Chapter 3
Pathophysiology / metabolism / integrative physiology

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Section A. Introduction

Type 2 diabetes mellitus is manifested by progressive defects in pathways controlling glucose metabolism in tissues such as skeletal muscle, adipose tissue and liver, such that they become insulin resistant. Insulin resistance is a condition in which normal amounts of insulin are inadequate to produce an appropriate glucose response with increased uptake in fat and muscle and decreased production from liver cells. Insulin resistance is one of the fundamental defects in type 2 diabetes and may appear many years prior to the onset of clinical disease; it is also a problem in type 1 diabetes. Thus, understanding the pathogenesis of insulin resistance may provide clues to prevention and treatment of diabetes.

Many people with type 2 diabetes are also obese, thereby linking these two metabolic disorders since obesity is closely associated with insulin resistance. Insulin resistance is also found with a cluster of medical conditions including stroke, non-alcoholic fatty liver disease, polycystic ovary syndrome, lipodystrophies, asthma, some cancers, and Alzheimer's disease. Importantly, the incidence of type 2 diabetes increases with age, which is a major concern in Europe where longevity is increasing. The cause of the vast majority of cases of insulin resistance is currently unknown, but multiple organs are involved. Over the last decades, substantial progress has been made in defining the molecular steps and important tissues controlling insulin action. However, an integrated approach is warranted to resolve the complex physiology governing the metabolism of important energy sources, including glucose, lipid, and protein, and the mechanisms by which these fuels contribute to whole body metabolic balance. By identifying the steps controlling insulin action on glucose, lipid, and protein metabolism, new targets for development of pharmacological strategies for prevention and treatment of diabetes and associated disorders can be explored.

Research activities directed towards identifying the cause of type 2 diabetes have now transcended into the post-genomic era in which genetic information is examined in multiple healthcare situations throughout the lives of individual people with diabetes. Thus, a physiological assessment or 'metabolic fingerprint' of novel diabetes risk genes is now possible. Clearly, genetic factors play a role in the manifestation of type 2 diabetes; however, diabetes-related polymorphisms may only explain a fraction of the variance in quantitative glycaemic traits. Environmental factors, such as diet and exercise, can thus have a profound impact on metabolic pathways controlling glucose and lipid metabolism. More research is needed to determine the mechanism for altered glucose and energy homeostasis in type 2 diabetes and understand better why obesity and inactivity are strong risk factors for insulin resistance in diabetes.

Cellular communication or 'signalling' is critical for all biological functions and ensures proper growth and survival of all cells and organs in the body. Intracellular signalling as well as more distant communication within and between organs is fundamental for the control of glucose and lipid uptake and metabolism in both type 1 and type 2 diabetes. Insulin resistance arises from disturbances in communication within and between beta cells, liver, adipose tissue and skeletal muscle. Signalling defects that modify insulin secretion and action thus underlie the manifestations of diabetes, with elevations in blood glucose and ultimately the development of complications in the microvascular and macrovascular systems. One important goal of future research is to systematically identify the inter- and intracellular signalling pathways controlling cellular and whole-body glucose and lipid homeostasis. Through the discovery of key regulatory proteins in glucose and energy homeostasis, new diabetes prevention and treatment targets can be identified and validated.

Obesity, inappropriate quantity and quality of food, and a lack of physical activity are major risk factors for the development of type 2 diabetes, and consequently this is becoming a major challenge for the European healthcare system. Studies have shown that physical exercise training and diet are among the most powerful physiological interventions known to maintain or improve insulin

sensitivity. However, the specific genes and pre- and post-natal environmental factors that influence the effects of exercise or diet on insulin sensitivity and the intra-individual variations in metabolic health are incompletely understood. Remarkably, physical exercise enhances insulin sensitivity, and bariatric surgery in obese patients with diabetes 'cures' diabetes even before major weight loss is achieved, but the mechanism(s) for these improvements is unknown. Also, the underlying mechanism(s) by which alterations in energy intake and expenditure contribute to peripheral insulin sensitivity and the prevention of diabetic complications in type 2 diabetes is unclear. Such information is likely to be valuable for developing therapies, as well as improving public health.

Through the use of large cohorts of people with and without diabetes with insulin resistance, DNA/tissue banks can be developed to help identify novel genetic, hormonal or lifestyle-related factors that impact the development of type 2 diabetes. The prospective design of clinical studies allows for the validation of biomarkers and predictors of type 2 diabetes, as well as mechanistic and clinical insight into novel pharmaceutical and physiological approaches to prevent the conversion from impaired glucose tolerance to type 2 diabetes.

Although we recognise that it is the progressive decline of beta cell function and mass that underlies type 2 diabetes (*see* Chapter 2), insulin resistance is certainly a major contributing factor to the disease and that should be addressed in any therapeutic strategy. This chapter focusses on the integration of whole body and cellular physiology to understand the complex mechanisms involved in the development of insulin resistance in diabetes. The ultimate goal is to identify disease markers and both physiological and pharmacological therapeutic strategies, such as diet/exercise and anti-diabetic drug therapy respectively, to improve insulin sensitivity, correct whole-body glucose and energy balance and thereby to prevent and treat the complications of diabetes.

Section B. Scientific advances and major challenges

Integrated physiology approaches for understanding tissue-specific and whole body metabolism

Over the past decade, new insight into the tissue-specific role of key insulin-sensitive organs, including skeletal muscle, adipose tissue, and liver, has been offered through the generation and characterisation of tissue-specific transgenic and knock-out mice, rodent models of insulin resistance and model organisms (i.e. *C. elegans*, *Drosophila melanogaster* and Zebrafish). Basic research efforts using diabetic animal models have brought forward a new understanding of the causes of insulin resistance, and this has uncovered new pathways that may be amenable to diabetes prevention and treatment. Translation of these basic findings to the clinical setting has been quite successful in Europe, specifically with support from the European Commission FP6 programmes including the Network of Excellence Programme EUGENE2 (European Network on Functional Genomics of Type 2 Diabetes) and large integrated projects such as EXGENESIS (Health benefits of exercise: identification of genes and signalling pathways involved in effects of exercise on insulin resistance, obesity and the metabolic syndrome) and HEPADIP (Hepatic and Adipose Tissue and Functions in the Metabolic Syndrome), which also integrate genomic approaches. It is however most unfortunate that there is no existing mechanism to allow for continued funding of such highly successful European projects, decreasing the return for investment.

Insulin orchestrates a series of intra- and intercellular signalling events to promote fuel utilisation including fat storage in adipose tissue and glucose uptake in skeletal muscle as well as inhibiting glucose output from the liver. Mitochondria are the seat of fatty acid oxidation and recent evidence highlights the importance of mitochondrial dysfunction in the development of peripheral insulin resistance. The capacity to switch between glucose and lipids as primary fuel sources under fed and fasting conditions respectively has been characterised as metabolic flexibility. Lipid overload or 'lipotoxicity' is one example of the tight communication between organs, such that in times of nutrient overload, when adipose tissue cannot store excess lipids, fat is accumulated inappropriately in skeletal muscle and liver. This build-up of lipids and lipid intermediates causes insulin resistance and metabolic inflexibility. Another example of organ-organ communication is the interaction between the insulin-producing beta cells of the pancreas with peripheral tissues to control whole body insulin sensitivity. Indeed, evidence from genome-wide association studies designed to identify type 2 diabetes susceptibility genes reinforces the notion that impaired insulin secretion is critical to the development of the disease.

In addition to the peripheral control of whole-body glucose homeostasis, central regulatory mechanisms have been identified, highlighting the brain as an important organ in the control of insulin sensitivity and appetite/energy homeostasis. Intestinal flora, nutrient absorption and gut hormones have also been shown to feed back on central and peripheral tissues. Studies that identify the molecular determinants of tissue-to-tissue communication may have therapeutic repercussion for individuals with diabetes and other age-related diseases. A greater integration and assimilation of the tissue-specific contributions to the development of insulin resistance is required. Such efforts will unify genetic, molecular, biological, physiological and clinical information to harness an integrative view of the physiological mechanisms controlling metabolic and gene regulatory pathways involved in glucose and energy homeostasis. Through a better understanding of the complex physiology that underlies the development of insulin resistance and diabetes, future treatment strategies may be fine-tuned to treat defects in specific tissues in an effort to tailor individual patient therapy.

