

Metabolic diseases: the fight is on



During the last few years, the increased prevalence of metabolic diseases (such as obesity, type 2 diabetes and atherosclerosis) has largely contributed to a rise in cardiovascular-related illness and death. Understanding the mechanisms responsible for the onset of metabolic and cardiovascular dysfunctions and the relationship between these disorders has become a major research endeavour today.

Metabolic and cardiovascular diseases are a major cause of death and illness in France, as it is in other industrialized countries. Changes in lifestyle, including less physical activity, poor diet and longer life spans mean that there will be a dramatic increase in metabolic and cardiovascular diseases in the population, which will represent a major public health issue.

This issue of the magazine will focus on the research of laboratories working on metabolic diseases. In a future issue, we will present topics developed in the field of cardiovascular diseases.

The obesity epidemic

The increased prevalence of obesity and type 2 diabetes, now considered of epidemic proportions, has led to the development of research on these diseases. This has allowed



steady progress in the fields of metabolic diseases over the last 15 years. The discovery in 1994 of leptin, a hormone produced by adipose tissue that controls food intake, as well as other proteins secreted by adipocytes, revealed the endocrine activity of adipose tissue and the complexity of its functions. Recently, new molecules controlling lipid metabolism and novel interactions between intestine and insulin activity have been identified. Finally, large population studies have allowed us to identify the major risk factors responsible for cardiovascular diseases, which show significant regional differences.

For many years now, Toulouse has had a national and international reputation in the field of research on metabolic and cardiovascular diseases. Research at Toulouse mainly concentrates on risk factors and cardiovascular complications and covers molecular and cellular aspects, pre-clinical, clinical and epidemiological studies. Clinical research benefits from a close partnership with the hospital departments of diabetology, metabolic diseases, cardiology, nutrition and sports medicine. These clinical departments, at the Rangueil and Larrey University hospitals, are situated close to the research teams at the Institut de Médecine Moléculaire de Rangueil. The Inserm-Toulouse Hospitals Clinical Investigation Center, at Purpan University Hospital, has developed a unique know-how in France in the field of metabolism. The clinical biochemistry laboratories here are also strongly involved in translational research. Epidemiological

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>>> Dominique LANGIN, professor at UPS, hospital physician, head of research on obesity at the Institut de Médecine Moléculaire de Rangueil (I2MR, Inserm/UPS) and Angelo PARINI, professeur at UPS, hospital physician, director of I2MR.

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>>> The new Institute of Molecular Medicine building, inaugurated in January 2008. ©CNRS Cyril Frésillon

>>> research is supported by a partnership between Inserm and Toulouse Hospitals and pre-clinical research benefits from core facilities attached to the Toulouse genopole, notably the animal core facilities, and the gene expression and lipidomics platforms. During the next four-year contract, starting in 2011, metabolic diseases will be further studied with the arrival of teams at l'Institut de Médecine Moléculaire de Rangueil, currently located at Purpan University Hospital.

*I2MR: Institut de Médecine
Moléculaire de Rangueil /
Rangueil Institute of
Molecular Medicine*

*CPTP : Centre de
Physiopathologie de Toulouse
Purpan/ Research Center for
Pathophysiology
at Toulouse Purpan*

*Laboratoire d'Epidémiologie et
d'Analyses en Santé
Publique/Public Health
Epidemiology and Analysis
Laboratory*

Collaboration with industry

Different academic laboratories are strongly involved in national and European research networks funded by the National Grant Agency and the European Commission (6th and 7th framework research programmes in the field of diabetes, obesity and nutrition).

Collaboration with the pharmaceutical industry (including Sanofi-Aventis, Pierre Fabre, Servier, GlaxoSmithkline and Beecham) is also very active. Moreover, these academic

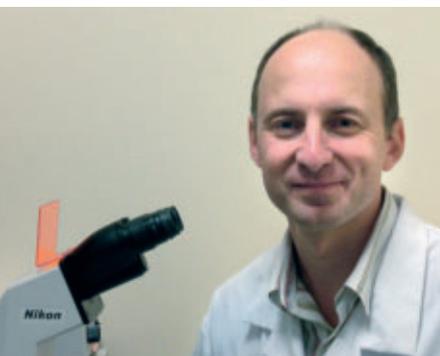
laboratories are taking part in the development of several local biotech companies.

