8.° SIMPOSIO CIENTÍFICO

NUEVAS APROXIMACIONES AL SÍNDROME METABÓLICO

Director invitado:
Dr. José Antonio Gutiérrez
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## Contents

**8th SCIENTIFIC SYMPOSIUM**

**NEW APPROACHES TO THE METABOLIC SYNDROME**

**Invited editor:**
Dr. José Antonio Gutiérrez

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This supplement has been sponsored by Fundación Lilly.

This publication shows the conclusions, findings and comments of the authors and mentions clinical studies that could have indications/dosages/administration forms of currently unauthorized medicinal products in Spain. It is stressed that any drug mentioned should be used in accordance with the Data Sheet in force in Spain.
Three years ago, at the 2nd Fundación Lilly Scientific Symposium entitled “The Metabolic Syndrome on its 80th anniversary”, we remembered the merits and honoured the memories of two physicians, Eskil Kylin, from Goteborg and Gregorio Marañón, from Madrid. They both presented eighty years before, patients in which high arterial blood pressure and glucose intolerance or diabetes mellitus of adult type were coincident in one clinical picture, suggesting a common mechanism for development.

Eskil Kylin (1889-1975), was a Swedish specialist in internal medicine, most of his time working as a head of department at the general hospital in Jönköping, southern Sweden. He interested himself in every aspect of arterial hypertension, but most of all the close associations between hypertension and diabetes mellitus as well as other metabolic disorders. In his early publication from 1923 (Zeitschrift für Innere Medizin) he delineated a metabolic syndrome, including hyperglycemia, arterial hypertension and hyperuricemia. From his papers it is clear that he often referred to similar works and hypotheses from the great Spanish endocrinologist Gregorio Marañón (1887-1960). Therefore both Kylin and Marañón should be jointly acknowledged for their early and accurate views on what, passed the time, would be recognized as the metabolic syndrome (MetS).

The frequent simultaneous presence of obesity, hyperlipidemia, diabetes mellitus and arterial hypertension was first described in 1965 by Avogaro et al. In this work, they reported the high risk of coronary artery disease in carriers of this cluster of metabolic and vascular abnormalities. The association of these factors was subsequently described in 1977 by Haller et al, who first used the term “Metabolic Syndrome” and described the association with atherosclerosis. In 1980 Vague suggested the concept that fat mass per se has little effect on the progression from obesity to diabetes mellitus, but it is the predominance of fat in the upper part of the body that leads to diabetes mellitus and atherosclerosis. As a matter of fact, insulin and cortisol secretion in obese patients are correlated with central obesity. Later on, the coining by Reaven in 1988 of the term “Syndrome X” renewed the impetus to conduct research concerning this syndrome. In his description of this syndrome, Reaven considered the following abnormalities: resistance to insulin-stimulated glucose uptake, glucose intolerance, hyperinsulinemia, increased VLDL triglycerides, decreased HDL cholesterol, and arterial hypertension. Other metabolic abnormalities that have been considered as part of the syndrome include abnormal weight or weight distribution, inflammation, microalbuminuria, hyperuricemia, and abnormalities of fibrinolysis and of coagulation.

Today, the term “Metabolic Syndrome” is generally used to indicate a clinical situation in which different degrees of arterial hypertension, impaired glucose tolerance, atherogenic dyslipidemia, central fat accumulation, insulin resistance, as well as prothrombotic and proinflammatory states, cluster together in the same individual. Such a concurrence of disorders increases the probability of suffering from cardiovascular disease or type 2 diabetes mellitus, possibly more than what the sum of the single risk factors would predict. Sometimes, the “whole” really is greater than the “sum” of its parts. Such is the case with MetS.

During the last decade, the MetS has progressively become a major public health problem both in wealthy societies and in developing countries. MetS is now approaching epidemic proportions worldwide. A total 115 million individuals suffer from this syndrome in the US, Japan, France, Germany, Italy, Spain and the UK, a number which is set to increase rapidly, fuelled by the rising obesity and diabetes mellitus epidemic. Its spreading prevalence is strictly associated with the adoption of a “westernized” lifestyle, characterized by lack of physical activity, excessive food intake, a combination of factors leading to overweight and obesity. In fact, obesity, particularly visceral obesity, seems to be a major determinant of insulin resistance, hence preparing the path to the clustering of metabolic and non-metabolic factors embraced under the descriptive term of MetS. Significant though it is, the MetS patient population remains poorly diagnosed.

The prevalence of MetS depends on gender and several socioeconomic, ethnic and geographic factors. It is estimated in the USA to be approximately 22.7% of the general population with important differences between ethnic groups within the same socio-geographic areas1, whereas in Europe MetS prevalence results in 23% and 12% for male and female populations, respectively, with ample north-south and east-west geographic variations2. To appreciate the whole impact of the problem on population health, it must be considered that not only cardiovascular mortality but all-cause mortality are increased in people with the MetS3. And what is even a matter of greater concern the prevalence of MetS in children and adolescence is on the increase worldwide.

This reality is requiring an increasing effort of the scientific community in detecting the etiopathogenic mechanisms and, consequently, to elaborate interventional initiatives to counteract such escalating health crisis. This mounting involvement of the biomedical community is well represented by the exponential trend in the number of scientific papers on “metabolic” and “insulin resistance” syndromes published in the literature in the last 3 decades.
Pathways leading directly from adiposity to the genesis of dislipidemia and arterial hypertension have been elucidated. Recent knowledge implies a role for fat-derived “adipokines”, including TNFα and adiponectin, as pathogenic contributors or protective factors. Current therapies include diet and exercise as well as agents indicated for the treatment of individual components of the syndrome. Future therapies may accrue from the aggressive pursuit of newer molecular drug targets that have the potential to prevent or treat multiple aspects of the MetS.

Aim of this symposium was to provide the participants with first-hand cutting-edge information (from molecular pathophysiology to genetic epidemiology) on a crucial component of the MetS as obesity, but also on the newer components, such as inflammation molecules, prothrombotic state, endothelial dysfunction or non-alcoholic fatty liver disease.

A special effort has been done to present a complete and updated overview, enriched with several original contributions that we are confident fulfilled all participants' expectations in this 8th Fundación Lilly Scientific Symposium.

REFERENCES
2. The European Group for the Study of Insulin Resistance (EGIR). The frequency of the WHO metabolic syndrome in European cohorts, and an alternative definition of the insulin resistance syndrome. Diab Metab. 2002;28:364-76.
There is increasing evidence that the clinical disorder termed nonalcoholic fatty liver disease (NAFLD) is closely associated with the metabolic syndrome. It is currently under investigation whether NAFLD represents a major risk factor for type 2 diabetes mellitus and cardiovascular disease. Several pathophysiologic mechanisms may underlie the development of NAFLD. Among them insulin resistance of peripheral tissues, hepatic insulin resistance and an imbalance in serum adipocytokines are the predominant ones. Recently genetic variability in candidate genes of lipid metabolism was identified to be closely associated with the accumulation of intrahepatic lipids.

It is accepted that an imbalance between the enzymes that promote uptake and synthesis of fatty acids and those that promote the oxidation and export of fatty acids exists, and that this results in NAFLD. This imbalance may be caused by whole-body insulin resistance. Insulin resistance, particularly insulin resistance of the adipose tissue leads to increase in lipolysis, which results in increased circulating fatty acids.

Another factor contributing to lipid storage in the liver may be hyperinsulinemia. It results from insulin resistance and may lead to increased uptake and storage of fatty acids in hepatocytes. Insulin is stimulatory to synthesis of glycogen in the liver. However, as glycogen accumulates to high levels (roughly 5% of liver mass), further synthesis is strongly suppressed. When the liver is saturated with glycogen, any additional glucose taken up by hepatocytes is shunted into pathways leading to synthesis of fatty acids. Thus, insulin resistance of the adipose tissue may be the primary factor leading to increase in fatty acid release into the portal vein, resulting in hepatic steatosis and consecutively in hepatic insulin resistance. Fatty acids in parallel increase insulin resistance of the skeletal muscle leading to hyperglycemia followed by hyperinsulinemia (fig. 1).

A different mechanism has begun to emerge from studies of mice with targeted gene mutations, namely that the liver and the beta-cells are primary sites of insulin resistance. Liver-specific insulin receptor knockout mice exhibit insulin resistance, severe glucose intolerance, and a failure of insulin to suppress hepatic glucose production. In addition regulation of hepatic gene expression was impaired. These alterations are paralleled by hyperinsulinemia due to a combination of increased insulin secretion and decreased insulin clearance. These mice also showed further characteristics of the metabolic syndrome including alterations in lipid metabolism. LIRKO mice had a threefold increase in low-density lipoprotein cholesterol compared with the control mice, with normal total cholesterol and high-density lipoprotein cholesterol.

Based on other knock-out and transgenic animal models an important role of the insulin receptor-protein 2, sterol regulatory element-binding protein 1c, suppressors of cytokine signalling in the liver for the accumulation of fat in the liver was also found. These data suggest that impaired insulin signalling in the liver results in accumulation of fat in hepatocytes.

In humans, imaging procedures such as computed tomographic scanning, magnetic resonance tomography (MRT) and proton magnetic resonance spectroscopy (1HMRDS) are adequate tools for non-invasive detec-
tion and classification of NAFLD. Particularly early and non-invasive detection of NAFLD when serum liver enzymes are not elevated yet, may be important for early intervention to prevent disturbances in glucose and lipid metabolism.

With 1HMRS and MRT we could investigate the role of visceral adipose tissue, adipocytokines and genetic variability in the pathophysiology of NAFLD. We found that visceral fat was a strong determinant of fatty liver both in males and in females. Adiponectin, the adipocytokine that has multiple beneficial effects on glucose and lipid metabolism, was also found to play an important role for NAFLD. Adiponectin plasma levels were not only associated with liver fat in cross-sectional analyses but adiponectin plasma levels at baseline also predicted change in liver fat during a lifestyle intervention in obese subjects. Further we found that polymorphisms in the adiponectin receptor-1 gene are also predictive for the change in insulin sensitivity and liver fat.