Insulin resistance and insulin action

The identification and characterisation of cell surface and nuclear receptors, as well as signalling proteins including protein kinases, phosphatases, histone acetylases and deacetylases, has afforded a more thorough understanding of the mechanism by which insulin controls glucose and lipid metabolism. Critical nodes or hubs of communication in the insulin signalling cascade have been identified at the cellular and whole-body level, through the use of pharmacological inhibitors and molecular tools such as small interfering ribonucleic acid (siRNA) to specifically silence gene expression, or transgenic/knockout technology in small animal. Researchers have identified the isoform-specific roles of the insulin receptor substrates (IRS) and distal (feed-forward) signalling elements, including protein kinase B (Akt), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) isoforms. For example, through a series of protein phosphorylation events involving the PI 3 kinase/PKB pathway and possibly via the Rab guanosine triphosphate (GTP)ase-activating proteins, insulin promotes vesicle trafficking of

glucose transport proteins from a specific intracellular compartment to the cell surface to facilitate glucose uptake in skeletal muscle and adipose tissue.

Inhibitory (feed-back) signals emanating from the insulin receptor have also been implicated in the development of insulin resistance. Indeed, both a negative and a positive role of serine phosphorylation events have been shown to control insulin signalling. Several protein kinases, including MAPK (JNK, ERK), PKC isoforms, and suppressor of cytokine signalling (SOCS) proteins, have been shown to negatively regulate insulin signalling by inhibiting IRS signalling. In addition to the growth-promoting effects of insulin, insulin-like growth factors (IGFs) play a role in growth/differentiation. However, despite efforts from numerous laboratories, the mechanism by which insulin primarily affects metabolism, whereas IGFs primarily affect growth under 'physiological' conditions, is still incompletely understood. Recent advances link nutrient sensors including 5' adenosine monophosphate-activated protein kinase (AMPK), a master regulator of energy metabolism, insulin sensitivity, and exercise- or stress-mediated responses, as well as the amino acid-sensitive mTOR/p70 S6 kinase pathway in the control of insulin sensitivity. Indeed, elevated levels of nutrients (i.e. free fatty acids, glucose or amino acids), hormones [insulin, IGF, triiodothyronine (T3)] or even cytokines can negatively impact insulin signalling. This complex protein network forms multiple signalling pathways that communicate important information from the external environment to promote growth and metabolism. Ultimately these pathways control the metabolic health of the individual with diabetes, as they are involved in the sensing of excess glucose and lipids in the diabetic environment and, more specifically, the circulation.

Defects in insulin sensitivity and energy homeostasis are not only hallmarks of type 2 diabetes, but they also represent significant risk factors for the development of aging-related diseases such as Alzheimer's disease and cognitive impairment. Furthermore, a narrow interrelation exists between pathways and tissues that regulate lifespan and insulin action at the cellular level, as exemplified by indications that sirtuins play a role in energy metabolism, insulin sensitivity and longevity. Thus, evidence is emerging for a highly coordinated interaction between hormones and nutrients to fine tune signalling pathways controlling metabolic and gene regulatory events. Through these basic research efforts, a link between diabetes and neurological disease is also appreciated, which may have an impact for our ageing population.

Inflammation and insulin sensitivity

Clinical and experimental evidence indicates that inflammation, provoked by macrophages, leads to insulin resistance and type 2 diabetes. Chronic inflammation in adipose tissue triggers the release of various cytokines that cause insulin resistance in skeletal muscle, adipose tissue and liver. High levels of cytokines [e.g. tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β] inhibit insulin signalling at the cellular level and ultimately impair whole body insulin-mediated glucose uptake. In addition to macrophages, a variety of tissues including adipocytes, liver and skeletal muscle have been shown to secrete proteins into the circulation (tissuekines), which in turn act on other peripheral tissues to modify insulin sensitivity. These tissuekines are also biomarkers that can be used to identify individuals at high risk of developing type 2 diabetes. Inflammation and insulin resistance are frequently associated factors that are likely to contribute to the development of both type 1 and type 2 diabetes and diabetic complications, particularly macrovascular outcomes. The causal relationship between these abnormalities is incompletely understood. Interestingly for any understanding of the integrated pathophysiology of diabetes, we now appreciate that inflammation also arises in islets in type 2 diabetes and that beta cells can secrete cytokines. It will be important to discover more about the tissue-specificity of inflammation in type 2 diabetes, and how this impacts inter-organ crosstalk and ultimately metabolism.

The inflammatory cytokine TNF-α was one of the first factors shown to cause insulin resistance in fat cells. Thus, inflammatory stress contributes to peripheral insulin sensitivity in diabetes. Stress-activated signals involved in inflammatory responses and insulin resistance include the signalling molecule inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β) and the transcription factor NF-κβ. Molecular or pharmacological inhibition of IKK-β or other protein kinases, including JNK, or MAP4K4 for example, prevents TNF-α-induced insulin resistance. An oral delivery of a therapeutic dose of siRNA designed against specific protein kinases in the inflammatory stress signalling cascade can prevent TNF-α-induced insulin resistance. Other adipokines, including adiponectin and in some cases IL-6, have a positive impact on peripheral insulin sensitivity, whereas elevated levels of serum retinol binding protein are negatively correlated with insulin resistance in type 2 diabetes. Thus, emerging

evidence indicates that type 2 diabetes and obesity are low-grade inflammatory states. The highly regulated dialogue between serum factors, including tissuekines, and metabolically active insulin-sensitive peripheral organs highlights a dynamic regulation of glucose and energy homeostasis. These findings underscore the deleterious cycle between increased abdominal fat mass, increased cytokine production, and a worsening of the metabolic profile in patients with diabetes due to increased obesity.

Environmental factors in type 2 diabetes pathogenesis

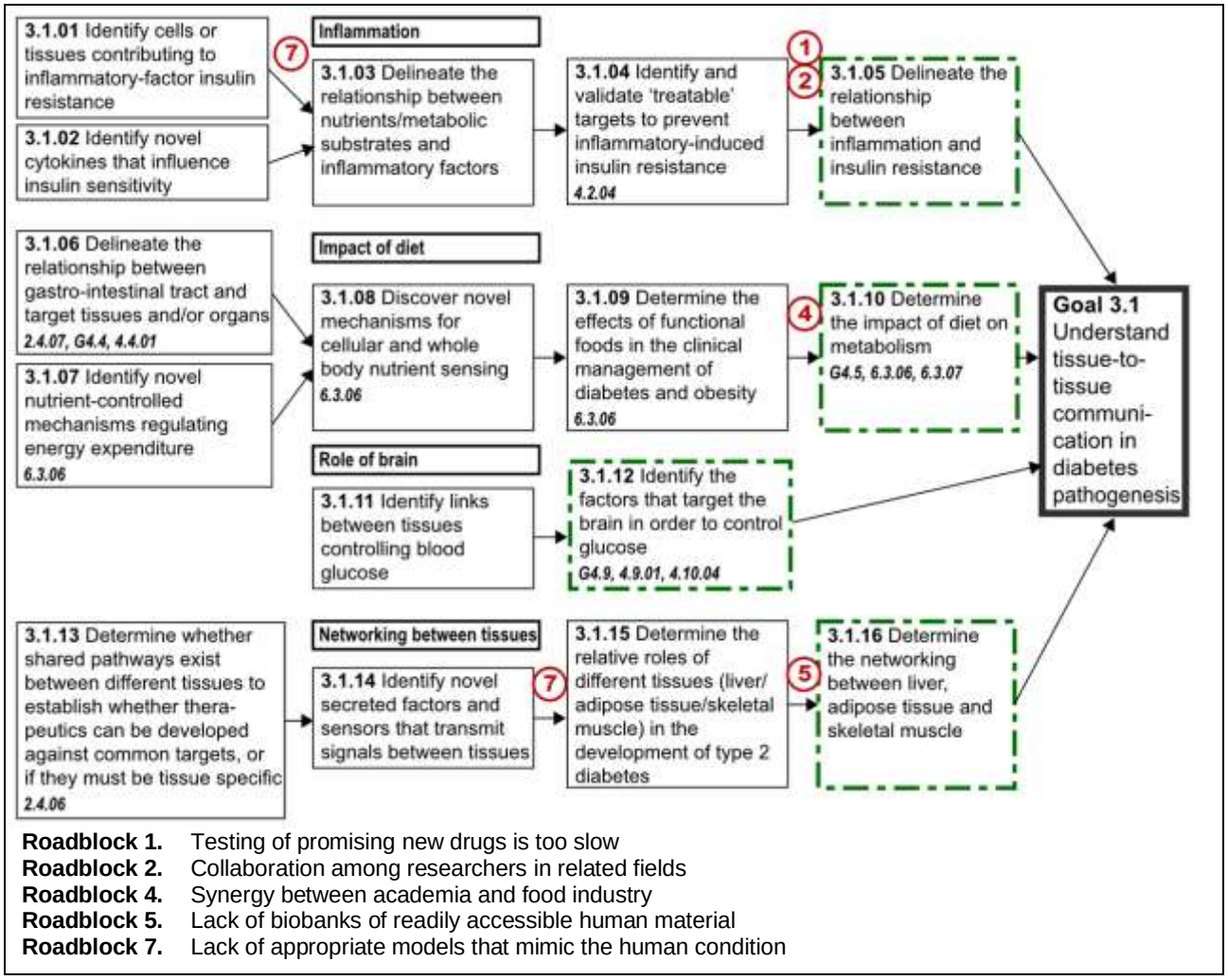
Researchers have made progress in understanding the important influence of gene-environment interactions on metabolic pathways that govern insulin sensitivity and energy homeostasis. Calorie rich high-fat diets, coupled with a sedentary lifestyle, constitute major environmental factors linked to the development of peripheral insulin resistance and type 2 diabetes. Regular exercise improves insulin sensitivity in type 2 diabetes and reverses some of the deterioration in functional capacity that has traditionally been considered an inevitable part of ageing. The identification of exercise-response proteins has provided insight into novel and conventional pathways that regulate glucose and energy homeostasis. For example, exercise and cellular stress increase glucose transport and metabolism via an insulin-independent pathway. Exercise training can also promote mitochondrial biogenesis and lipid metabolism, which in turn reduces body weight and enhances insulin sensitivity. Research into the basic biochemical and molecular adaptations to lifestyle factors, including diet and exercise training, will pave the way for evidence-based dietary and exercise recommendations for the prevention and treatment of insulin resistance in diabetes. Increased knowledge in proper nutrition programmes aimed at the prevention of insulin resistance may also influence the public sector to improve food choices, production, and service with the aim of preventing obesity. Increased knowledge of physical exercise training in the treatment of diabetes may influence the sports and fitness industry, as well as the public educational school systems to increase awareness of lifestyle modifications.