In this issue, we have described the contributions of teams representing the various fields at Toulouse. These descriptions are not exhaustive but do provide a first glimpse at the various aspects of research on metabolic diseases in the region. These research activities have the potential to lead to the development of novel diagnostic, therapeutic and nutritional approaches.

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Obesity, so simple to diagnose but very difficult to treat

Studying how fat accumulation leads to body dysfunctions, researchers start to unravel new tracks for treatments.



>>> Dominique LANGIN, professor at UPS, hospital physician, head of research on obesity at the Institut de Médecine Moléculaire (I2MR, joint Inserm/UPS laboratory).

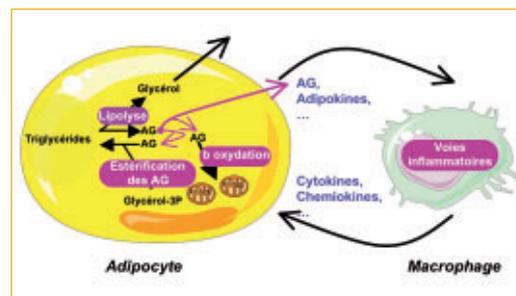
Obesity is a growing problem across the world. According to the World Health Organization, more than 300 million people were obese in 2006. In France, 12.6% of the population is obese, which is more than twice the number in the 1980s. The increase in childhood obesity is increasing too, a particularly worrying trend.

Obesity is defined as the accumulation of fat in the body with negative consequences for health. The condition leads to a large number of complications, the most frequent being cardiovascular diseases, diabetes, respiratory disorders and certain types of cancer. The mechanisms that link an excess of fat mass to metabolic disturbances are partly known thanks to studies in cellular and animal models. However, their relative importance in humans still needs to be defined.

Metabolism and inflammation

Adipose tissue plays an essential role in regulating energy balance through its ability to store and mobilize energy. Adipose tissue is complex and contains adipocytes (fat cells), vessels, nerves and immune cells, especially macrophages involved in obesity-associated adipose tissue inflammation. The dynamics of the various cell populations control the functions of adipose tissue. The cells release fatty acids and proteins, named adipokines, into the bloodstream that contribute to insulin resistance, which is at the origin of metabolic disturbances linked to obesity.

Our team works on the biology of adipose tissue using a translational approach from in vitro work on human fat cells and characterization of transgenic mouse models to clinical research. We study fatty acid metabolism in adipocytes to identify dysfunction in obese subjects and propose new therapeutic targets. Paradoxically, we have shown that obese patients are less able to mobilize fat. This adaptation could be a protective mechanism to limit an excessive concentration of fatty acids circulating in the bloodstream and the development of insulin resistance. Protocols are being carried out in mice and humans to evaluate the effect of molecules blocking lipolysis.



>>> Adipose tissue contains adipocytes, where fatty acid metabolism takes place (storage, utilization and release) and macrophages, which play a role in inflammatory reactions.

Another therapeutic strategy is to increase the use of fatty acids as energy substrates within the fat cell. Expression or activation of transcriptional regulators leads to a modulation of adipocyte metabolism. We are also interested in the relationship between adipose tissue lipolysis and inflammation. Indeed, we have already shown that the stimulation of fat mobilization modulates the production of pro-inflammatory cytokines in human adipose tissue.

Nutrition and physical exercise

In humans, these pathways are studied during dietary interventions and different types of physical exercise. These lifestyle modifications are a mandatory component in the management of obese patients. The influence of macronutrient composition (proteins, lipids and carbohydrates) is studied in energy restricted diets but also in weight maintaining diets that often fail. A similar approach is used to assess the effect of various types of training. A better understanding of how the body adapts is crucial for more individualized and efficient treatments of patients.

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Adipokines as promising pharmacological targets



>>> Philippe VALET, professor at UPS, "adipocyte secretions, obesity and associated diseases" team at the Institut de médecine Moléculaire (I2MR, joint Inserm/UPS laboratory)

Obese people are potentially more prone to developing diabetes, cancer or cardiovascular diseases. The main factors suspected are fat cells and their secretions. Numerous targets for future treatments.

What role do fatty tissues play in the development of obesity-related diseases (diabetes, cancer or cardiovascular pathologies)? To address this question, the "adipocyte secretions, obesity and associated diseases" team in the I2MR is studying the endocrine and/or nutritional control of adipocyte secretions. These adipokines are, in fact, the main link between adipose tissue and target organs (adipose tissue, muscle, heart, tumours). We have developed various complementary cell biology approaches on cell lines and adipose tissue explants but have also created several mice models (wild type and transgenic) that show varying degrees of obesity and/or diabetes. In this context, we study the role and the regulation of several adipokines discovered in the lab.