We also identified a polymorphism in the hepatic lipase gene to predict fatty liver. Moreover, we could show that this effect was modulated by the important Pro12Ala polymorphism in the peroxisome proliferator-activated receptor-γ gene.

In summary, NAFLD is associated with characteristics of the metabolic syndrome. Whether it is a primary pathophysiological state of the liver or secondary to peripheral insulin resistance and/or an imbalance in plasma adipokines (fig. 1) is still under investigation. Nevertheless, early screening of subjects for this phenotype, while the complete picture of the metabolic syndrome was not manifested, is necessary to early direct these subjects toward an intervention. This includes lifestyle intervention with diet and increase in physical activity and possibly pharmacological therapy.

REFERENCES
Dietary habits, body weight and insulin resistance in nonalcoholic fatty liver disease

GIULIO MARCHESINI AND REBECCA MARZOCCHI


Nonalcoholic fatty liver disease (NAFLD) includes a wide spectrum of hepatic alterations of metabolic origin, significantly associated with the metabolic syndrome (MS) and its individual features. A lot of data support this association: a high proportion of NAFLD patients have MS as a systemic disease, and a high proportion of cases with MS have NAFLD as its specific hepatic disease. Both conditions have common pathogenic mechanism(s) and share the same complications and treatment.

The sequence of events leading to liver fat accumulation and disease progression are not completely understood; a possible unified theory is depicted in the figure 1. Genetic and acquired factors contribute the first “hit”, leading to hepatic fat deposition through accelerated lipolysis and increased hepatic flux of free fatty acids, mainly derived from the visceral adipose tissue, through mechanism(s) which are incompletely understood. Insulin resistance has a pivotal role, favoring fatty acid (FFA) flux from adipose tissue to the liver and driving hepatic triglyceride production. Hyperinsulinemia and hyperglycemia also promote de novo lipogenesis, and in turn both hepatic triglyceride accumulation and high circulating FFA levels contribute to hepatic and peripheral insulin resistance. Accordingly, patients with NAFLD are more insulin resistant than age, gender and body mass index (BMI) matched controls without hepatic steatosis. When tested by the “glucose clamp” technique, nearly all NAFLD patients demonstrate a lower-than-normal insulin-mediated glucose disposal, and there is evidence that the severity of liver disease is associated with progressively increased insulin resistance. This defect, however, is not limited to overweight/obese subjects. A recent study in non-diabetic, non obese NAFLD patients pointed that also normal weight NAFLD cases are characterized by a reduced insulin activity on both glucose and lipid metabolism. Peripheral glucose disposal was markedly decreased in a 2-step euglycemic insulin clamp at the low and high insulin doses, due to impaired glucose oxidation and glycogen synthesis. Compared with controls, glycerol appearance and lipid oxidation were significantly increased in the basal state, and were suppressed by insulin to a lower extent. Lipid oxidation was significantly related to endogenous glucose production (EGP), glucose disposal, the extent of hepatic steatosis, and LDL oxidability. The correlation existing between hepatic steatosis and lipid oxidation suggests that fat accumulation can result from an increased lipid delivery to the liver, due to a reduced antilipolytic effect of insulin in adipose tissue coupled with defects in re-esterification, promoting enhanced oxidation. Steatosis per se may generate insulin resistance, further contributing to metabolic imbalance. Tikkainen et al compared subjects with high and low liver fat, selected on the basis of similar BMI, subcutaneous and visceral fat. High liver fat was associated with higher insulin, a marker of insulin resistance, as well as higher triglyceri-
magnetic resonance spectroscopy, Thomas et al. reported carried out by a magnetic resonance imaging and proton weight loss showed a larger decrease in hepatic fat and a low liver fat, subjects with high liver fat submitted to a probably regulated by dietary fat. Compared with subjects with low liver fat, subjects with high liver fat submitted to a weight loss showed a larger decrease in hepatic fat and a more marked decrease in insulin concentration. In a study carried out by a magnetic resonance imaging and proton magnetic resonance spectroscopy, Thomas et al. reported that intra-hepatocellular lipids increase by 22% for any 1% increase in total adipose tissue, by 21% for any 1% increase in subcutaneous adipose tissue, and by 104% for 1% increase in intra-abdominal adipose tissue.

Hepatic lipid accumulation is not the sole factor responsible for hepatocellular injury. Increased hepatic FFA oxidation can generate oxygen radicals promoting lipid peroxidation, cytokine secretion and mitochondrial dysfunction. FFAs may also cause hepatocyte apoptosis, the final mechanism of cellular injury in NAFLD.

Diet may be partly responsible for steatosis and oxidative stress. In animals, the liver has been shown to have a high capacity to accumulate triglycerides, and the size of this pool can change several folds within hours. Recent studies in humans have shown that up to 20% of dietary fatty acids are secreted as VLDL triglycerides within 6 hours after a meal. The habitual diet of NASH patients is rich in saturated fat and cholesterol and poor in polyunsaturated fat, fiber, and vitamin C and E, and is associated with a lower sensitivity to insulin and with other aspect of the metabolic syndrome. In overweight non-diabetic women, changes in dietary fat can change liver fat, independently of any change in body weight. Free fatty acid concentration, intra-abdominal or subcutaneous fat mass, or rate of carbohydrate, lipid or protein oxidation. Reducing dietary fat from 36% to 16% systematically reduces the percentage of hepatic fat content, which is systematically increased by crossing over to a diet containing 56% fat. Changes in liver fat were paralleled by changes in fasting serum insulin concentration.

The major breakthrough in the relation between diet, insulin resistance and liver fat is a very recent study, first showing that the amount of fat in the diet regulates the hepatic expression of endocannabinoid receptors. Endocannabinoids (anandamide) are novel lipid mediators that modulate appetitive behavior, increasing consumption of palatable substance, through the activation of central cannabinoid (CB1) receptors, involved in the development of obesity. This receptor is widely expressed, in hepatocytes and in adipocytes, as well as in the hypothalamus, limbic forebrain, and peripheral sensory nerve terminals. CB1 stimulation affects fat metabolism by regulating the level of adiponectin, by increasing lipoprotein lipase activity, and by contrasting the activity of leptin. Osei-Hyiaman et al. demonstrated that endocannabinoids also target the liver, where activation of CB1, results in increased de novo fatty acids synthesis through the induction of the lipogenic transcription factor steroidal regulatory element binding protein-1c (SREBP-1c) and its target enzymes acetyl-CoA carboxylase-1 and fatty acids synthase. A high fat diet increases hepatic anandamide owing to a major reduction in its degradation by fatty acid amidohydrolase, the enzyme responsible for the metabolism of anandamide. The activation of this pathway by endogenous anandamide in the liver has a key role in the development of diet-induced obesity and fatty liver. These findings suggest that CB1 antagonists may be effective not only as anti-obesity agents, but also in preventing/reversing the development of fatty liver and its progression to cirrhosis of metabolic origin.

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Marchesini G et al. Dietary habits, body weight and insulin resistance in nonalcoholic fatty liver disease.
Strategies for the evaluation of nonalcoholic steatohepatitis

ARTHUR J. MCCULLOUGH


Non alcoholic fatty liver disease (NAFLD) is a disease of our generation. This disease currently impacts virtually all fields of clinical medicine and will continue to do so. NAFLD is the most common form of chronic liver disease in Western societies and its prevalence is increasing in all other areas of the world as well1. NAFLD and its most severe form—nonalcoholic steatohepatitis (NASH)—are common, expensive to society, adversely affect quality of life and cause cirrhosis and liver related death in a significant but still imprecisely known percentage of patients.

The available data, which are based on screening population studies using the diagnostic modalities of ultrasound and liver function tests, now indicate the prevalence rate for both NAFLD and NASH have increased from previous estimates. They are now estimated to be in the range of 17-33% for NAFLD and 5.7-16.5% for NASH. Because NAFLD and NASH are associated with insulin resistance and obesity, these prevalence rates are expected to increase world wide concurrent with the pandemic of obesity and type 2 diabetes mellitus.

The importance of these observations stems from the fact that NASH is a progressive fibrotic disease, in which cirrhosis and liver related death occur in up to 20% and 12% of these patients, respectively over a 10 year period. This is of particular concern given the increasing recognition of NAFLD in children. Therefore, the diagnosis of this disease has become an extremely relevant topic in clinical hepatology.

DEFINITION OF NAFLD

When discussing the diagnosis of NAFLD it is important to define precisely this disease.

Histology

It should be emphasized that NASH should be considered as the most severe form of a larger spectrum of NAFLD with histologic findings ranging from fat alone to fat plus inflammation to fat plus hepatocyte injury (ballooning degeneration) with or without fibrosis, polymorpho nuclear cells or Mallory hyaline. Only fat plus hepatocyte injury with or without fibrosis should be considered NASH. The significance of these histologic categories rests not only on the fact that the prevalence varies by histology with steatosis alone with or without inflammation being more common than NASH, but clinical outcomes also vary by histologic category. Therefore, it is important to reliably distinguish NASH from other histologic types of NAFLD.

As shown in figure 1, cirrhosis develops in 15-25% of NASH patients2-5 and once developed, 40% of these patients may experience a liver related death.
death over a 10-year period with mortality rates similar to or worse than cirrhosis associated with hepatitis C. NASH is also now considered the major cause of cryptogenic cirrhosis. NASH associated cirrhosis can also decompensate into subacute liver failure, progress to hepatocellular carcinoma and re-occur post-transplantation.

In contrast, steatosis alone is reported to have a more benign clinical course, although progression of fibrosis in cirrhosis has occurred in 3% of those patients with steatosis alone.