State-of-the-art technologies, including gene array and proteomic studies, have provided evidence that exercise has a profound effect on expression of messenger RNA and proteins involved in metabolism and growth responses. Low-calorie nutritionally balanced diets improve insulin sensitivity. Moreover, severe calorie restriction can also reduce the risk of heart attack, stroke, and diabetes. Dietary intake of macro- and micronutrients is also coupled with proper glucose control. Evidence is emerging that specific genetic factors may also influence an individual's response (or lack thereof) to exercise and diet. Functional genomics have been important to link novel exercise- or diet-sensitive molecular markers with specific phenotypes and genotypes. Proper integration of these factors will allow for the design of individualised programmes to improve metabolic health. A systematic integration of genetic and environmental factors, in particular exercise and diet, is needed to define lifestyle intervention programs to prevent or treat insulin resistance in patients with diabetes.

Section C. Road map reports

Links between Goals and Milestones in the DIAMAP report are noted within the text and also in the diagrams in *italics*. Roadblocks are shown in red circles, listed below the diagrams and described at the end of the chapter. Goals and Milestones considered a priority are indicated with a broken green line.

Goal 3.1. Understand tissue-to-tissue communication in diabetes pathogenesis



Roadblock 1. Testing of promising new drugs is too slow
Roadblock 2. Collaboration among researchers in related fields
Roadblock 4. Synergy between academia and food industry
Roadblock 5. Lack of biobanks of readily accessible human material
Roadblock 7. Lack of appropriate models that mimic the human condition

Introduction and background

Nearly all tissues of the body are involved in the development of insulin resistance and diabetes. A major challenge in the development of successful diabetes treatments is to identify the complex network between the various tissues and to understand the communication between tissues in order to prevent and treat the condition successfully. Even beyond the classical tissues that are currently recognised as playing a key role in the control of glucose and energy homeostasis, future work should be expanded to include the role of brain in the control of blood glucose and feeding behaviour, as well as the role of the taste and smell centres, sensation perception and chemical recognition. This research track can be crosslinked with both Chapter 1 and Chapter 2. There is great interest from the diabetes and obesity branch of the biotechnology/pharmaceutical sector to learn about these mechanisms and their dysregulation in metabolic disease to develop improved therapy. The role of the food industry and fast-food chains should be considered since nutritional factors can be linked to insulin sensitivity and energy homeostasis. Thus, synergy between academic and industry research efforts may present an opportunity to stimulate progress in diabetes care and treatment. Four research tracks are proposed to achieve this Goal as outlined below.

Track 1. Delineate the relationship between inflammation and insulin resistance to identify factors and therapeutic approaches that influence the metabolic health of the diabetic patient

Insulin resistance, i.e. the situation when insulin cannot exert its correct effect and diabetes subsequently develops, has recently been linked to inflammation, and obesity-linked insulin resistance as well as type 2 diabetes are now considered to be low-grade inflammatory states. Research to unravel the pathophysiological events leading to onset of the inflammatory response, and to prevent this inflammatory-induced insulin resistance, would allow for new and improved therapeutic approaches. Results obtained with animal models should be carefully validated in humans, since the immune system, inflammatory processes and the nature of the secreted factors (i.e. adipokines secreted from adipose tissue; myokines secreted from muscle) can differ between humans and animal models. This should be considered, since the crosstalk between tissues revealed in animal models could be absent or different in humans. Caution should be considered when extrapolating results from rodents to humans.

Milestone 3.1.01. Identify cells or tissues contributing to inflammatory-factor insulin resistance

The cells or tissues that contribute to inflammatory-factor insulin resistance must first be identified since the targeted therapy against the causative tissue may improve blood glucose control. Based largely on studies in animals, adipose tissue has been shown to be an important tissue for promoting the inflammatory response associated with insulin resistance, although the nature of the specific adipose tissue (white, brown) or the anatomical location (visceral, subcutaneous, atypical sites) of its depots must be identified. The exact cellular origin of this inflammation and more specifically the exact macrophage phenotype, requires unambiguous identification, particularly in people with diabetes, since the majority of the evidence to date is derived from studies of morbidly obese rodents. The role of other tissues should also be considered since this will identify whether tissue-specific or whole body treatment approaches need to be designed.

Milestone 3.1.02. Identify novel cytokines that influence insulin sensitivity

Cytokines (adipokines, myokines) that influence insulin sensitivity must next be identified since earlier studies provide strong experimental and clinical evidence that for example, communication between the macrophages or adipose tissue and the hypothalamus or skeletal muscle/liver by leptin, TNF-α, and adiponectin can control body weight and blood glucose. Elucidation of the exact mechanisms involved in the secretion of these cytokines, the pathways involved in their action, the tissues upon which they act, the role of the transcription factors involved and the involvement of all types of stress (such as endoplasmic reticulum stress, oxidative stress) are important steps between the inflammatory response, secretion of cytokines and the development of insulin resistance in diabetes.

Milestone 3.1.03. Delineate the relationship between nutrients/metabolic substrates and inflammatory factors

The relationship between nutrients, metabolic substrates (including lipid intermediates), and inflammatory-factor insulin resistance requires clarification, as does the relationship between the immune system and the cells responsible for utilising the metabolites. This is important because dietary factors may influence the inflammatory response either directly or indirectly due to obesity, and this may have an impact on healthy food choices for the individual with diabetes. This is also an expanding area for the food industry, where research into functional foods may offer an opportunity for future development of nutritional interventions to prevent or treat insulin resistance.

Milestone 3.1.04. Identify and validate 'treatable' targets to prevent inflammatory-induced insulin resistance

The preceding Milestones should identify new pathways and factors in the inflammatory process linked to obesity and diabetes, first on a molecular level, then at the whole-body level in order to evaluate whether inflammation is a cause or a consequence of insulin resistance. If this is a primary cause, then it is mandatory to determine whether therapies that specifically target inflammation are effective in preventing diabetes from becoming established. A major challenge will be to define the 'window of opportunity' for intervention before irreversible diabetes sets in, for prevention approaches with lifestyle and/or drugs. If successful therapies can be identified, this could potentially reduce the disease burden for the person with diabetes and society at large (*see also* Milestone 4.2.04).