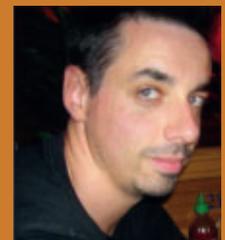
New molecules

We have described the secretion of Autotaxin and the production of Lyso-Phosphatidic Acid (LPA) by adipocytes, its role in adipogenesis processes, its link with obesity-related diabetes and more recently its effect on the development of renal fibrosis. We have also identified an amine oxidase sensitive to semicarbazide (SSAO) in the plasma membrane of adipocytes and described its ability to generate H_2O_2 (hydrogen peroxide). The last adipokine we have studied is called Apelin and we have shown that this peptide is secreted by adipocytes in response to insulin and/or TNF in mice and humans. We have also shown that it is involved in angiogenic processes inside adipose tissue.

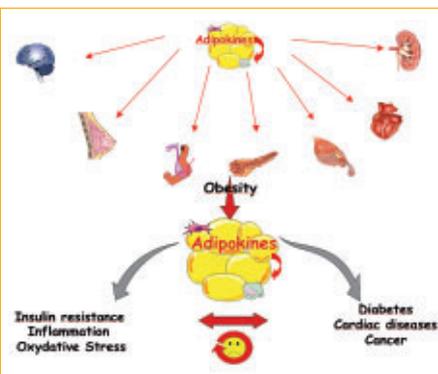
We are developing new drugs in collaboration with pharmaceutical companies and thus propose novel therapeutic approaches for treating obesity-linked diseases.

Apelin, a promising treatment for diabetes

We have observed a clear decrease in blood glucose levels in mice when they are injected with apelin. The effect, obtained with a low dose of apelin, has no impact on cardiovascular function. During a hyperinsulinic euglycaemic clamp, apelin is able to increase glucose utilization in oxidative muscles and adipose tissues. After having demonstrated the presence of apelin receptor (APJ) in both organs, we explored the effects of apelin on isolated adipocyte and muscle. In both cell types, apelin increases glucose transport in basal or insulin-stimulated conditions. The intracellular pathways involved in the glucose uptake have been depicted using both pharmacological approaches and transgenic mice models. In diabetic mice, apelin increases both glucose tolerance and glucose uptake by muscle and adipocytes when insulin is no longer efficient, (Dray et al. Cell Metabolism, 2008). Our current research focuses on the long term effect of apelin treatment in diabetic animals. The apelin/APJ system is therefore a very interesting target in the pharmacological control of metabolic disorders and diabetes.



>>> CASTAN-LAURELL and Claude KNAUF, assistant professors at UPS and researchers at the Institut de médecine Moléculaire (I2MR, joint Inserm/UPS laboratory)



>>> Adipocyte hormones interact with a number of tissues in an organism. They are also involved in the development of obesity-related diseases.

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The unexpected behaviour of fat

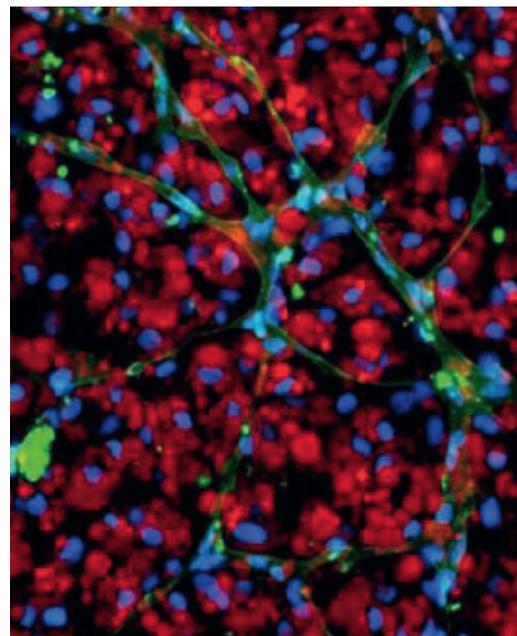
More than just being an inert and loose connective tissue, adipose tissue is now recognized as being a true organ endowed with endocrine and paracrine functions.

