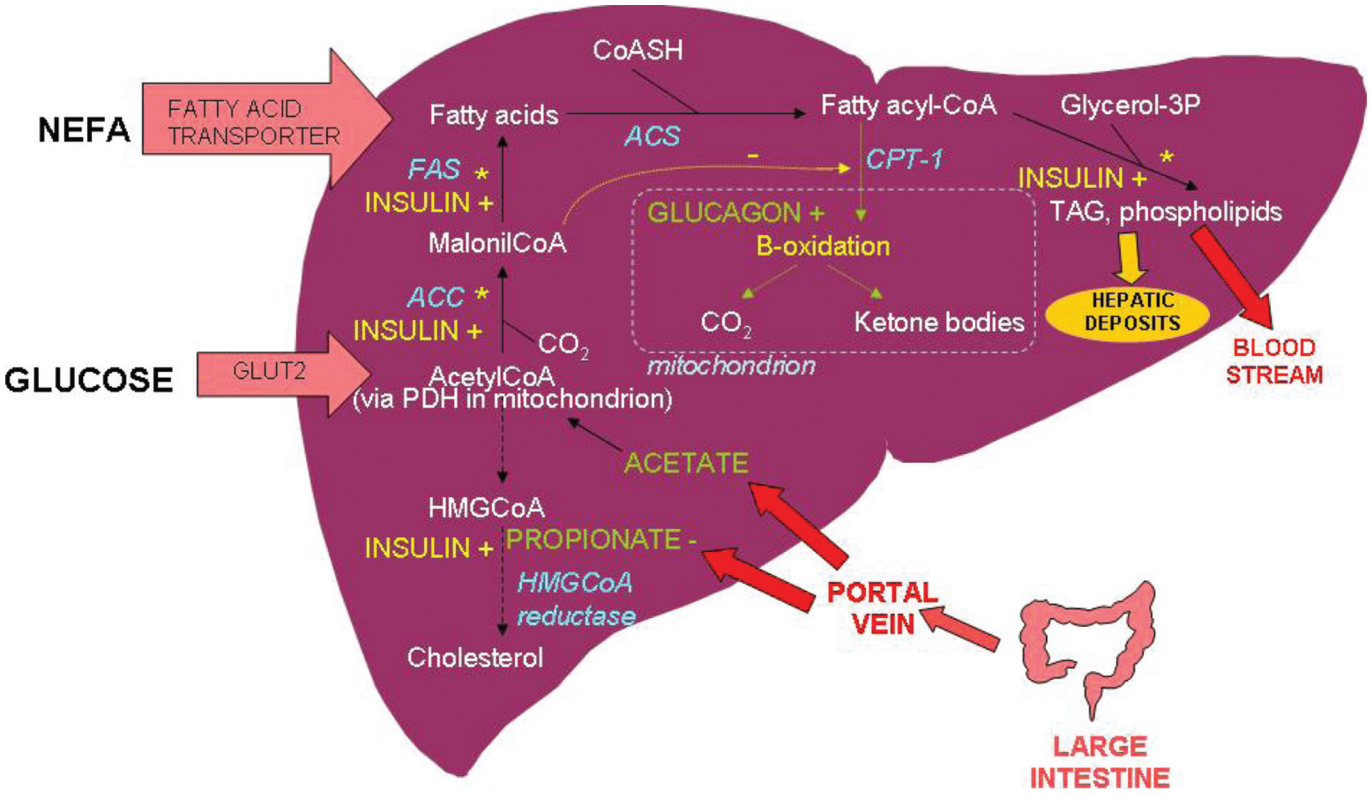
| Function | Definition of characteristic |
|---|---|
| Central obesity | Waist circumference ≥95 cm for Europid men and ≥80 cm for Europid women, with ethnicity-specific values for other groups |
| *Plus any two of the following four factors:* | |
| Raised triacylglycerol (TAG) level | ≥150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality |
| Reduced high-density lipoprotein (HDL)-cholesterol | <40 mg/dL (1.03 mmol/L) in males and <50 mg/dL (1.29 mmol/L) in females, or specific treatment for this lipid abnormality |
| Raised blood pressure (BP) | Systolic BP ≥130 or diastolic BP ≥85 mm Hg, or treatment of previously diagnosed hypertension |
| Raised fasting plasma glucose | ≥100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes |

*The table summarises the characteristics that a person must show to be defined as having MS

## Table 4: Additional metabolic parameters that appear to be related to the metabolic syndrome (MS) and which are used in research studies as risk factors of the MS (adapted from Alberti *et al.* 2006)

| Additional metabolic parameter for MS | Associated biomarker |
|---|---|
| Abnormal body fat distribution | General body fat distribution |
| | Central fat distribution |
| | Adipose tissue biomarkers (e.g. leptin, adiponectin) |
| | Liver fat content |
| Atherogenic dyslipidaemia (beyond elevated TAG and low HDL cholesterol level | ApoB (or non-HDL cholesterol) |
| | Small LDL particles |
| Dysglycaemia | Glucose tolerance by oral glucose tolerance test (OGTT) |
| | Fasting insulin/proinsulin levels |
| | Homeostasis model assessment of insulin resistance |
| | Insulin resistance by Bergman minimal model |
| Insulin resistance (other than elevated plasma glucose) | Elevated free fatty acids (fasting and during OGTT) |
| | M value in the euglycaemic hyperinsulinaemic clamp technique for determining insulin sensitivity |
| | Measurement of endothelial dysfunction |
| Vascular dysregulation (beyond elevated BP) | Microalbuminuria |
| | Elevated C-reactive protein |
| | Elevated inflammatory cytokines (e.g. TNF-$\alpha$, IL-6) |
| Proinflammatory state | Decrease in adiponectin plasma levels |
| Prothrombotic state | Fibrinolytic factors (e.g. PAI-1) |
| | Clotting factors (e.g. fibrinogen) |
| Hormonal factors | Pituitary-adrenal axis |

Figure 1: Hepatic fatty acid metabolism and *de novo* lipogenesis (adapted from Frayn 2003a)




Non-esterified fatty acids (NEFA) from the plasma enter the hepatocyte through a carrier-mediated process and can follow two major pathways: formation of TAG and phospholipids or oxidation. Fatty acids are esterified with the reduced form of Coenzyme A (CoASH) by the enzyme AcylCoA synthase (ACS) and can enter the mitochondrion through the action of the enzyme carnitine-palmitoyl transferase-1 (CPT-1), where they follow the β-oxidation pathway. Alternatively, fatty acids may be incorporated into glycerolipids. In conditions of an excess of carbohydrate glucose may be utilised for *de novo* lipogenesis. Plasma glucose enters the hepatocyte through the glucose transporter 2 (GLUT2) and it generates AcetylCoA from pyruvate, via pyruvate dehydrogenase (PDH) in the mitochondrion. AcetylCoA is subsequently converted into Malonyl-CoA by the enzyme AcetylCoA carboxylase (ACC) and finally into fatty acids by the enzyme fatty acid synthase (FAS). Cholesterol synthesis from AcetylCoA involves formation of 3-hydroxy-3- methylglutaryl-CoA (HMGCoA) by the enzyme HMGCoA synthase and further reduction of HMGCoA by HMGCoA reductase. Insulin up-regulates both the synthesis of lipids from NEFA and the *de novo* lipogenesis. Acetate, derived from colonic fermentation, may be used as substrate for lipid synthesis from AcetylCoA. Propionate may inhibit the incorporation of acetate into fatty acids (mechanism not shown) and the activity of HMGCoA reductase.

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