Milestone 3.1.05. Delineate the relationship between inflammation and insulin resistance

The culmination of the research activities along this track will establish the links between inflammation and insulin resistance. This may refocus clinical treatment of diabetes to include correcting inflammatory processes since there is an evolving notion that insulin resistance and diabetes have

immunological components such that anti-inflammatory strategies may prove to be efficacious to treat the metabolic consequences of excess adiposity commonly seen in adult diabetes.

Track 2. Impact of diet on metabolism

Milestone 3.1.06. Delineate the relationship between the gastrointestinal tract and target tissues and/or organs

The role of the gastrointestinal tract in insulin resistance should be defined, both because it is the first organ that controls food absorption, and then as an organ with an endocrine role [the incretin concept, especially the gut hormone glucagon-like peptide 1]. The potential role of intestinal flora in obese patients in modifying nutrient absorption and efficacy should be elucidated since this may be important for proper weight balance. Obesity surgery (bariatric surgery) has now been proposed as a potential treatment for diabetes linked to obesity, but the mechanism remains elusive. Therefore, mechanisms for the therapeutic anti-diabetic effect of the surgery need to be understood in order to reduce the increase in obesity and the ensuing surge in insulin resistance and diabetes that comes as a consequence (*see also* Milestones 2.4.07, 4.4.01 and Goal 4.4).

Milestone 3.1.07. Identify novel nutrient-controlled mechanisms regulating energy expenditure

Research should be directed to a better understanding of nutrient-controlled mechanisms because cellular energy sensors may influence energy expenditure and weight management. The previously identified pathways such as AMPK (a master regulator of energy metabolism) and mTOR should be studied in detail. Emphasis should be put on the role of diurnal variation on gene expression, which impacts on food intake, appetite control, and metabolism due to the recent evidence that there is a certain 'circadian' rhythm for several biological functions that modify growth and metabolism. This may be important for the treatment of diabetes because of the link between obstructive sleep apnoea and increased type 2 diabetes risk.

Milestone 3.1.08. Discover novel mechanisms for cellular and whole-body nutrient sensing

We must understand better how nutrient signalling interferes with insulin action and insulin sensitivity. Some metabolites appear crucial in the development of insulin resistance, such as free fatty acids, glucosamine and ceramides, although the actual list is unlikely to be complete. This will require large-scale metabolomic studies of the chemical 'fingerprint' of blood and tissues involved in diabetes pathogenesis to be performed in clinical material from people with different stages and forms of diabetes. Research will aim at a more perfect understanding of the relationship between cellular and whole body nutrient sensing. This may improve food and dietary recommendations in order to ensure better quality of life for the person with diabetes.

Milestone 3.1.09. Determine the effects of functional foods in the clinical management of diabetes and obesity

Functional foods are those intended to be consumed as part of the normal diet but which contain certain ingredients that enhance health or reduced the risk of disease. Functional foods are foods or dietary components that may provide a health benefit beyond basic nutrition. Such foods should be developed and tested for their ability to reduce the risk of developing diabetes, both in the general population and in high-risk sub-groups, and also as a means of improving diabetes treatment. In this regard, the role of the food industry and fast-food chains should be considered since nutritional factors can be linked to insulin sensitivity and energy homeostasis.

Milestone 3.1.10. Determine the impact of diet on metabolism

A healthy diet is a crucial part of managing diabetes. Diet directly impacts on glucose and lipid metabolism, and this may impact body weight and diabetes. Diet is particularly important in the development of diabetes and plays a role in the management of the condition. Since it is more difficult for cells to interact with insulin in a person who is overweight, a low-fat diet is important for people with diabetes. Large-scale nutritional studies in humans should be undertaken to determine the impact of diet (and of functional foods) on metabolism and diabetes risk. Studies should be performed in the ageing population since nutritional requirements may vary with advanced ageing (*see also* Goal 4.5). Dietary recommendations to improve blood glucose levels, manage body weight, and lower cardiovascular risk factors such as low density lipoprotein (LDL) cholesterol and blood pressure should be developed. The potential role of metabolites and pollutants in the development of obesity and diabetes should be considered since there is some evidence that people who are exposed to high levels of certain pollutants either in the food we eat or the air we breath may be at an increased risk of developing type 2 diabetes (*see also* Goal 4.5 and Milestone 6.3.07).

Track 3. Role of the brain in the control of blood glucose

Milestone 3.1.11. Identify links between tissues controlling blood glucose
The brain is intimately linked to the control of blood glucose through communication with the liver and peripheral tissues. For example, the liver and peripheral tissues secrete hormones and metabolites into the blood stream as a form of communication or 'signal' that interacts with the brain to deliver information about the metabolic state of the body. This may occur when blood glucose levels or circulatory lipid levels rise or fall in response to a meal. The physiology behind this complex interplay between the brain and the rest of the body is still incompletely described. The molecular and structural equivalents of the central regulators of blood glucose need to be identified to fully understand the manner in which the brain influences metabolic responses for proper blood glucose control in the person with diabetes and to understand how relevant regions of the brain (cerebral hemispheres, hypothalamus) interact with peripheral tissues.

Milestone 3.1.12. Identify the factors that target the brain in order to control glucose
The fundamental biological mechanisms underlying the processes by which the central (brain) and the peripheral tissue (rest of the body) interact should be outlined in animal models and translated to non-invasive studies in humans so that these basic research efforts can be translated into improved clinical treatment of diabetes. Advanced neuroimaging techniques, such as nuclear magnetic resonance imaging (MRI), are required for such studies. Future studies in humans should address the cause versus the consequence of the impairments in blood glucose control in patients with diabetes, for example by separating genetic from environmental influences, interventional studies, and investigations on cerebral processes. Specifically, structural remodelling (such as neuronal sprouting, neurogenesis, and/or angiogenesis) in response to pharmacological blood-glucose lowering interventions must be understood to improve upon current diabetic therapy, which is still sub-optimal in stabilising blood glucose level throughout the day. Multimodal brain imaging techniques using different magnetic resonance technologies [voxel-based morphometry (VBM), diffusion tensor imaging (DTI), functional MRI, perfusion MRI, MR-spectroscopy], and electroencephalography (EEG), magnetoencephalography (MEG), positron emission tomography (PET), transcranial magnetic stimulation (TMS), and optical imaging will be essential. Endocrinologists, neurologists, neuroimaging specialists and neuroscientists must collaborate in this effort because it is impossible and likely unethical to obtain brain biopsies from living humans for mechanistic studies to understand the role of the brain in blood glucose control (*see also* Milestones 4.9.01, 4.10.04 and Goal 4.9).

Track 4. Networking and communication between tissues

Milestone 3.1.13. Determine whether shared pathways exist between different tissues in order to establish whether therapeutics can be developed against common targets, or if they must be tissue-specific
Insulin exerts its effect in adipose, muscle, and liver, but the pathways stimulated by the hormone are not completely identical. The common (e.g. glucose uptake by adipocytes and muscle cells) as well as the different pathways (e.g. those specific to exercise in muscle) need to be characterised in fine detail; the points of difference need to be identified. The role of counter-regulatory hormones (such as glucagon in liver, catecholamines in liver/muscle) should be evaluated as these hormones participate in 'tissue-specificity' and modulate insulin action in diabetes (especially glucagon). The role of orphan hormones should be addressed (*see also* Milestone 2.4.06).

Milestone 3.1.14. Identify novel secreted factors and sensors that transmit signals between tissues
Gut, liver, muscle and adipose tissue do not function independently but have reciprocal influences, and it is important to identify the link between these tissues and beyond (i.e. beta cells, *see also* Chapter 2). This research will involve identifying factors secreted by each of these tissues, which act as endocrine factors on the others to control glucose and lipid metabolism. Although the list of such secreted factors is continuously increasing, it is probably far from complete because results from the human genome study predict additional secreted proteins are expressed in our genome. Current factors include gastrointestinal and other hormones (*see also* Milestone 3.1.02), neuropeptides and sympathetic nerve activity. Understanding the role of these factors may reveal new points of entry in the treatment of diabetes.