Adipose tissue has been long thought of as an inert specialized connective tissue consisting mainly of specific lipid-laden cells (adipocytes) surrounded by a collagenous matrix, storing and releasing energy depending upon the body's demands. The recent discovery that adipocytes can release factors, such as leptin, regulators of food intake and energy expenditure, have shed light on adipose tissue's real functions. Indeed, together with its metabolic activity, adipose tissue shows endocrine and paracrine activity and we, and other teams, have recently shown that adipose tissue cell composition is more complex than expected. Indeed it contains adipocytes but also various and distinct cell populations, the so-called stroma vascular fraction that we characterize in terms of phenotype and function.

Using cell imaging and/or cell immunoselection/depletion approaches, we showed that human adipose tissue contains immune cells, the number of which increases with obesity. Moreover, the plasma concentrations of inflammatory markers have been shown to increase in obese patients. Taken together, the present data has led us to believe that obesity is associated with chronic low-grade inflammation. The present data also reveals that dramatic changes in cell composition can occur in adipose tissue, thus suggesting that blood and adipose compartments do exchange cell populations. We are trying to investigate and characterize the mechanisms underlying such cell exchanges.

Adipose tissue as a reservoir of progenitor/stem cells?

By employing magnetic cell sorting methods, we demonstrate that adipose tissue contains a cell population that exhibits progenitor/stem cell-like features. They display self renewal in vitro but also in vivo. Depending on cell culture conditions, the adipose stem/progenitor cells alter their morphology and express adipogenic and angiogenic potentials. Finally, in vivo, in mice, adipose cells seem to repair damaged vascular tissue.



>>> Angiogenic (green) and adipogenic (red) potentials of human adipose tissue progenitor cells.

Adult adipose tissue therefore appears to represent an accessible and rich source of adult progenitor/stem cells that could, on one hand, support adipose tissue development and on the other exhibit properties suitable for tissue engineering and regenerative medical applications. We are interested in understanding how to control adipose tissue stem/progenitor cell fate (proliferation regulation, differentiation and migration processes).

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>>> From left to right. Top: Max Lafontan Inserm Senior Scientist, Anne Bouloumié Inserm research scientist, Jean Galitzky Inserm research scientist, Coralie Sengenès, Inserm research scientist, Marie Maumus, PhD student, Pauline Decaunes, assistant scientist. Bottom: Aurélie Caria, Carine Duffaut, Sandra De Barros, PhD students, Virginie Boulier, assistant professor UPS, Alexia Zakaroff-Girard, study scientist UPS, all members of the Avenir team at the Institut de Médecine Moléculaire de Rangueil (I2MR, joint UPS/Inserm laboratory)

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Lipid transport and how it contributes to cardiovascular risk



Understanding how lipids are absorbed by the intestine is very important because lipid absorption varies greatly among individuals and because many patients do not respond to existing therapies.

Our team is mainly involved in research on the different stages of lipid transport, starting from absorption in the intestine, then metabolism of plasma lipoproteins to cholesterol delivery to liver cells for further excretion in bile.

The importance of postprandial lipemia (following a meal) and cardiovascular risk has long been underestimated. Absorption of lipids and of fat-soluble vitamins results from the complex interplay of selected lipid transporters, located in the apical membrane of enterocytes, their expression being regulated in response to fat ingestion. Selected transporters facilitate cholesterol and triglyceride absorption, while others do just the opposite, promoting a backward sterol efflux toward the intestinal lumen. Moreover, we have recently observed that some transporters traffic intracellularly, along with the absorbed lipids, moving from apical to basolateral membranes, prior to lipoprotein excretion into the lymph, and then into the bloodstream. A better understanding of how those molecular transporters act together would enable us to identify new pharmacological targets for the control of plasma cholesterol levels.

cholesterol from cell membranes, thanks to the activity of selected lipid transporters (ABC-A1), and HDL particles are further taken up by hepatocytes, the major liver cells.