Definition of nonalcoholic

By definition, excessive alcohol consumption excludes the diagnosis of NAFLD. However, the definition of excessive has been elusive and a wide range of alcohol has been allowed in previous reports. Early studies allowed no alcohol use, while more recent studies have allowed 40 g weekly or up to 140 and 210 g weekly for women and men respectively. Confounding this issue is a recent study describing endogenous alcohol production in NASH patients related to the degree of obesity as well as the protective effect of moderate alcohol intake in the prevention of diabetes and the development of NASH in morbidly obese patients undergoing bariatric surgery. Although there is no consensus regarding the definition of “nonalcoholic” in NAFLD patients, it seems reasonable to exclude patients from this diagnosis if current or past (within 5 years) daily alcohol intake has exceeded more than 10 g in women and 20 g in men. However, recent data suggest remote or cumulative alcohol use is associated with NASH in up to 15% of patients. Since there is no clinical feature or laboratory test sufficiently sensitive to detect this amount of alcohol intake, a careful history from the patient, the patient’s family and other health care providers involved in the patient’s management is paramount.

DIAGNOSIS OF NAFLD

It should be emphasized that this discussion deals predominantly with NAFLD associated with insulin resistance and the metabolic syndrome. This form of NAFLD is often referred to as primary NAFLD. Other secondary forms, which must be sought and excluded from the diagnosis of primary NAFLD, are provided.

Clinical presentation

History

The most common presentation is the patient with abnormal enzymes often performed during routine screening or for an abnormal ultrasound, which was performed for abdominal pain. However, the typical patient will be asymptomatic, although some patients will complain of right upper quadrant pain or progressive fatigue. The fatigue is usually vague and thought related to distention of Glisson’s capsule. Other entities associated with NAFLD and insulin

### TABLE 1. Patient demographics

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<th>Author (year)</th>
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resistance include Obstructive Sleep Apnea and Polycystic Ovary Syndrome (PCOS)33.

**Physical examination**

In the absence of cirrhosis, most patients usually have an unremarkable physical exam. Hepatomegaly may be present in 50% of patients27. The majority of patients will be overweight [body mass index (BMI) > 25] or have increased visceral adiposity and an increased waist circumference36. If PCOS is present, female patients may have hirsutism and increased acne. Attention should be paid to fat distribution because either congenital or drug induced lipodystrophies may be present.

Intermittent dysconjugate gaze24 and acanthosis nigricans25, which result from mitochondrial injury26 and insulin resistance, respectively, may also be present. When present the typical stigmata of chronic liver disease; including spider telangiectasia, caput medusa, ascites, palmar erythema, and gynecomastia, suggest the likelihood of cirrhosis.

**Patient demographics**

Table 1 provides patient demographic information obtained from a number of different studies2,5,22,23,37,43. Most cases of NAFLD occur in the fifth and sixth decades of life, although of considerable concern is the occurrence of NAFLD in children44-47. Ten of the studies in table 1 describe an overall patient demographic consistent with the original typical NAFLD patient2. Cases occurred more frequently in females (51% to 83%) and there was a high prevalence in both type 2 diabetes mellitus (28% to 55%) and obesity (47% to 90%). The prevalence of dyslipidemic disorders is highly variable, ranging between 4% and 92%. It is important to emphasize, however, that three studies2,42,43 indicate that the typical clinical profile needs to be expanded to include male patients with normal weight and without abnormalities in either glucose nor lipid metabolism. In fact, such male patients existed in the other studies listed in table 1 but were not the majority and were not emphasized. Although there appears to be little difference histologically between the expanded and original profiles, one report found male patients to have less steatosis and more stainable iron on liver biopsy than females25.

Nonalcoholic fatty liver disease has been reported in all ethnic groups, with preliminary data48 suggesting an over representation of Caucasians and Hispanics. A cross-sectional study suggested a low prevalence of NAFLD among African Americans49, but the National Health and Nutrition Examination Survey (NHANES) III indicates that NAFLD may be more common in African Americans than in Caucasians50. There may be a familial component also. One study38 found 16 of 90 patients with NAFLD had a first-degree relative with the disease also. Another study51 found that among eight families, 18 family members were affected.

**Serum chemistries**

Increase aminotransferase activities are the most common abnormality reported in patients with NASH38,31,39. Usually, ALT or AST are elevated only mildly to moderately in the range of a two- to fivefold elevation2,5,23,33,36. Alkaline phosphatase may be abnormally elevated two- to threefold, in fewer than half of patients25. Serum albumin levels are almost always normal, and bilirubin levels are usually abnormal2,38 unless cirrhosis has developed.

Many studies have reported elevated serum ferritin in approximately 50% of NAFLD patients2,23,38,62,63, without evidence of hepatic iron overload. Two studies25,26 noted that heterozygosity for the HFE gene is increased in NAFLD patients, with a trend toward more severe hepatic fibrosis in NASH patients with a genetic basis for hepatic iron overload. The authors acknowledged, however, that hepatic iron overload occurred in only a minority of their NASH patients.

The AST/ALT ratio is reported to be less than 1 in 65% to 90% of NAFLD patients2,22,23,64,66. When the AST/ALT ratio is greater than 1, it suggests that there is an advanced fibrotic form of NAFLD25. However, this ratio is almost never greater than 25.

Hematologic measurements are usually normal, unless cirrhosis has led to hypersplenism. Several small selected case studies have reported positive tests for antinuclear antibody in 10% to 46% of patients with NAFLD17,38,64,71. The significance of this observation is unclear, however.

Finally, it should be emphasized that data questioning the accuracy of standard liver function tests have been reported37,72-74. Although liver function tests usually are elevated mildly in NAFLD58, values can be normal, and the degree of abnormalities does not correlate with the degree of steatosis or fibrosis77,78. What is considered an abnormal value also has been questioned, since the normal limits for ALT in population studies have been revised downward, with values individualized by gender and for individuals with obesity or the metabolic syndrome59. However there are limitations in the accuracy of serum chemistries for diagnosing fatty liver. The limitations of this approach has been discussed72,74 and include the following: a) lack of specificity; b) although normally mildly elevated75,76, values can be normal, and the degree of abnormalities does not correlate with the degree of steatosis or fibrosis77,78, and d) the normal limits for ALT have been revised downward and individualized by gender for patients with NAFLD as well as individuals with obesity or a dysmetabolic syndrome59.

**Radiologic methods**

Ultrason, CT scan, magnetic resonance imaging (MRI), and proton magnetic spectroscopy (1H MRS) have all been used to assess hepatic fat deposition in the liver79,80. While some studies have described superiority of a particular modality30,82,84,87; a recent study82 demonstrated ultrason, CT scan and MRI have similar diagnostic accuracy for quantitating the severity of steatosis when fat deposition is > 33% of the liver volume. 1H MRS has greater sensitivity that the other 3 modalities and has been shown to detect as little as 5% fat deposition in the liver80. MRI is useful for confirming the nature of hepatic steatosis when it occurs focally rather that its usual diffuse pattern82 and calibrated CT scans may be useful in monitoring hepatic fat content over time81. However, differences between NASH and steatosis are not apparent with any of the radiologic modalities79,85. Even though two studies83,84, have evaluated test characteristics for ultrasound and found that ultrasound leads to an incorrect diagnosis of fatty liver in 15-33% of patients, the most recent data as well as cost considerations85, have made ultrasound the most common radiologic modality used for evaluating hepatic steatosis.

**LIVER BIOPSY**

Although radiologic techniques and serum liver function tests are useful, they remain only indirect surrogate markers of fatty liver. Liver biopsy is the only currently available method for differentiating NASH from steatosis with or without...
TABLE 2. Prediction acronyms for advanced fibrosis in NAFLD

<table>
<thead>
<tr>
<th>Predictor</th>
<th>BARD</th>
<th>BARG</th>
<th>BAAT</th>
<th>HAIR</th>
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</thead>
<tbody>
<tr>
<td>BMI</td>
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<td>AGE</td>
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<td>X</td>
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<tr>
<td>AST/ALT</td>
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<td>X</td>
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<tr>
<td>DIABETES</td>
<td>X</td>
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<tr>
<td>HgA1C</td>
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<td>TRIGLYCERIDES</td>
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<td>TG'S</td>
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<td>HYPERTENSION</td>
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<td>INSULIN RESISTANCE</td>
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<td>INDEX</td>
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BAAT: BMI (≥ 50), ALT (≥ 2 x normal), and triglycerides (TG’s) (≥ 1.7 mmol/l); BARD: BMI (≥ 30), Age (≥ 45), Ratio of AST/ALT (≥ 1), and diabetes mellitus; BARG: BMI (≥ 28), Age (≥ 50), Ratio of AST/ALT (≥ 0.8), and HgA1C (≥ 5.2); HAIR: Hypertension, ALT (≥ 4), and Insulin resistance (≥ 5.0 defined by Quicki).

hout inflammation79,82, despite the issue of sampling error. However, the role of liver biopsy remains unclear with its advantages and disadvantages.

There are a number of certain situations that a liver biopsy has significant clinical importance. These include: suspected subreptitions alcohol use, a positive anti nuclear antibody, possible medication effect, unexplained elevated serum ferritin concentrations, or positive serology for hepatitis C.

PREDICTORS OF ADVANCED FIBROSIS

In addition to histology (the presence or absence of NASH), a number of risk factors have been identified as predictors for the development of progressive fibrosis and cirrhosis. These include: obesity23,93, diabetes mellitus23,28, age23,39, arterial hypertension23,39, AST/ALT ratio23,56,96, triglycerides, elevated ALT23,39, iron93, extent of steatosis56, and the grade of inflammation23,93,94.

Table 2 displays combinations of the strongest predictive factors along with their acronyms that have been used by different investigators to predict fibrosis in patients with fatty liver23,29,94. The presence of either obesity and/or type 2 diabetes mellitus are the most robust predictors of fibrosis23,28,39,94, Age (≥ 45 or 50) is also a strong predictive factor for cirrhosis23,56,96, which probably reflects the duration of time that steatosis is at risk for a subsequent second hit. An elevated ALT level23,94, an AST/ALT ratio > 0.823,96, arterial hypertension23,39, triglycerides94 and a high insulin resistance index94.