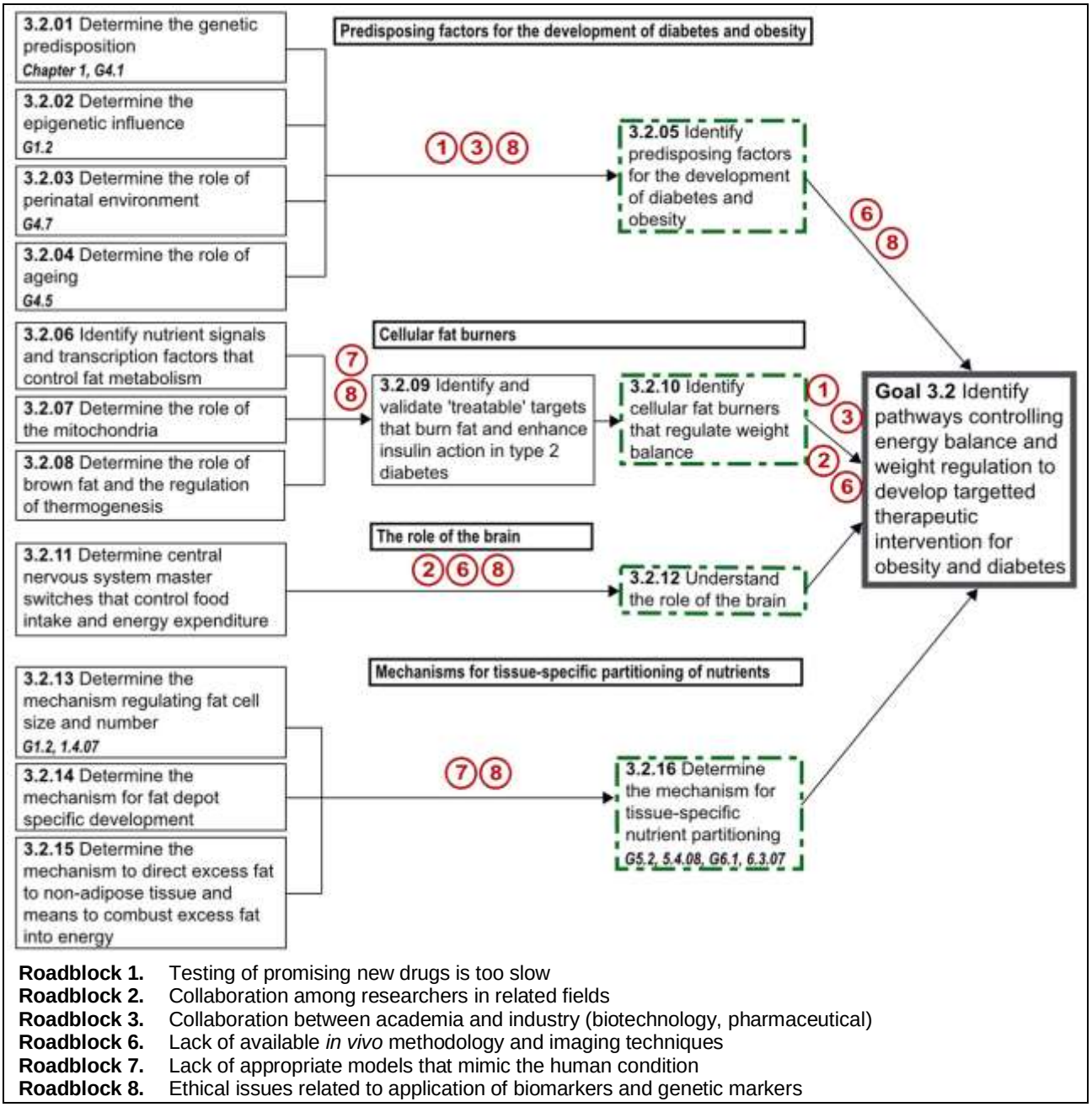
Milestone 3.1.15. Determine the relative roles of different tissues (liver, adipose tissue, skeletal muscle) in the development of type 2 diabetes
Insulin resistance does not develop simultaneously in all tissues, and not all pathways are insulin resistant. These puzzling observations need clarification, and a better understanding of those

pathways is crucial before further research is undertaken because this will help identify which tissues/organs and their specific insulin signalling pathways should be treated. For example, the manifestation of defects in glucose metabolism compared with lipid metabolism in the different insulin-sensitive tissues needs to be determined. Also important is the observation that insulin resistance affects essentially only the metabolic action of insulin and not the growth promoting actions. This is very relevant in the context of the current issue of a potential line between cancer and diabetes. *In vivo* approaches must be used in mouse models and humans to study the behaviour of the different insulin-sensitive tissues. Mouse models are relevant because they can be genetically modified or manipulated in ways that would be otherwise impossible in humans. Human studies are needed to confirm the physiological relevance of eventual discoveries from animal studies and to move closer to patient care.

Milestone 3.1.16. Determine the networking between liver, adipose tissue, and skeletal muscle

Relative roles of these tissues in the development of type 2 diabetes can be determined by using integrative *in vivo* approaches in diabetic animal models and humans. Understanding tissue-to-tissue communication requires determination of the networking between what are currently considered to be the major tissues for glucose turnover i.e. liver, muscle and adipose tissue. This will be important for future pharmacological efforts to develop tissue-specific therapy and for understanding the basic physiology of metabolic regulation.

Goal 3.2. Identify pathways controlling energy balance and weight regulation to develop targeted therapeutic intervention for obesity and diabetes



Roadblock 1. Testing of promising new drugs is too slow
Roadblock 2. Collaboration among researchers in related fields
Roadblock 3. Collaboration between academia and industry (biotechnology, pharmaceutical)
Roadblock 6. Lack of available *in vivo* methodology and imaging techniques
Roadblock 7. Lack of appropriate models that mimic the human condition
Roadblock 8. Ethical issues related to application of biomarkers and genetic markers

Introduction and background

The following tracks consider established research lines addressing the control of energy balance and weight regulation. They all have in common major research activities on more or less isolated issues that are successfully ongoing, but what is missing and essential is the translation to therapy (including drug development) to benefit people with diabetes. This is important because current therapies are insufficient to normalise blood glucose and enhance insulin sensitivity. There is a need for appropriate models that mimic the human condition (especially neurobehavioural aspects). Furthermore, species-to-species differences need to be considered since comparative physiology may enhance the understanding of the (patho)physiological regulation and treatment strategies to control energy balance and weight regulation. Thus, direct access to pathways of fat oxidation, mitochondrial function and fat tissue (trans)differentiation and functionality in humans is mandatory, which – despite most recent technical and methodological developments

- is still limited. This requires intensive synergistic efforts between experimental and clinical researchers both at academic and industry levels as a perfect model of translational research.

Track 1. Predisposing factors for the development of diabetes and obesity

Milestone 3.2.01. Determine genetic predisposition
Information regarding the genes associated with type 2 diabetes and obesity will continue to become available at an ever-increasing rate because several genome-wide association studies have been performed with new studies in progress. However, identification of truly causative genetic variants for diabetes and physiological data are trailing behind, and a complete picture is unlikely to evolve soon. This research track can be crosslinked with Chapter 1 since genetic and epigenetic factors, as well as changes in chromatin and DNA methylation, may contribute to the development of diabetes and obesity. Technological advances, such as next-generation gene sequencing, will provide a platform for tracking down functional variants. Ultimately, studies with sufficiently large numbers of subjects (cohorts) with relevant sub-groups with specific clinical characteristics (glucose and insulin parameters, energy metabolism) are required to understand the pathophysiological role of the variants and make them available for drug development, prevention and prediction of specific therapies (important for personalised medicine) (*see also* Goal 4.1 and Chapter 1).

Milestone 3.2.02. Determine the epigenetic influence
Ideally, gene-environment interactions will be uncovered by measuring the role of as many environmental/nutritional factors as possible on gene expression profiles. This research track can be cross-linked with the larger population-based studies highlighted in Goal 1.2 with molecular mechanistic studies as proposed in this track.

Milestone 3.2.03. Determine the role of the perinatal environment
There is evidence that the environment that the fetus experiences *in utero* can influence diabetes risk later in life. Whether the mother is obese or has diabetes may leave a lasting imprint on the fetus, which carries over into adult life. Large birth cohorts and prospective long-term follow-up studies would provide an opportunity to illuminate the role of the perinatal environment (i.e. the developmental period of the fetus) relative to the genetic factors from the parents. Of relevance is the clinical observation that an 'adverse/unfavourable' intrauterine environment is associated with fetal growth retardation and low birth weight, which leads to increased risk of diabetes in adult life. Through the identification and prevention of diabetes risk factors during the fetal development phase, diabetes may be prevented later in life (*see also* Goal 4.7).