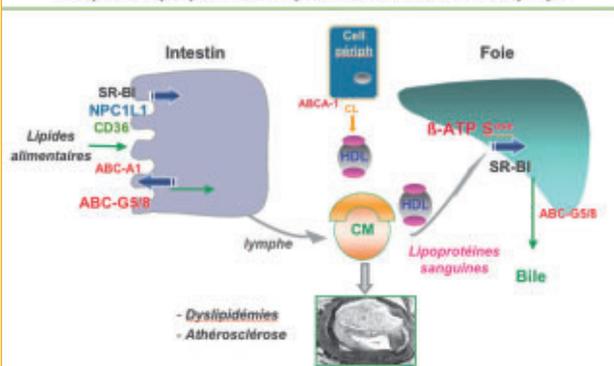
Our team has contributed to deciphering the mechanisms of this last step. Most surprisingly, we have observed that components of the mitochondrial ATP-synthase, an enzymatic complex involved in the synthesis of ATP, the major source of energy in living cells, are also present at the cell surface. More precisely, the (F1) catalytic part of ATPS is found at the cell surface, where it triggers the reverse reaction, that is, the hydrolysis of ATP into ADP. The generated ADP further interacts with a specific purinergic receptor named P2Y13. Recently, we have deciphered the signalling mechanisms set off by P2Y13 that trigger HDL endocytosis in hepatocytes.

These experimental approaches have provided us with new pharmacological tools, useful in modulating this new metabolic pathway and to check its physiological relevance in animal models. However, an assessment of its relevance in humans is also required, both in physiological and pathological conditions, as regards its impacts on HDL levels and on cardiovascular risk. To this end, we have set up collaborative studies with the cardiovascular epidemiology group (Pr Jean FERRIÈRES, Inserm/UPS), in order to develop, new biochemical and genotypic markers for this new pathway in clinical studies: the membrane F1-ATPS/P2Y13 sequence.

As well as its role in reverse cholesterol transport, the original finding that F1-ATPS is also present on the cell surface opens up new perspectives, both in cell biology and with regards to other physiological functions it might be involved in.

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Transporteurs lipidiques : de l'absorption intestinale à l'élimination hépatique



>>> Lipid transporters: from intestinal absorption to liver delivery.

The final step of cholesterol metabolism within the organism involves reverse transport to the liver, prior to its secretion in bile. This reverse cholesterol transport is mediated by a defined family of lipoproteins, called “high-density lipoproteins” or HDL, that are often labelled as “good cholesterol” owing to their function in sterol clearance. Epidemiological studies have emphasized the role of HDL as actually protecting against atherosclerosis. High-density lipoproteins take up excess

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The role of intestinal microflora in metabolic disease



>>> Rémy BURCELIN, Inserm Senior Scientist, at the Institut de Médecine Moléculaire, (I2MR, joint UPS/Inserm laboratory).

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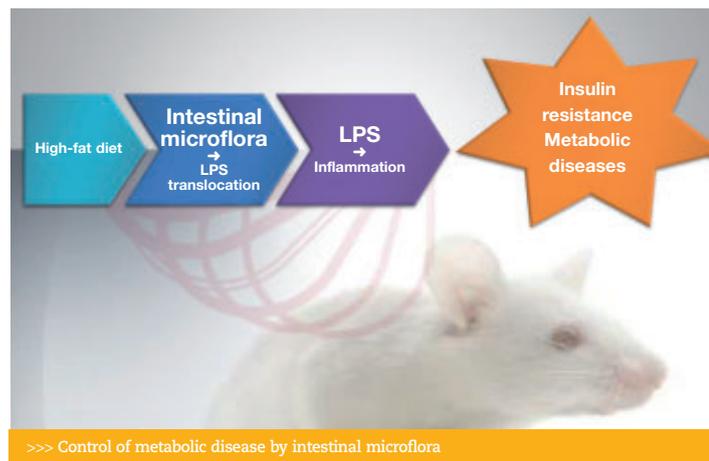
Communication between the intestine and brain, which regulates metabolism in organisms, can become disrupted with an unhealthy diet. A new mechanism for this disruption has now been proposed.

Unhealthy diets that contain an increased amount of fat, at the detriment of dietary fibre, are certainly an important factor in the increased prevalence of diabetes and obesity in western Europe and in the United States. In spite of extensive studies regarding the molecular mechanisms linking fat in food to metabolic disease no unifying hypothesis had clearly emerged over the last decades - until recently that is. We and other colleagues have now shown that intestinal microflora play an important role in the mechanisms responsible for the control of glycaemia and increase in body weight.

Our research team in collaboration with Pr J Amar at the Ranguel Hospital has recently discovered that, in humans and rodents, the plasma concentration of Lipopolysaccharides (LPS) increases when fed a high-fat diet. Thus, by metagenomic analyses and antibiotic treatments, we have shown that intestinal microflora is dramatically affected by a fat-enriched diet. Indeed, such a diet reduces the amount of Gram positive bacteria and increases the amount of Gram negative bacteria, which produce LPS. Injecting healthy mice with small doses of LPS leads to gain in body weight, hyperglycaemia and insulin resistance - that is, the ensemble of characteristics defining obesity and diabetes.