Data from the BARD or BARG acronyms (table 2), for example, would predict a patient with fatty liver on ultrasound who is < 45 years old, has neither obesity or diabetes and an AST/ALT ratio < 0.8, has only a minimal risk for developing significant fibrosis. In contrast, almost two thirds of patients with diabetes or obesity, age ≥ 45 years, and an AST/ALT ratio > 0.8 will have significant fibrosis.

This information can be used to determine the usefulness of performing a liver biopsy in patients with fatty liver by targeting a population with a high likelihood for having NASH.

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McCullough AJ. Strategies for the evaluation of nonalcoholic steatohepatitis

Nonalcoholic fatty liver disease (NAFLD) is emerging as a common cause of chronic liver disease in Western countries. NAFLD is considered the hepatic manifestation of the metabolic syndrome, a cluster of metabolic abnormalities related to insulin resistance, including obesity, hyperglycemia, dyslipidemia, and hypertension. NAFLD is more frequent among people with diabetes and obesity, and it is almost universal among morbidly obese people with diabetes. Steatohepatitis is present in 18.5% of markedly obese patients and 2.7% of lean patients. A recent cohort study clearly demonstrated chronological ordering between body weight gain, hypertransaminasemia, and insulin resistance-related clinical features in a healthy population. NAFLD incorporates a wide spectrum of liver change ranging from simple steatosis to steatosis plus necroinflammatory activity (nonalcoholic steatohepatitis or NASH), to cirrhosis and ultimately liver failure. Some clinical variables have been identified as predictors for advanced fibrotic disease including obesity, diabetes, age > 45 years and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio > 1. The risk of cirrhosis-related death or hospitalization appears to be increased among persons with a central fat distribution that might be related to insulin resistance and hepatic steatosis. Patients with NAFLD and diabetes are at risk for the development of aggressive outcome, such as cirrhosis and mortality.

PATHOGENESIS

Several hypotheses have been proposed to explain the pathogenesis of NAFLD. The most accepted theory is the “two hit” hypothesis, in which the first hit involves the development of hepatic steatosis, rendering the liver more susceptible to a second, as yet undefined, hit, resulting in more severe liver damage (fig. 1). Current evidence points toward insulin resistance playing a key role, since it may influence several intracellular metabolic pathways. As a result of insulin resistance, there is increased free fatty acids (FFA) flux to the liver. Impairment of fatty acid oxidation or decreasing apolipoprotein formation or microsomal formation of VLDL, which allows triglycerides to accumulate in the liver also occurs. The progression of steatosis to steatohepatitis is associated with increasing oxidative stress within hepatocytes. Hepatocytes handle the increased FFA load by increasing FFA β-oxidation, thus contributing to generation of reactive oxygen species with subsequent cytokine induction (i.e. TNFα) that eventually leads to mitochondrial dysfunction.

TREATMENT

Treatment strategies for NAFLD have been focused on improvement in underlying insulin sensitivity, the management of associated meta-
Charatcharoenwitthaya P et al. Targets for therapy and current status of treatment of nonalcoholic fatty liver disease

![Fig. 1. “Two-hit” hypothesis: the first hit involves the development of hepatic steatosis, rendering the liver more susceptible to a second hit resulting in more severe liver damage.]

...bolic conditions, and protection of the liver from oxidative stress. Pharmacotherapy should be aimed to slow the progression of NAFLD and is therefore restricted to those patients with NASH, at highest risk of developing complications.

Weight reduction with diet and exercise leads to improved insulin sensitivity and therefore should be an initial approach in the management of patients with NASH. However, there are no randomized clinical trials of weight control as treatment for NAFLD. The National Heart, Lung and Blood Institute (NHLBI) and National Institute of Diabetes and Digestive Kidney (NIDDK) expert panel clinical guidelines for weight loss recommended that the initial target for weight loss should be 10% of baseline weight within a period of 6 months. This can be achieved by losing approximately 1-2 lb/week. Huang et al. demonstrated that one-year intense nutritional counseling for improving insulin sensitivity resulted in histological improvement in NASH patients with mean weight reduction of 2.9 kg.

Recently, bariatric surgery for morbid obesity has become more popular. Restrictive procedure (gastric bypass, gastroplasty) to achieve weight loss are safer than malabsorptive procedures (jejunoileal bypass). Dixon et al. obtained repeat liver biopsies in 23 obese patients with NASH who underwent laparoscopic adjustable gastric banding for weight loss. After losing a mean of 34 kg within 25.6 months, NASH resolved in 82% of these patients. Major improvement was seen in steatosis, necroinflammation and fibrosis.

Pharmacological agents used for weight reduction have also been evaluated in small trials. Orlistat, a reversible inhibitor of gastric and pancreatic lipases, is currently approved for weight loss. Small pilot study conducted on obese patients with NASH has demonstrated significant improvement in serum aminotransferases, hepatic steatosis, necroinflammatory activity, and fibrosis.

Clofibrate revealed no significant biochemical or histological improvement in 16 NASH patients treated for 12 months. Gemfibrozil was evaluated in a short duration, randomized trial of 46 NASH patients, demonstrating significant biochemical improvement compared with no treatment.

Thiazolidinediones improve insulin sensitivity by activating the peroxisome proliferators-activated receptor gamma and have shown promise in pilot studies involving patients with NASH, although weight gain has been a troublesome side effect. Troglitazone was withdrawn from the market because of hepatotoxicity. The second-generation thiazolidinediones, rosiglitazone and pioglitazone appear to be safer. Rosiglitazone has been evaluated in an open-label trial of 4 mg twice daily for 48 weeks in 30 NASH patients. Insulin sensitivity and ALT levels improved significantly with post-treatment biopsy showing a significant improvement of necroinflammatory activity and perisinusoidal fibrosis. Recently, a randomized placebo-controlled, multicenter clinical trial with pioglitazone in 40 patients appears to confirm the beneficial effects of thiazolidinediones in NASH.

Metformin improves insulin sensitivity through decreased hepatic glucose and triglyceride production. An open labeled study of metformin 20 mg/kg for 1 year in 15 NAFLD patients demonstrated a transient improvement in serum aminotransferase levels and insulin sensitivity remained steady without further improvement. Recently, an open-label randomized trial of metformin 2 g/day for 12 months versus either vitamin E 800 IU/day or a prescription, weight-reducing diet in 55 NAFLD patients showed that long-term metformin treatment significantly reduce average ALT levels and increased the chances to have ALT within the normal range as well as histological improvement in comparison to control treatment.

Given the role of oxidative stress in the pathogenesis of NASH, numerous studies have focused on the use of antioxidants for NASH treatment. A small pilot study in adult NASH patients treated with vitamin E 300 mg/day for one year showed significant biochemical and histological improvement. Subsequently in a placebo-controlled trial, NASH patients were treated with vitamin E 1,000 IU/day plus vitamin C 1,000 mg/day for 6 months in comparison with placebo that showed decreased fibrosis within the treatment groups, whereas there was no significant difference in placebo group between groups.

Ursodeoxycholic acid (UDCA), the non-hepatotoxic epimer of chenodeoxycholic acid, has multiple hepatoprotective activities as well as immunological effects. Early pilot studies of UDCA in NASH patients revealed promising results, however, a recent multicenter, randomized trial in 166 NASH patients demonstrated that UDCA 13-15 mg/kg/day for 2 years led to no significant difference in the biochemical or histological improvement between the UDCA and placebo groups.

Betaine, N-acetylcysteine, pentoxyphylline, and lorcaserin have shown promise in small pilot trials. Other promising potentially useful nutritional approaches to NAFLD patients include metadoxine, folic acid, alanine, oligofructose, omega 3 fats, acarbose, and probiotics. Further studies to assess potential beneficial effects of these novel findings are warranted.

CONCLUSIONS

A better understanding of the pathogenesis leading to fat accumulation and oxidative balance impairment in steatotic livers is greatly expected to improve the therapeutic approach of NAFLD. Currently, treatment is limited to weight reduction and the control of associated metabolic conditions. Attractive pharmacological therapy with insulin-sensitizing agents and antioxidants hold promise, but only small short-term pilot studies have been assessed. Further studies are required to identify agents with adequately powered randomized controlled trials evaluating fibrotic progression or clinical complication as end points.
REFERENCES

Globalization of occidental way of life is leading to increasing prevalence of obesity and type 2 diabetes, the greatest pandemic of the XXI Century. Obesity-associated insulin resistance has an important role in acute phase response and inflammatory pathways. Chronic subclinical inflammation develops in subjects with abdominal obesity and has been proposed as a part of the metabolic syndrome. The study of the factors which regulate the acute phase response in apparently healthy obese subjects has yielded consistent results implicating cytokines and growth factors in the pathophysiology of obesity, insulin resistance and its complications.

It should be kept in mind that humans live in close association with vast numbers of microorganisms that are present on the external and internal surfaces of our body. The ability to mount a prominent inflammatory response to pathogens confers a continuous advantage in our fight against pathogens. All metazoan organisms have evolved complex immune defense systems, used to repel invasive microbes that would parasitize or kill them. Innate immunity is the most universal and the most rapidly acting. Most organisms survive through innate immune mechanisms alone. After any trauma or infection, the organisms mount a homeostatic response to injury called acute-phase response, a highly complex process. In the acute phase, the acute phase response is protective because it counteracts the effects of injury and improves survival. A continuous and permanent equilibrium exists between proinflammatory factors and anti-inflammatory molecules. The maintenance of this equilibrium will be very important for an adequate eradication of injury without chronicification of the process.