Milestone 3.2.04. Determine role of ageing
The influence of ageing on metabolic health will be determined by studying people at different stages of life (childhood, puberty, old age). With ageing, the beta cells fail, and peripheral insulin resistance develops. As our population grows older due to increased longevity, it will be important to determine the mechanism for ageing-induced insulin resistance so that successful preventive measures can be implemented (*see also* Goal 4.5). In parallel, specialised animal models whereby specific genes have been either over-expressed or omitted need to be developed, and specific cell lines will be necessary to reveal the biological pathways important for the effect of ageing on insulin action. Finally, epigenetic changes and whether they can be inherited across generations need to be addressed in the field of diabetes.

Milestone 3.2.05. Identify predisposing factors for the development of diabetes and obesity
The overall aim is to identify and validate genetic and epigenetic factors that directly contribute or increase risk for the development of diabetes and obesity. Identifying factors associated with early and later programming, including the role of the perinatal environment and the ageing process will also help decipher the mechanism for the development of diabetes and obesity. The collective goal is to uncover the gene/environmental influence on insulin sensitivity and secretion and energy homeostasis to reduce the risk for diabetes and obesity.

Track 2. Cellular fat burners

Milestone 3.2.06. Identify nutrient signals and transcription factors that control fat metabolism
Studies of the global profile of circulating and tissue-specific metabolites (i.e. the 'metabolome') are needed to elucidate the abundance of amino acids, fatty acids and other compounds and the extent to which differences exist in diabetes. This may also allow for identification of biomarkers for diabetes (*see also* Chapter 1). Nutrient-specific sensing pathways need to be studied in a tissue-specific manner because of the specialised role of insulin-sensitive organs in the regulation of whole-body glucose and lipid control. Subsequently, tailored dietary interventions should be tested in different cohorts in order to develop genotype/phenotype-specific approaches to

prevention and treatment of obesity and diabetes because this may be one way to identify sub-groups of patients with diabetes based on the metabolomic profile and may impact future diabetes treatment (*see also* Chapter 1).

Milestone 3.2.07. Determine the role of the mitochondria
Mitochondria and endoplasmic reticulum are considered to be central to the (cellular) inflammatory responses to fat cell expansion in obesity, metabolic alterations in diabetes, and also in the development of diabetic complications. The relative roles of inherited versus acquired factors controlling the cellular regulation of mitochondrial function need to be identified in order to determine the causative role in mitochondrial impairments in diabetes. Methods for assessing mitochondrial function in humans require novel development, because non-invasive tools are lacking. Some of these new methods will take advantage of stable isotopes and metabolic imaging [magnetic resonance spectroscopy (MRS) and positron emission tomography (PET)]. In particular, the impact of ageing and related pathways requires attention in humans. By modifying transcription factors and the effects of small molecules on compounds of the electron transport chain and mitochondrial enzymes, it will be easier to identify pathophysiological events and develop therapeutic approaches to fat burning to prevent fat storage and obesity-induced insulin resistance.

Milestone 3.2.08. Determine the role of brown fat and the regulation of thermogenesis
Brown adipose tissue for regulating thermogenesis has been identified in humans. But there is no information on its distribution and physiological role in various human genotype/phenotypes, which require refined methods of metabolic imaging and molecular medicine. In humans, fat tissue development and (trans)differentiation in the direction of brown adipose tissue in adults is an important challenge because, based on studies in rodents, it may contribute to the regulation of lipid oxidation. This is important since people with diabetes have defective lipid oxidation, which may contribute to obesity and a worsening of the metabolic profile. By increasing brown versus white adipose tissue, defects in lipid oxidation may be corrected, and obesity may be prevented in the patient with diabetes.

Milestone 3.2.09. Identify and validate 'treatable' targets that burn fat and enhance insulin action in type 2 diabetes
Recent evidence suggests that brown adipose tissue and skeletal muscle arise from the same lineage, and several genes may be expressed in both organ types. Ideally, treatable targets that burn fat and enhance insulin action will be uncovered by measuring gene expression profiles in brown adipose tissue and skeletal muscle in different states of differentiation. These factors can be further validated in cell and animal models to determine whether they are directly involved in fat burning. The ultimate goal is to identify proteins that can burn fat to enhance insulin action and improve glucose control.

Milestones 3.2.10. Identify cellular fat burners that regulate weight balance
Excessive accumulation, pathological distribution and abnormal function of fat tissue and fat oxidation in tissues such as liver, skeletal muscle and heart are now considered central in determining the risk for obesity, type 2 diabetes and premature atherosclerosis. The overall aim is to identify and validate 'treatable' targets that burn fat to reduce abdominal (stomach) body fat to treat obesity and early atherosclerosis, which are becoming common features of diabetes.

Track 3. Role of the brain

Milestones 3.2.11. Determine central nervous system master switches that control food intake and energy expenditure
The master switches regulating food intake and energy expenditure are located in the brain, and evolution has biased homeostasis, hedonic behaviour and reward systems towards a positive energy (and central glucose) balance. It is necessary to identify the molecular and structural equivalents of the central 'set points' for body weight, satiety and glucose and to understand how on an individual basis the relevant regions of the brain (cerebral hemispheres, hypothalamus) promote feeding and distribute nutrients to various tissues in the body like liver, skeletal muscle and adipose tissue. It is important to understand the fundamental biological mechanisms underlying these processes (in animal models). Moreover, the ever-increasing gap to understanding human physiology/pathophysiology and behavioural biology must be bridged such as understanding the enterohepatic-brain axis, the role of central insulin resistance, central nutrient sensing and appetite control, the cognitive and emotional evaluation of food stimuli because overeating and obesity are deleterious to metabolic health, especially in people with diabetes. It is crucial to advance neuroimaging techniques for use in metabolism beyond applications in neurology and neurosurgery because invasive studies are not possible to perform in humans.

Milestone 3.2.12. Understand the role of the brain
Future studies in humans should address causal relationships, metabolic and cellular mechanisms, for example by separating genetic from environmental influences, interventional studies, and investigations on cerebral processes. Structural remodelling (such as neuronal sprouting, neurogenesis, and/or angiogenesis) in response to interventions must be understood to improve patient care. Multimodal brain imaging using different magnetic resonance technologies [voxel based morphometry (VBM), diffusion tensor imaging (DTI), functional MRI, perfusion MRI, MR-spectroscopy], electroencephalogram (EEG), magnetoencephalography (MEG), PET, transcranial magnetic stimulation (TMS), and optical imaging will be essential. Endocrinologists, neurologists, neuroimaging specialists and neuroscientists must collaborate towards this Goal because this is a multifaceted area of biology involving all these fields, and current interactions among these potential partners are weak.

Track 4. Mechanisms for tissue-specific partitioning of nutrients

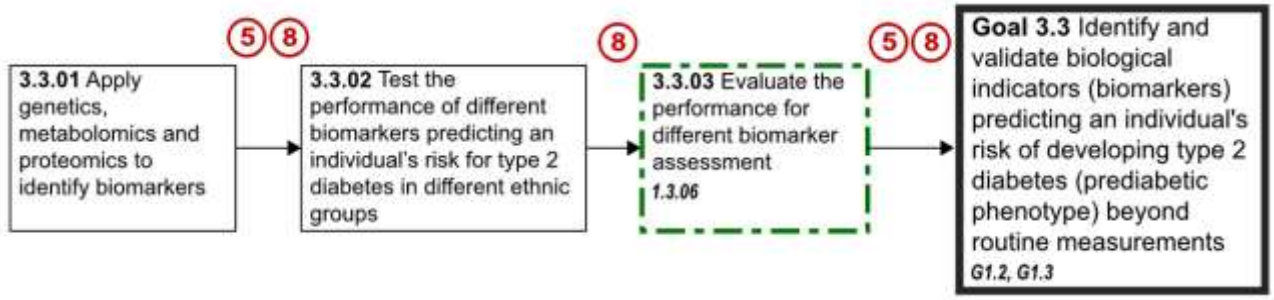
Milestone 3.2.13. Determine the mechanism regulating fat cell size and number
Adipose tissue dysfunction is a primary defect in obesity and includes enlarged fat cell size, increased fat cell number, alterations in fat cell turnover and partitioning of fat (visceral, ectopic and intracellular fat deposition). This area is important to study because adipose tissue dysfunction contributes to the increased risk of obese people for type 2 diabetes, hypertension, coronary heart disease, stroke, and several types of cancer (*see also* Milestone 1.4.07 and Goal 1.2).