The molecular mechanism of LPS-induced metabolic diseases involves an inflammatory reaction leading to insulin resistance and adipose tissue plasticity. Consequently, metabolic disease and probably vascular disease, both characterized by inflammation, could have impaired intestinal microflora at their origin.

Our innovative research includes studying which molecular mechanisms, controlled by intestinal microflora, regulate metabolic function. Hence, dialogue between the host (our body) and intestinal



microflora is certainly very important. To study this further, we have been looking at the role of gut hormones such as the Glucagon Like peptide one, which is secreted during a meal and which contributes to increased insulin secretion. We showed that the mechanism is related to the activation of the autonomic nervous system, which relays nutritional information to the endocrine pancreas for the control of insulin and glucagon secretion. This process is also involved in the control of muscle glucose utilization and vascular blood flow. These functions are altered during diabetes induced by a high-fat diet. Our main research direction is to now validate our hypotheses generated in animal models and in humans, and close collaborations have been set up between medical departments at the Ranguel Hospital in Toulouse and our laboratory. Ideally, we shall be able to put forward therapeutic and preventive strategies in the near future based on probiotics and prebiotics to reprogram intestinal microflora and reduce the impact of an unhealthy diet.

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Cardiovascular heart disease risk factors

We now know that five major risk factors account for the majority of coronary heart disease cases. However, this information still needs to be exploited to protect vulnerable populations.



>>> Jean FERRIÈRES, professor of medicine, hospital physician, Vanina BONGARD, assistant professor, hospital physician, and Jean-Bernard RUIDAVETS, hospital physician, researchers at the Laboratoire d'Epidémiologie et d'Analyses en Santé Publique (joint Inserm/UPS laboratory).

The impact of risk factors in both men and women has only been established very recently. The first cohort study was carried out in 1962 in Framingham (USA). Since then, a great number of studies have been conducted, including the PRIME Study in Toulouse, Strasbourg, Lille and Belfast. These showed that the majority of cases were linked to five main risk factors: smoking, high LDL-cholesterol, low HDL-cholesterol, high blood pressure and diabetes. Public health workers thus now have the capacity to develop prevention programmes adapted to target populations at risk.

North/South cardiovascular risk split

While the main causes of atherosclerosis were discovered before 1980, coronary heart disease was only described at the European level after the MONICA (Monograph and multimedia sourcebook) Study. Great variations in risk were observed within relatively limited geographic scales. For instance, coronary mortality ranged from 1 to 4 between Toulouse and Glasgow. The MONICA study showed a North/South gradient in death risk and risk of coronary heart disease linked to atherosclerosis.

The French paradox

The well known French Paradox was put into context by the MONICA study. This paradox is a very superficial explanation of the low coronary risk observed in Southern European populations, with a rather caricatured example being France. Actually, we have demonstrated that consuming a little alcohol, eating fruit and vegetables and taking physical exercise may account for this paradox.

Atmospheric pollution

When epidemiological studies published during the last 40 years are analysed, the question concerning the impact of new risk factors of atherosclerosis in men remains unanswered. What is the role played by inflammatory, immune, hormonal and environmental risk factors? Our work shows that atmospheric pollution may encourage cardiovascular disease. The role played by these new risk factors is important in trying to understand the occurrence of cardiovascular disease not

MONICA
Monograph and
Multimedia Sourcebook
World's largest study of heart disease, stroke,
risk factors, and population trends
1979–2002

accounted for by classical risk factors and to improve our comprehension of all types of coronary heart disease. However, these new risk factors are not as important in the sense that the five main risk factors account for some 80% of the coronary cases in Toulouse. Nevertheless, these new risk factors will need to be included in care-giving strategies because, unlike classical risk factors, they concern the whole population and not just individuals. Human atherosclerosis is now, unlike experimental models, in the political arena and in the hands of decision and policy makers in charge of public health strategies at a country level.

The future

Until now, the study of atherosclerosis in men and women attempted to study factors accelerating or favouring the development of the disease. Yet, epidemiology has shown that some countries or regions are relatively protected. Among protective factors, HDL-cholesterol, nutritional and behaviour factors play a major role. The last twenty years in cardiovascular epidemiology have been characterized by improvements in genetics without any substantial benefit in terms of management. In the years to come, we will have to discover gene-environment interactions targets that could be true therapeutic weapons.

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