Long-term exposure to stressful stimuli (mucositis, aging, increased fat intake, periodontitis…) may result in disease (insulin resistance, atherosclerosis) rather than repair. There exist two arms of innate immunity: the sensing arm (those mechanisms involved in the continuous sensing and perception of infection) and the effector arm, the sophisticated processes aimed at eradicate infection and tissue repair. Each of these arms may be subdivided in humoral and cellular processes tightly coordinated in the inflammatory process. This system constitutes the first line of body’s defense and is constituted by different barriers (epithelia, adipose tissue), and different blood and tissue components as macrophages, and neutrophils. This innate immune system generates the acute phase response in which different acute phase proteins and cytokines are produced in response to different aggressions as infections and traumatisms.
Intense exercise
Chronic infections (periodontitis, etc.)

Trauma
Cytokines

Acute phase response
Tobacco

Acute phase changes
Insulin resistance
Alterations in carbohydrate metabolism
Hyperlipidemia

Cellular Sensing of the Afferent Arm of Innate Immunity and Insulin Action

From a historical point of view, the cellular effector arm was the first to be characterized and the best understood in this context. The discovery that TNFα was expressed in adipose tissue and modulated insulin action in animal models is perhaps the cornerstone that led to an in-depth knowledge of the interactions between immune system and metabolism.

It is not enough for the host to sense microbes. It must kill microbes as well. In vertebrates, innate immunity is largely dependent upon myeloid cells: professional immunocytes that engulf and destroy pathogens. Myeloid cells include monoclonal phagocytes and polymorphonuclear phagocytes. The mononuclear phagocytes are the macrophages, derived from blood monocytes. A higher peripheral white blood cell count has been associated with insulin resistance and with atherosclerosis. Peripheral white blood cell count correlated significantly with insulin-mediated glucose disposal during an euglycemic clamp. In subsequent studies, it was demonstrated that neutrophil and lymphocyte count correlated positively with several components of the insulin resistance syndrome, and that plasma insulin concentration was specifically associated with the number of lymphocytes and monocytes.

In a recent study we substantiated these relationships at the molecular level, providing evidence that a particular defense against infection bacterial and permeability increasing protein (BPI) runs in parallel to insulin sensitivity among healthy subjects. We found that circulating BPI concentration was significantly different across categories of glucose tolerance: BPI was significantly lower in patients with type 2 diabetes. In subjects with glucose intolerance we found the strongest associations between plasma BPI and central obesity, glucose metabolism, insulin sensitivity and components of the metabolic syndrome.

We also tested the functional significance of these findings. Bioactive lipopolysaccharide (LPS) was significantly and negatively associated with circulating BPI concentration. This finding suggests that, with decreasing BPI, the ability to buffer LPS is impaired. We further substantiate this hypothesis by studying the effects of the insulin sensitizer-metformin, on circulating BPI. In patients receiving metformin, but not in those receiving placebo, we observed improved insulin sensitivity and raised circulating BPI concomitantly. How can all these associations be explained? Insulin action may lead to increased plasma BPI concentration. A recent study has demonstrated that insulin is a strong regulator of the main neutrophil functions in nondiabetic, healthy subjects. The cellular functions of human neutrophil, including bactericidal activity require energy derived from glucose. Whereas insulin does not stimulate hexose transport in this immune cell, previous reports have clearly shown that this hormone is able to regulate glucose metabolism in neutrophils.

Cellular Sensing of the Afferent Arm of Innate Immunity

The chronic inflammatory process of insulin resistance is triggered and sustained by unknown factors. Among the candidate triggers are oxidized or enzymatically modified low-density lipoproteins, heat shock proteins, and infectious pathogens. Interestingly, in the last years it has become clear that all these triggers and ligands could be recognized and sensed by the same cell and the same receptor. Macrophages (again) play a primary role in host defense against infection, utilizing a range of receptors to recognize microbes by opsonic as well as direct interactions. The term “pathogen-associated molecular patterns” (PAMPs) was coined to describe those microbial principles that triggered an innate immune response. PAMPs were said to act via “pattern recognition receptors” (PRR), i.e. those sensors that could recognize a pattern on a microbe. Binding of targets via PRR results in phagocytosis and killing. Macrophages express a broad repertoire of PRR (e.g., scavenger and lectin-like). In this sense, the amount of lipid retained in macrophages during the atherosclerotic process depends on the unregulated uptake of oxidized lipoproteins by scavenger receptors, counterbalanced by degradation and cholesterol efflux. This scavenger receptor also plays a major role in microbial uptake in the absence of opsonins. The principal signaling receptors of the innate immune system—through which the greater part of the host awareness of infection is processed—are the toll-like receptors (TLR) family of transmembrane molecules. The best understood TLR, both in terms of ligand binding and signal transduction, is the lipopolysaccharide (LPS) receptor, TLR4.

Interpretation

Evolution pressures have led to survival of the fittest individuals, those with genetics that allows the best defense against infection and periods of famine. The advantages of increased inflammatory responses, hypersecretion of proinflammatory cytokines (TNFα, interleukin [IL]-1β, IL-6, IL-18), or decreased anti-inflammatory molecules (adiponectin, certain TNFα isoforms, sCD14, etc.), would lead to chronic inflammation conditions, such as obesity and type 2 diabetes, leading to cardiovascular disease.

Increasing evidence is reported according to which chronic inflammation precedes these conditions. The knowledge of how these metabolic pathways interact with the inflammatory cascade will facilitate new therapeutic approaches. Anti-inflammatory drugs are only the first step of this new approach.
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Macronutrient intake induces oxidative and inflammatory stress while insulin causes suppression of reactive oxygen species generation and inflammation

PARESH DANDONA


Following our original observation that the intake of 75 g of glucose in normal subjects induces an increase in reactive oxygen species (ROS) generation by mononuclear cells (MNC), we have shown that glucose, equicaloric amounts of fat (eaten as cream) and a mixed fast food meal (900 calories) induce not only an increase in ROS generation by MNC but also cause an increase in p47phox expression. In addition, there is an increase in intranuclear NF-κB binding, a fall in IkBa expression and an increase in IKKa and IKKb expression. There is a concomitant increase in TNFα mRNA in the MNC. Two other pro-inflammatory transcription factors, activator protein-1 (AP-1) and early growth response-1 (Egr-1), were also induced by glucose intake. There was an increase in matrix metalloproteinases (MMP) MMP-2, MMP-9, tissue factor (TF) and Plasminogen activator inhibitor-1 (PAI-1).

Thus, there occurs a comprehensive oxidative and inflammatory stress response following macronutrient intake. Consistent with this concept, the state of obesity, associated with increased macronutrient intake, is characterized by an increase in oxidative stress and chronic low grade inflammation. As would be expected, caloric restriction in the obese results in a marked reduction in ROS generation by MNC and other indices of oxidative stress, like lipid peroxidation and protein carbonylation. A 48 hour fast in normal subjects leads to a reduction in ROS generation by 50% and a parallel reduction in p47phox. In contrast to macronutrient intake, a low dose insulin infusion (2 units per hour), results in a significant reduction in ROS generation by MNC, p47phox expression, intranuclear NF-κB binding with an increase in IkBa expression (fig. 1). In addition, there is a suppression of AP-1 and Egr-1, MMP-2, MMP-9, PAI-1 and TF. The anti-inflammatory effect of insulin was further confirmed by in patients with acute myocardial infarction who were treated with a low dose insulin infusion in addition to the standard thrombolytic therapy. Insulin infusion led to a significant fall in C-reactive protein (CRP), serum amyloid (SAA), PAI-1, MMP-1 and oxidative stress. In addition, insulin had a significant suppressive effect on the increase in plasma creatine kinase (CK), CK-MB and myoglobin concentrations in these patients, consistent with a cardioprotective action. This effect of insulin on CRP and SAA has now been confirmed both in acute myocardial infarction and in patients undergoing coronary artery bypass surgery.

These facts allow us to conclude that there exists a novel relationship between macronutrient intake and insulin, the hormone secreted in response to macronutrient intake. This relationship extends beyond the clas-
The New Paradigm

**Fig. 2. Relationship between macronutrient intake, insulin, and oxidative/inflammatory stress.** Adapted from: Dandona et al.

- AP-1: activator protein-1; Egr-1: early growth response-1; NF-κB: nuclear factor Kappa-B; T2D: thiazolidinediones.