Milestone 3.2.14. Determine the mechanism for fat depot specific development
The mechanisms causing adipose tissue dysfunction (gene-environment/nutrient-behaviour interaction, hypoxia, different stresses, inflammation, impaired vascularisation and others) are not well understood because studies in humans are lacking. The causative genetic and nutrient factors that underline the enlarged fat cells (including specialised lipid droplet proteins such as perilipin, FSP27, CIDE protein family members) and the mechanism regulating the increased fat cell number (such as recruitment of fat cell precursors, apoptosis, renewal of fat cells) will be critical to understand so that diet and pharmacological therapies can be developed to curtail the enlarging fat mass in people with diabetes, which is known to worsen their metabolic condition. Diabetogenic and atherogenic fat distribution (impaired adipose tissue endocrine function and impaired cross-talk with other tissues such as muscle, liver and brain, lipodystrophies) should be identified. This line of research will allow development of novel therapeutic approaches for targeting and reversing adipose tissue dysfunction in obesity and diabetes.

Milestone 3.2.15. Determine the mechanism to direct excess fat to non-adipose tissue and means to combust excess fat into energy
Directions for the development of pharmaceutical therapy include the search for mechanisms which: a) direct excessive fat to non-adipose tissue (trans-differentiation of adipose tissue, fat-muscle cross-talk), b) combust excessive fat into energy (role of brown fat in human obesity, mitochondrial dysfunction, nutrient signalling, role of food contaminants), and c) act specifically in adipose tissue to improve insulin sensitivity in people with diabetes.

Milestone 3.2.16. Determine the mechanism for tissue-specific nutrient partitioning
Tissue-specific insulin action may provide a mechanism for nutrient partitioning between liver, skeletal muscle, adipose, and other organs in the patient with diabetes. With diabetes, nutrients such as lipids are inappropriately partitioned from adipose tissue to skeletal muscle and liver. Redirecting lipid storage to adipose tissue rather than skeletal muscle or liver for oxidation may treat obesity and insulin resistance in patients with diabetes, thereby lowering blood glucose levels. New research is needed to identify the mechanism by which nutrients such as glucose, lipids and amino acids are partitioned or directed to specific tissues and how this impacts on insulin sensitivity in people with diabetes. This track can be cross-linked with Milestone 5.4.08 and Goal 5.2 in terms of drugs targeted towards the kidney to enhance glucose excretion and with Milestone 6.3.07 and Goal 6.1 for macrovascular complications, especially the cardiovascular system.

Goal 3.3. Identify and validate biological indicators (biomarkers) for predicting an individual's risk of developing type 2 diabetes (prediabetic phenotype) beyond routine measurements



Roadblock 5. Lack of biobanks of readily accessible human material
Roadblock 8. Ethical issues related to application of biomarkers and genetic markers

Introduction and background

The vast majority (80-90 percent) of all cases of diabetes are type 2 diabetes. Therefore, it is essential to identify 'prediabetic' individuals who are at high risk of developing this disease. In this regard, research to enhance prediction of diabetes has been highlighted in Chapter 1, while the goal here is to understand the biology as a means to identifying new biomarkers of prediabetes. Among risk factors predicting type 2 diabetes are obesity, central obesity (large waist circumference), family history of diabetes, previous history of gestational diabetes or mild elevation of fasting or 2-hour glucose in an oral glucose tolerance test, lipid disorders (high total triglycerides, low high-density lipoprotein cholesterol), smoking, and high blood pressure. Limited information is available on the significance of biomarkers (i.e. biological markers associated with disease) for predicting an individual's risk beyond these measurements. Thus, simple markers to identify disease risk are lacking. For example, methods of proteomics and metabolomics are still under development for the screening of large numbers of subjects. This is a major limitation since the assessment of novel markers based on these methods should be done in large population cohorts. Special effort should be taken to develop inexpensive assays suitable for large-scale screening. One commonly used biomarker of diabetes is an elevation in fasting blood glucose; however, this marker does not predict the disease, rather it is a sign of the disease. Recent advances in the genetics of type 2 diabetes have indicated that large prospective population-based studies, including thousands of subjects, are needed to estimate the role of genetic variants as risk factors for type 2 diabetes. Therefore, pan-European resources are needed to fund large population-based studies to reach numbers needed to test biomarkers. Crucial information on ethnic-specific differences will also be required. This research track will emphasise molecular and cellular studies and will synergise with the clinical studies described in Goals 1.2 and 1.3. Through the identification and molecular validation of biomarkers that predict diabetes, clinical efforts can be focussed on preventive medicine to reduce diabetes incidence in the general public.

Milestone 3.3.01. Apply genetics, metabolomics and proteomics to identify biomarkers

Only a few studies have investigated the effects of different single-nucleotide polymorphisms (SNPs) on the risk of type 2 diabetes beyond clinical routine measurements. In these studies the interaction between genetic markers and lifestyle and environment was not investigated. Furthermore, 'epigenetic' changes, for example chromatin remodelling and DNA methylation of the promoters, could modify the risk of type 2 diabetes. MicroRNAs that regulate gene expression and direct silencing machinery to promoters could also potentially modify the risk of type 2 diabetes. RNA expression is useful in identifying genetic determinants of human metabolic traits. For example more than 50 percent of the transcriptional network in adipose tissue is associated with human obesity, and an extensive genetic component underlies gene expression traits in the adipose tissue. There is direct overlap here with Goals 1.2 and 1.3, and this research effort must be well coordinated.

Metabolomics means studies of the metabolome, i.e. a set of metabolites in cells, tissues and biofluids while proteomics is the corresponding study of proteins in such samples. Changes in the concentrations of metabolites or proteins signify amplified responses in biological systems to the interaction between genes and the environment.

New analytical methods combined with information technology provide a sensitive way to measure the extended metabolome and proteome in readily accessible human samples including plasma or urine. This will allow exploration of the mechanisms of many complex diseases and identification of putative biomarkers. Lipids, a subset of the metabolome, play key structural, energy storage and signalling roles in biological systems. New emerging mass spectrometry approaches afford sensitive detection of individual lipid molecular species.

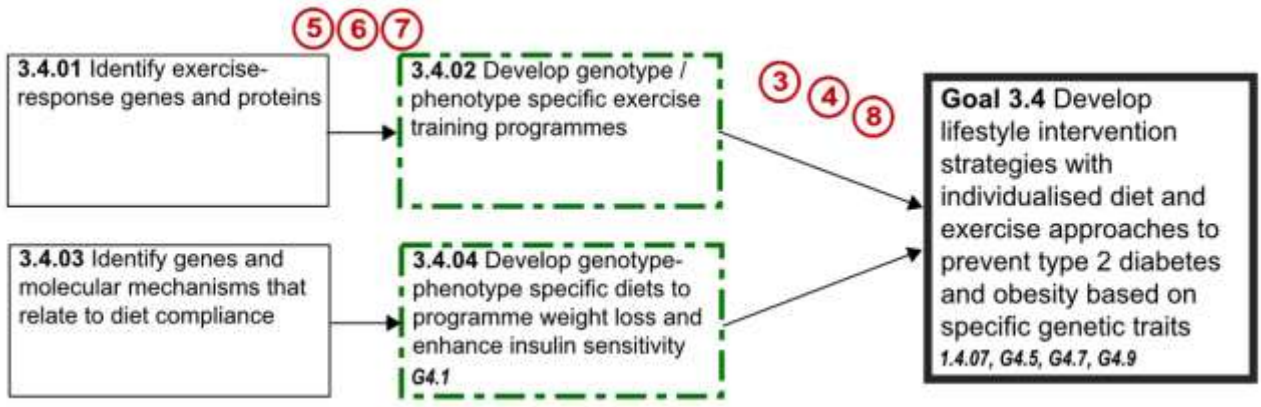
Milestone 3.3.02. Test the performance of different biomarkers predicting an individual's risk for type 2 diabetes in different ethnic groups

All these new measurements should be evaluated in large population-based cohorts and in different ethnic groups for their performance to predict type 2 diabetes. The risk of type 2 diabetes differs between ethnic groups (for example between people of European descent and Asians), and therefore the performance of biomarkers as risk indicators for the disease is likely to differ between them. For example, *TCF7L2* gene variants are the best markers of genetic risk of type 2 diabetes in people of European descent, but not in Asians, because of a low frequency of risk alleles. It is possible that metabolomic and proteomic markers similarly differ among ethnic groups, but no information is currently available in this respect. Emphasis should be placed on understanding the basic biology and molecular mechanisms of action of new predictive biomarkers for type 2 diabetes risk.