The purpose of this talk was to update understanding about the connection between fibrinolysis, mainly plasminogen activator inhibitor 1 (PAI-1) and the metabolic syndrome. Fibrinolysis is a controlled enzymatic cascade that, when activated, generates a trypsin like protease, plasmin. Remarkably, the same enzyme functions in blood to breakdown fibrin and maintain vessel patency and in tissues to breakdown extracellular matrix and control cell adhesion and migration. That is to say that this system is broadly used in human physiopathology. Activation of this system is accomplished by the release of two plasminogen activators from cell, tPA and uPA, in response to signals such as inflammation. The regulation of fibrinolysis is achieved primarily by PAI-1 which prevents the escape of this potentially destructive protease system. It is quite easy to propose that excess of PAI-1 could contribute to the atherothrombotic process by increasing fibrin and extracellular matrix deposit into the vascular wall. This has been well demonstrated in mice. For example, transgenic mice that express a stable form of human PAI-1 develop spontaneous coronary arterial thrombosis. These mice exhibit a high level of circulating PAI-1 and most of them developed spontaneous occlusions of coronary arteries after six months evolution, with histological evidence of subendocardial infarction. In humans, plasma PAI-1 levels have been associated with risk of developing coronary events. Remarkably the power of the association was strongly reduced after adjustment for the factors which belong to the metabolic syndrome. Thus, it appears that most of the predictive ability of PAI-1 on cardiovascular events in humans depends on the metabolic syndrome. The link between PAI-1 and the metabolic syndrome was established many years ago. We have previously shown that the more severe the metabolic syndrome with a high number of criteria, the higher the level of plasma PAI-1. From the nuclear families of the Stanislas Cohort, we have quantified this association and observed that the metabolic syndrome explained a major part of PAI-1 variability, this relationship being stronger in males than in females (45 vs 26%). Attempts to understand the mechanisms leading to the increase in plasma PAI-1 levels in the metabolic syndrome come up against the complexity of this syndrome. The first approach was to propose that the metabolic disturbances observed during the metabolic syndrome directly affect PAI-1 synthesis. Most cell culture experiments confirm that hyperinsulinemia, excess of free fatty acid, angiotensin II, cortisol directly increase PAI-1 synthesis. But clinical observations do not always support such a direct effect. Although PAI-1 belongs to the serpin group, it does not hold all its properties. PAI-1 gene expression is inducible and has the features of an immediate early gene. It is short lived and synthesized by a wide variety of cells and tissues on condition that the environment is favourable. The main environmental conditions found in the literature are inflammation and tissue remodelling. By transposing these
situations to the metabolic syndrome one can ask oneself whether the subinflammatory state, well described during obesity, explains the increase in plasma PAI-1 levels and/or whether the remodelling of adipose tissue explains the PAI-1 increase observed. From 1996 until today several groups have connected adipose tissue to PAI-1. A PAI-1 production by adipocyte cell lines, human adipose tissue explants has been described. Using immunohistochemistry and in situ hybridization we found PAI-1 antigen mainly localized in the stromal compartment of the adipose tissue. PAI-1 antigen was detected in purely stromal area and was also found in small cells in direct contact with adipocytes as monocytes (or macrophages). The pattern of PAI-1 localization differs from that observed for von Willebrand factor (vWF) antigen and from leptin. Double labelling confirmed that few small cells in close contact with adipocytes expressed both PAI-1 and CD14, a monocyte marker. During human adipocyte differentiation we observed a transitory increase in the production of PAI-1 antigen but we found a continuous decrease in PAI-1 mRNA despite the presence of insulin and dexamethasone in the medium. Using freshly collected tissues we have separated mature adipocytes from stromal cells and found PAI-1 mRNA mainly in the stromal fraction of the tissue. These results are in accordance with those of Fain et al., who find a PAI-1 release by adipocytes at levels far lower than those of non fat cells. Accumulation of fat in the central part of the body is one of the features of the metabolic syndrome. Rather than a global fat accumulation the metabolic syndrome is the witness of fat redistribution. This fat redistribution is thought to reflect a deficiency in the peripheral fat storage process with a reorientation of fat in ectopic territories such as visceral fat, liver, muscle, vessels causing lipolysis, lipo-toxicity. Several groups have stressed the exclusive association between high plasma PAI-1 levels and visceral obesity. For example the change in plasma PAI-1 levels during a weight reducing program was well correlated with that of the visceral but not that of the subcutaneous fat depot. Fat redistribution has also been recognized in patients with HIV infection associated with the features of the metabolic syndrome. Interestingly in this population the only significant predictor of PAI-1 was the waist to hip ratio although it partially explains the rise in PAI-1. This prompted us to examine the PAI-1 expression in ectopic fat depots mainly the visceral adipose tissue and the liver. We found that ectopic visceral expressed 5 fold more PAI-1 than subcutaneous tissue. Ectopic fat accumulation in human liver was also associated with a strong expression of PAI-1 close to fat. All these results suggest that circulating PAI-1 levels are not closely dependent on fat mass but reflect rather a fat redistribution and could be considered as a biomarker of the ectopic fat storage state. But several questions remained unanswered: is there a direct ectopic fat mass effect, an indirect connection between ectopic fat and PAI-1 through a mediator or a common ground with a parallel evolution of PAI-1 and ectopic fat without true connection? Literature has supplied some data on the intervention of possible mediators. For a long time inflammatory cytokines and growth factors have been shown to play a key role in PAI-1 regulation mainly in vitro. TNF and TGFβ, appeared to be candidates of choice. Indeed TNF has been involved in the mechanism of insulin resistance. TGFβ represents an inducer of choice in the context of tissue remodelling. The group of Loskutoff was the first to emphasize the potential contribution of TNF in PAI-1 regulation during obesity. In ob/ob mice deletion of both TNF receptors (R1 and R1) led to significant reduction of plasma PAI-1 as well as adipose tissue PAI-1 mRNA levels. In these animals the use of TNF neutralizing antibodies leads to an immediate decrease in plasma PAI-1 level proving a direct link between TNF and PAI-1 during obesity. The invalidation of both TNF receptors decreases TGFβ expression in the adipose tissue, suggesting that the TNF and TGFβ pathways are connected within adipose tissue and could both control PAI-1 expression. In humans similar associations were evidenced. We found, within the adipose tissue, a strong relationship between both TNF Rs, TGFβ, and PAI-1. This result reinforces the possible connection between the TNF/TGFβ pathway and PAI-1 within adipose tissue. Thus the increased PAI-1 expression observed during the metabolic syndrome may reflect a particular kind of inflammatory state localized in ectopic fat tissues and in response to tissue aggression. Apart from the contribution of the inflammatory process we could not exclude the contribution of other inducers involved at the same time. Clinical observations have highlighted the link between glucocorticoids and obesity. We have previously shown that dexamethasone and cortisol are potent inducers of PAI-1 synthesis by 3T3 cells and human adipose tissue. Interestingly cortisol can be produced within the adipose tissue through the action of the 11β-hydroxysteroid dehydrogenase (11β-HSD). The only isoform expressed in adipose tissue, acts predominantly as an oxoreductase to generate cortisol from inactive cortisone. Its expression is elevated in the visceral tissue. We observed that the expression levels of this enzyme followed the same evolution of PAI-1. Using adipose tissue explants we found that cortisol and inactive cortisone stimulated PAI-1 secretion, but coincubation with a specific 11β-HSD inhibitor prevented the effect of cortisone whereas this effect was not produced with cortisol. This suggests that the local conversion of cortisone to cortisol may be involved in the increase of PAI-1 expression in adipose tissue.

The contribution of PAI-1 to the development of the metabolic syndrome has recently been proposed. Several studies have indicated this direction. High PAI-1 levels may help to identify a high-risk population with the potential to prevent both atherosclerotic disease and type 2 diabetes. Indeed, Festa et al. showed that high plasma PAI-1 levels predict the development of diabetes. Interestingly in a logistic regression model that included a lot of parameters increased PAI-1 levels still remained significantly related to incident type 2 diabetes (OR [95% CI] for 1 SD increase, 1.61 [1.20-2.16]; p = 0.002). A direct connection between PAI-1 and the action of insulin has been shown in vitro. Addition of vitronectin to fibroblasts cooperates with insulin to induce protein kinase B phosphorylation. PAI-1 was able to prevent this cooperation in a vitronectin-dependent manner. We thus suspect that PAI-1 may interfere with insulin signalling in adipocyte and may control the development of obesity or the metabolic syndrome. A first result was that obtained by the group of Liang et al. Wild type and PAI-1 deficient adipocytes were used after 10 days of differentiation. It was shown that PAI-1 deficiency enhanced glucose uptake at the basal state and under insulin stimulation. Interestingly the same group studied the effect of PAI-1 on adipocyte differentiation. Inhibition of PAI-1 with a neutralizing antibody promoted 3T3 adipocyte differentiation. Similar results were obtained with PAI-1 deficient adipocytes although less pronounced. Conversely overexpression of PAI-1 by adenovirus-mediated gene transfer inhibited differentiation. Differentiation markers were controlled in parallel. PAI-1 deficiency increased expression of CCAAT/enhancer binding protein alpha, CEBPα and fatty acid binding protein (aP2). Remarkably PAI-1 deficiency was able to prevent the deleterious effect of TNF on insulin sensitivity. So in the light of these results an interesting aim was then to
look at these effects starting from the whole organism. Our group was interested in the analysis of the effect of PAI-1 excess. Mice overexpressing murine PAI-1 (PAI-1 Tg) under the control of the aP2 promoter were constructed in order to develop a model with high PAI-1 expression within adipose tissue. This model has not only increased adipose tissue PAI-1 levels but led to 10 fold higher plasma PAI-1 antigen levels. Under high fat diet (HFD) transgenic mice exhibited a lower feeding efficiency with a significant lower body weight after 15 weeks HFD. This leads to the conclusion that PAI-1 overexpression has impaired adipose tissue growth. This result could be in accordance with the effect of PAI-1 on adipocyte differentiation but this possibility needs to be confirmed. We then looked at the metabolic parameters in these mice. It appears that the PAI-1 overexpression worsens the metabolic profile. Transgenic mice maintained on standard fat diet exhibit higher insulin and triglyceride levels despite lower body fat. Glucose and insulin tolerance tests did not reveal significant differences between both genotypes. We then performed euglycemic clamp and again we did not find any difference between the groups. More precise analysis at the tissues levels have been planned. Due to the improvement of insulin sensitivity induced by PAI-1 inhibition in vitro one could expect that PAI-1 deficiency will lead to higher subcutaneous fat accumulation in vivo. Surprisingly two groups found that fat accumulation was prevented in mice lacking PAI-1 in two different kinds of models a nutritionally induced and a genetic murine model of obesity. The protection against obesity was linked to an increase in metabolic rate, total energy expenditure and thermogenesis. Unfortunately our group did not reproduce these results in two different series of a nutritionally induced obesity. However we observed that pharmacological inhibition of active PAI-1 improves insulin sensitivity in mice. A synthetic low molecular weight PAI-1 inhibitor was added to the food of wild type mice for 4 weeks. Addition of this inhibitor did not significantly affect total body fat. After insulin injectionglycemia was lower in treated animals suggesting higher insulin sensitivity in treated mice. Overall these data support the concept that PAI inhibition has the potential to reduce obesity and its complications and may represent a new interesting therapeutic target.

REFERENCES
Endothelial dysfunction in the metabolic syndrome

ANGELO AVOGARO


The metabolic syndrome (MS) has reached epidemic proportions. Substantial clinical and experimental studies suggest that key components of the MS include, in addition to National Cholesterol Education Program (NCEP) criteria, chronic inflammation, procoagulation, and impaired fibrinolysis. The metabolic hallmark of MS is the presence of insulin resistance, i.e., a decreased sensitivity or responsiveness of peripheral tissue to the metabolic action of insulin. Insulin resistance per se and all the components of the MS are associated with altered functions of the endothelium, a dynamic autocrine/paracrine organ, that regulates vascular tone and the interaction of the vessel wall with circulating substances and blood cells. Endothelial cells secrete an array of mediators which can alternatively mediate either vasoconstriction or vasodilation. Nitric oxide (NO) is the major contributor to endothelium-dependent relaxation in conduit arteries. The MS substantially impairs the vasodilating properties of the endothelium and leads to the endothelial dysfunction which can thus be considered the first step in the progression of cardiovascular disease (CVD).

If we assume that the measurement of endothelial function represents a surrogate of endothelial NO availability, then endothelium dependent vasodilation could provide prognostic information in terms of future cardiovascular events, as clearly shown by several independent groups.

Oxidative stress is the common mechanistic damage by risk factors of the MS. Oxidation reactions are crucial in all the events that lead to atherogenesis, including endothelial dysfunction. The effect of oxygen derived free radicals (ROS) on vascular function depends critically on the amounts produced: when formed in low amounts ROS can act as intracellular second messengers, modulating the responses as growth of vascular smooth muscle cells and fibroblasts. Higher amounts of ROS can cause DNA damage, significant toxicity, or even cell apoptosis. Moreover, under the effect of risk factors, endothelial NO synthase (eNOS) becomes uncoupled and O$_2^{-}$ is made rather than NO. In endothelium exposed to agents that damage the vasculature there is stimulation of several enzymes that can produce ROS: among these enzymes, nicotinamide adenine dinucleotide/NADPH oxidase is a major vascular source of ROS. Hyperglycemia is the major causal factor in the development of diabetic vascular complications and can mediate their adverse effects through multiple pathways. One of those mechanisms is the activation of protein kinases (PKC) by hyperglycemia-induced increases in diacylglycerol (DAG) level, partly due to de novo synthesis. There is increasing evidence that PKC activation is important in diabetes-related endothelial dysfunction: impaired NO-vasodiliation...
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In hypertension there is activation of the renin-angiotensin system, a vasoconstrictor effect of angiotensin II and the mineralocorticoid effects of aldosterone. Angiotensin II has been shown to stimulate O$_2$ generation; it has also the capability to stimulate cell hypertrophy induced by angiotensin type 1 (AT1) receptor. Therefore in hypertension there is a condition of widespread oxidative stress that ultimately leads to endothelial dysfunction.

In vivo studies have convincingly shown that hypertension is associated with reduced endothelial function: defects have been detected either by infusing acetylcholine (Ach), methacholine, or by applying shear stress. Alterations in endothelial function have been shown at the conduit arteries level, in the microcirculation and in the subcutaneous circulation. Thus hypertension, a component of the MS, contributes significantly to the alteration of endothelial function in patients with this condition.

The atherogenic dyslipidemia associated with MS is characterized by hypertriglycerideremia, increase in VLDL secretion from the liver, increase in atherogenic small dense low density lipoprotein (LDL), and a decrease in antiatherogenic high density lipoprotein (HDL) cholesterol. High levels of LDL and the parallel low level of HDL generate ROS. In turn, oxidised LDL reduce NO synthesis and release, and can cause enhanced destruction of NO. Increased triglyceride concentration in the MS has also an important negative effect on endothelial function. There is evidence that the postprandial rather than the fasting triglyceride concentration play a negative role on endothelial function: this physiological phenomenon reflects changes in the composition and concentration of plasma lipoproteins that occur after ingesting a fatty meal.

Several studies in diabetic patients support the conclusion that postprandial hyperglycemia is a more powerful risk factor of cardiovascular disease than fasting hyperglycemia itself. It has been shown that an oxidative mechanism mediates endothelial activation induced by postprandial hyperlipidemia and hyperglycemia. This negative influence on endothelial function has been reported in both normal and diabetic subjects. Myocardial contrast echocardiography
using microbubbles may offer an approach to the noninvasive detection of endothelial disease using clinical ultrasound imaging techniques. We have shown that postprandial hyperglycemia determines myocardial perfusion defects in type 2 diabetic patients. These are secondary to deterioration in microvascular function causing a decrease in myocardial blood volume.

Each component of the MS alters the integrity of endothelium; however, this has the capability to renew itself at least partly through the action of the so-called endothelial precursor cells (EPCs), a subset of bone marrow-derived cells capable of originating mature endothelial cells. EPCs are believed to localize specifically at site of ischemia, where they should increase blood vessel growth: EPC ability to improve blood supply in peripheral and myocardial ischemia has been demonstrated in several experimental models. A substantial reduction in circulating EPCs has been demonstrated by our group in patients with MS. Thus, EPC reduction and dysfunction may be involved both in endothelial dysfunction, as an earlier event in the atherogenic process, and in impaired collateralization in the presence of vascular obstruction by a plaque, as an advanced event leading to clinical manifestation of the atherosclerotic disease. Multiple, interrelated mechanisms contribute to endothelial cell dysfunction in insulin resistance. All traditional risk factors for coronary heart disease (CHD), including insulin resistance, induce endothelial dysfunction which is the first key step in the pathogenesis of atherosclerotic lesions. Since patients with MS are at particular risk for developing CHD, endothelial dysfunction must be either prevented or corrected by modifying lifestyle and, if this is not adequate, by correcting each single risk factor without establishing hierarchic priority.

REFERENCES
Over the past decade we have witnessed a major increase in the scale of scientific activity devoted to the study of energy balance and obesity. This explosion of interest has, to a large extent, been driven by the identification of genes responsible for murine obesity syndromes, and the novel physiological pathways revealed by those genetic discoveries.

We and others have also recently identified several single gene defects causing severe human obesity. Many of these defects have been in molecules identical or similar to those identified as a cause of obesity in rodents. I will consider the human monogenic obesity syndromes that have been characterized to date and discuss how far such observations support the physiological role of these molecules in the regulation of human body weight and neuroendocrine function.

INTRODUCTION

The concept that body fat mass is homeostatically regulated emerged in the 1950s and was supported by the hypothalamic lesioning studies of Hetherington et al. and Anand et al. and the parabiosis experiments of Hervey. The subsequent emergence of several murine genetic models of obesity, and their study in parabiosis experiments by Coleman led to the consolidation of the concept that a circulating factor might be involved in the mediation of energy homeostasis. However, it was not until the 1990s when the precise molecular basis for the agouti, ob/ob, db/db and fat/fat mouse emerged, that the molecular components of an energy balance regulatory network began to be pieced together. The use of gene targeting technology has gone on to demonstrate the critical roles of certain other key molecules such as the melanocortin 4 receptor (MC4R) and melanin concentrating hormone (MCH) in that network.

A critical question raised by these discoveries is the extent to which these regulatory pathways are operating in the control of human body weight. Over the past few years a number of novel monogenic disorders causing human obesity have emerged. In many cases the mutations are found in components of the regulatory pathways identified in rodents.

CONGENITAL LEPTIN DEFICIENCY

In 1997, we reported two severely obese cousins from a highly consanguineous family of Pakistani origin. Both children had undetectable levels of serum leptin and were found to be homozygous for a frameshift mutation in the ob gene (ΔG133), which resulted in a truncated protein that was not secreted. We have since identified three further affected individuals from two other families (and unpublished observations) who are also homozygous for the same mutation in the leptin gene. All the families are of Pakistani origin but not known to be related over five gene-
rations. A large Turkish family who carry a homozygous missense mutation have also been described.[15] All subjects in these families are characterised by severe early onset obesity and intense hyperphagia.[14,16,17] Hypersinsulinaemia and an advanced bone-age are also common features.[14,16] Some of the Turkish subjects are adults with hypogonadotropic hypogonadism.[17] Although normal pubertal development did not occur there was some evidence of a delayed but spontaneous pubertal development in one person.[17]

We demonstrated that children with leptin deficiency had profound abnormalities of T cell number and function[14], consistent with high rates of childhood infection and a high reported rate of childhood mortality from infection in obese Turkish subjects[17]. However, there are some phenotypes where the parallels between human and mouse are not as clear-cut. Thus, while ob/ob mice are stunted[14], it appears that growth retardation is not a feature of human leptin deficiency[14,15], although abnormalities of dynamic growth hormone secretion have been reported in one human subject.[17] ob/ob mice have marked activation of the hypothalamic pituitary adrenal axis with very elevated corticosterone levels[19]. In humans, abnormalities of cortisol secretion are, if present at all, much more subtle[14]. The contribution of reduced energy expenditure to the obesity of the ob/ob mouse is reasonably well established[20]. In leptin deficient humans we found no detectable changes in resting or free-living energy expenditure[14], although it was not possible to examine how such systems adapted to stressors such as cold. Ozata et al[11] reported abnormalities of sympathetic nerve function in leptin deficient humans consistent with defects in the efferent sympathetic limb of thermogenesis.

**RESPONSE TO LEPTIN THERAPY**

Recently we reported the dramatic and beneficial effects of daily subcutaneous injections of leptin reducing body weight and fat mass in three congenitally leptin deficient children.[14] We have recently commenced therapy in the other two children and seen comparably beneficial results (personal observations). All children showed a response to initial leptin doses (that were) designed to produce plasma leptin levels at only 10% of those predicted by height and weight (i.e. approximately 0.01 mg/kg of lean body mass)[14].