Milestone 3.3.03. Evaluate the performance for different biomarker assessment

Clinical studies are required to evaluate the biomarkers as valid predictors of diabetes onset. The validation of biomarkers as early predictors of clinical disease can enhance health risk assessment and contribute to effective new disease prevention policies throughout Europe. Efforts along this track should be focussed on the validation of biomarkers at the biological level using molecular and cell biology tools, whereas efforts in Goal 1.3 should be focussed on the clinical evaluation of biomarkers.

Goal 3.4. Develop lifestyle intervention strategies with individualised diet and exercise approaches to prevent type 2 diabetes and obesity based on specific genetic traits



Roadblock 3. Collaboration between academia and industry (biotechnology, pharmaceutical)
Roadblock 4. Synergy between academia and food industry
Roadblock 5. Lack of biobanks of readily accessible human material
Roadblock 6. Lack of available *in vivo* methodology and imaging techniques
Roadblock 7. Lack of appropriate models that mimic the human condition
Roadblock 8. Ethical issues related to application of biomarkers and genetic markers

Introduction and background
The increased incidence of poor health associated with insulin resistance constitutes a major threat to global health. A cluster of clinically distinct, but metabolically related disorders, including type 2 diabetes and obesity, constitutes a syndrome that ultimately results in premature death. Physical exercise training is among the most powerful physiological interventions known to maintain insulin sensitivity. Accumulating evidence suggests that proper diet and nutrition can also have a positive impact on insulin sensitivity. However, the specific genes and pre- and postnatal environmental factors that influence the efficacy of the exercise- and nutrient-induced benefits on insulin sensitivity, and the intra-individual variations in metabolic health are incompletely understood. To translate discoveries to clinical care, discovery and validation efforts should be as close to human physiology as possible. In this regard, efforts should be taken to validate human genes/proteins identified in preclinical cellular and animal studies. Furthermore, animal models should be developed to match human physiology and specifically the response of humans to exercise and diet, as closely as possible. Emphasis should also be placed on the ageing population to understand the molecular mechanisms for the long-term health benefits of regular physical exercise. Moreover, the link between diabetes and non-classical diseases including cancer, Alzheimer's disease, and sleep apnoea should be considered (*see also* Milestone 1.4.07, Goal 4.5, Goal 4.7, Goal 4.9).

Milestone 3.4.01. Identify exercise-response genes and proteins
Identifying genes and proteins that respond to exercise would provide insight into novel and conventional pathways that regulate glucose, lipid and energy homeostasis in health and diabetes. This information can be used to prescribe exercise-training programmes to people with diabetes in order to promote health benefits, including enhanced insulin sensitivity.

Milestone 3.4.02. Develop genotype/phenotype specific exercise training programmes
Methodology and approaches to identify genes and gene products that relate to exercise compliance and response to lifestyle interventions are needed due to the observation that some individuals respond to exercise with positive effects, such as enhanced insulin sensitivity and reduced body fat, whereas other individuals show limited improvements. Individuals will be able to benefit from specifically developed genotype-phenotype exercise training programmes to enhance whole body insulin sensitivity and glucose metabolism and preserve muscle mass. This is important because exercise training can prevent and treat insulin resistance and muscle wasting in diabetes.

Milestone 3.4.03. Identify genes and molecular mechanisms that relate to diet compliance
Research is needed to identify genes and molecular mechanisms that relate to diet compliance and response to lifestyle intervention.

This is important because of the clinical observations that some individuals comply and respond well to diet programmes designed for weight loss, whereas others are either weight neutral (i.e. do not lose weight) or even gain weight. These genes should provide hints about the specific pathways that control weight loss and enhance insulin sensitivity. This research should form the basis for developing individual diabetes and obesity prevention programmes.

Milestone 3.4.04. Develop genotype-phenotype specific diets to programme weight loss and enhance insulin sensitivity

Individuals differ in their adherence and response to dietary intervention and by developing individual programmes adapted to different responses patients are more likely to be successful. Multidisciplinary approaches that capitalise on genetic, epigenetic, proteomic, physiological, and clinical disciplines are required to gain insight into factors behind the divergent effects of physical exercise training and diet on cellular and whole-body metabolism. State-of-the-art technologies are necessary to accelerate the translation of experimental and clinical discoveries to link novel targets and pathways with phenotype and genotype to define the exercise and diet response on metabolic health. Integrated approaches taking advantage of molecular biology, physiology, and clinical medicine are necessary to deconvolute the complex disease pathophysiology and to unmask exercise and dietary responsive and unresponsive individuals, both at the molecular and the physiological level. Availability of novel animal models to test gene-environment interactions is necessary since early experimental work to dissect molecular mechanism cannot always be performed in humans. Similar approaches are required to determine the effects of diet and specific nutrients on cellular and whole-body glucose and lipid homeostasis. Access to unique collections of genetic and physiological material from well-characterised individuals, including those at high risk for the development of type 2 diabetes (first-degree relatives, low birth weight, habitually sedentary), would greatly facilitate this Goal (*see also* Goal 4.1).

Academic efforts should be interfaced with the biotechnology/pharmaceutical sector to ensure the greatest impact. Comprehensive and multidisciplinary approaches are expected to generate new information on fundamental pathways that govern metabolic and gene regulatory responses, which can provide novel biological entry-points for industry efforts to develop therapeutic strategies that mimic the exercise response to prevent and treat insulin resistance in type 2 diabetes. The discovery of specific exercise responsive signatures and the implementation of individualised exercise-training programmes would facilitate European efforts to reduce the financial and public health burden of type 2 diabetes.

Roadblocks Chapter 3

Roadblock 1. Testing of promising new drugs is too slow
New ways are needed to promote synergy between industry and academia for accelerated testing of promising new drugs in the prevention of insulin resistance and type 2 diabetes, and to bring such drugs to market. Identification and validation of new targets in the inflammatory pathway with chemistry and high-throughput screening is an expensive, time-consuming line of research for academics and would be better conducted as a collaborative effort with industry. Industry is better equipped for drug development and possesses tools not usually available in academia.

Roadblock 2. Collaboration among researchers in related fields
Integrated research environments with multiple levels of expertise in basic and clinical research and approaches in diabetes and metabolism should be fostered to gain a better understanding of the pathological processes involved.

Roadblock 3. Collaboration between academia and industry (SMEs, pharmaceutical, food nutrition)
Academia-industry interaction and collaboration with experts in all research fields to translate basic and clinical research discoveries into prevention, treatment and care of diabetes do not have to be considered as blocks but rather opportunities. Interactions could be fostered among all key stakeholders (such as academia, industry, SMEs, government organisations, regulatory authorities). A recent model is the Innovative Medicines Initiative, which shows this type of collaboration is feasible.

Roadblock 4. Synergy between academia and food industry
Synergy between academia and the food industry is necessary to develop functional foods. Indeed, it is the task of the food industry to modify food to make it appropriate and palatable to human needs.

Roadblock 5. Lack of biobanks of readily accessible human material
The availability of readily accessible human material (biobanks of human tissues and samples for example from well-characterised subjects at rest and after physical training or diet interventions) is necessary to allow the transition from clinical physiology to cell biology in humans as effectively as possible.

Roadblock 6. Lack of available *in vivo* methodology and imaging techniques
Cellular and whole body imaging technology needs to be developed in order to study intact cells and human individuals under physiological conditions, such as exercise and dietary intervention, and their effects on metabolism, as well as gene/environment interactions.

Roadblock 7. Lack of appropriate models that mimic the human condition
Since molecular studies often require genetically modified cellular or animal models, the challenge will be to closely align these models with distinct characteristics of human physiology. This will require humanised animal models.

Roadblock 8. Ethical issues related to application of biomarkers and genetic markers
Increased awareness of ethical issues and privacy is needed whenever working with human material, biomarkers and especially genetic markers (DNA analysis).