The major effect of leptin was on appetite with normalisation of hyperphagia. Leptin therapy reduced energy intake during an 18 MJ ad libitum test meal by up to 84% (5 MJ ingested pre-treatment vs 0.8 MJ post-treatment in the child with the greatest response)[14]. We were unable to demonstrate a major effect of leptin on basal metabolic rate or free-living energy expenditure[14], but, as weight loss by other means is associated with a decrease in (BMR) basal metabolic rate[21], the fact that energy expenditure did not fall in our leptin deficient subjects is notable.

The administration of leptin permitted progression of appropriately timed pubertal development in the single child of appropriate age and did not cause the early onset of puberty in the younger children[14]. Free thyroxine and TSH levels, although in the normal range before treatment, had consistently increased at the earliest post-treatment time point and subsequently stabilized at this elevated level[14]. These findings are consistent with evidence from animal models that leptin influences TRH release from the hypothalamus[22-24] and from studies illustrating the effect of leptin deficiency on TSH pulsatility in humans.[25]

Throughout the trial of leptin administration, weight loss continued in all subjects, albeit with refractory periods which were overcome by increases in leptin dose[14]. The families in the UK harbour a mutation which leads to a prematurely truncated form of leptin and thus wild-type leptin is a novel antigen to them. Thus, all subjects developed anti-leptin antibodies after ~6 weeks of leptin therapy, which interfered with interpretation of serum leptin levels and in some cases were capable of neutralising leptin in a bioassay[14]. These antibodies are the likely cause of refractory periods occurring during therapy. The fluctuating nature of the antibodies probably reflects the complicating factor that leptin deficiency is itself an immuno-deficient state[26,27] and administration of leptin leads to a change from the secretion of predominantly Th2 to Th1 cytokines, which may directly influence antibody production.

**Is there a heterozygous phenotype?**

The major question with respect to the potential therapeutic use of leptin in more common forms of obesity relates to the shape of the leptin dose response curve. We have clearly shown that at the lower end of plasma leptin levels, raising leptin levels from undetectable to detectable has profound effects on appetite and weight[14]. Heymsfield et al administered supraphysiological doses (0.1-0.3 mg/kg body weight) of leptin to obese subjects for 28 weeks[28]. On average, subjects lost significant weight, but the extent of weight loss and the variability between subjects has led many to conclude that the leptin resistance of common obesity cannot be usefully overcome by leptin supplementation, at least when administered peripherally. We studied the heterozygous relatives of our leptin deficient subjects. Serum leptin levels in the heterozygous subjects were found to be significantly lower than expected for % body fat and they had a higher prevalence of obesity than seen in a control population of similar age, sex and ethnicity[29]. Additionally, % body fat was higher than predicted from their height and weight in the heterozygous subjects compared to control subjects of the same ethnicity[29]. These findings closely parallel those in heterozygous ob- and db/- mice[30,31]. These data provide further support for the possibility that leptin can produce a graded response in terms of body composition across a broad range of plasma concentrations.

All heterozygous subjects had normal thyroid function and appropriate gonadotropins, normal development of secondary sexual characteristics, normal menstrual cycles and fertility suggesting that low leptin levels are sufficient to preserve these functions[29]. This is consistent with the data of Ioffe et al[32] who demonstrated that several of the neuroendocrine features associated with leptin deficiency were abolished in low level leptin transgenic mice, which were fertile with normal corticosterone levels.

Our findings in the heterozygous individuals have some potential implications for the treatment of common forms of obesity. Whilst serum leptin concentrations correlate positively with fat mass, there is considerable inter-individual variation at any particular fat mass. Leptin is inappropriately low in some obese individuals and the relative hypo leptinemia in these subjects may be actively contributing to their obesity and may be responsive to leptin therapy[33]. Heymsfield et al[33] found no relationship between baseline plasma leptin levels and therapeutic response, however, study subjects were not preselected for relative hypo leptinemia. A therapeutic trial in a subgroup of subjects selected for disproportionately low circulating leptin levels would be of great interest.

**LEPTIN RECEPTOR DEFICIENCY**

A mutation in the leptin receptor has been reported in one consanguineous family with three affected subjects[34]. Af-
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affected individuals were found to be homozygous for a mutation that truncates the receptor before the transmembrane domain. The mutant receptor ectodomain is shed from cells and circulates bound to leptin. The phenotype has similarities to that of leptin deficiency. Leptin receptor deficient subjects were also of normal birthweight but exhibited rapid weight gain in the first few months of life, with severe hyperphagia and aggressive behaviour when food was denied. Basal temperature and resting metabolic rate were normal, cortisol levels were in the normal range and all individuals were normoglycaemic with mildly elevated plasma insulin similar to leptin-deficient subjects.

POMC

Two unrelated obese German children have been reported with homozygous or compound heterozygous mutations in POMC (pro-opiomelanocortin). Both children were hyperphagic and developed early-onset obesity presumably due to impaired melanocortin signalling in the hypothalamus. Presentation was in neonatal life with adrenal crisis due to isolated ACTH deficiency (POMC is a precursor of ACTH in the pituitary). The children had pale skin and red hair due to the lack of MSH function at melanocortin 1 receptors in the skin. Three further subjects with homozygous or compound heterozygous complete loss of function mutations of the POMC gene have been described. Recently, a number of groups have identified a heterozygous missense mutation (Arg236Gly) in POMC that disrupts the dibasic amino acid processing site between β-MSH and β-endorphin. This results in an aberrant β-MSH/β-endorphin fusion peptide which binds to MC4R (melanocortin 4 receptor) with an affinity identical to that of α-MSH. The mutant receptor ectodomain is shed from cells with an affinity identical to that of α- and β-MSH but has a markedly reduced ability to activate the receptor. Therefore, this cleavage site mutation in POMC may confer susceptibility to obesity through a novel molecular mechanism.

PROHORMONE CONVERTASE 1 DEFICIENCY

Further evidence for the role of the melanocortin system in the regulation of body weight in humans comes from the description of a 47 year old woman with severe childhood obesity, abnormal glucose homeostasis, very low plasma insulin but with elevated levels of proinsulin, hypogonadotropic hypogonadism and hypocortisolism associated with increased levels of POMC. She was found to be a compound heterozygote for mutations in prohormone convertase 1, which cleaves prohormones at pairs of basic amino acids, leaving C-terminal basic residues that are excised by carboxypeptidase E (CPE). The inability to cleave POMC is a likely mechanism for obesity in these patients. PC1 cleaves a number of other neuropeptides in the hypothalamus including glucagon-like-peptide 1, which may influence feeding behaviour. The phenotype of these subjects is very similar to that seen in the CPE deficient fat/fat mouse implicating this part of the pathway may be important in the control of body weight in humans.

HUMAN MC4R DEFICIENCY

Of the five known melanocortin receptors, the melanocortin 4 receptor (MC4R) has been most closely linked to control energy balance in rodents. Mice homozygous for a deleted MC4 receptor become severely obese; heterozygotes have body weights intermediate between wild type and homozygote null animals. In 1998, two groups reported heterozygous mutations in the MC4 receptor in humans which were associated with dominantly inherited obesity. Since then, heterozygous mutations in MC4R have been reported in obese humans from various ethnic groups. We have studied over 500 severely obese probands and found that approximately 5-6% have pathogenic MC4R mutations that are non-conservative in nature, not found in control subjects from the background population and co-segregate with obesity in families. MC4R deficiency represents the commonest known monogenic cause of human obesity. Some studies have observed a lower prevalence and this may be explained by the differing prevalence in certain ethnic groups although it is more likely to reflect the later onset and reduced severity of obesity of the subjects in these studies.

We have now studied over 100 MC4R mutant carriers in our clinical research facility. Alongside the increase in fat mass, MC4R mutant subjects also have an increase in lean mass that is not seen in leptin deficiency. Linear growth of these subjects is striking with affected children having a height standard deviation score (SDS) of +2 compared to population standards (mean height SDS of other obese children in our cohort ± 0.5). MC4R deficient subjects also have higher levels of fasting insulin than age, sex and BMI SDS matched children. The accelerated linear growth and the disproportionate early hyperinsulinaemia are consistent with observations in the MC4R KO mouse.

Affected subjects are objectively hyperphagic, but this is not as severe as that seen with leptin deficiency. Of particular note is the finding that the severity of receptor dysfunction seen in in vitro assays can predict the amount of food ingested at a test meal by the subject harbouring that particular mutation.

We have studied in detail the signalling properties of many of these mutant receptors and this information should help to advance the understanding of structure/function relationships within the receptor. Importantly, we have been unable to demonstrate evidence for dominant negativity associated with these mutants, which suggests that MC4R mutations are more likely to result in a phenotype through haploinsufficiency.

SUMMARY

The identification of molecules that control food intake has generated new targets for drug development in the treatment of obesity and related disorders. These considerations indicate that an expanded ability to diagnose the pathophysiological basis of human obesity will have direct applications to its treatment. A more detailed understanding of the molecular pathogenesis of human obesity may ultimately guide treatment of affected individuals.

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Farooqi IS. Monogenic human obesity syndromes


There is almost an epidemic increase in the occurrence of obesity in most countries. This is creating a growing health economy problem, mainly because of the metabolic complications that are associated with obesity and which, ultimately, lead to atherosclerotic disorders that promote disability and early death. Although a number of factors may explain the association between obesity and abnormal metabolism, the adipose tissue itself may be the worst culprit because the tissue produces a number of signalling molecules that influence other tissues. This review will focus on the role of adipose tissue for the metabolic complications to obesity paying particular interest to free fatty acids (FFAs), derived through adipocyte lipolysis, because those molecules are best characterized as regards a cause-effect relationship. Because of clinical concerns the review will predominantly deal with human adipose tissue. In the interest of space, review articles rather than original publications will be cited whenever possible.

ADIPOSE TISSUE AS A SECRETORY FACTOR

Previously, adipose tissue was just looked upon as a storage organ for FFAs, thus playing a role solely in energy homeostasis. This picture has markedly changed during the last 15 years or so because it has become increasingly apparent that adipose tissue produces and secretes a large number of proteins. Some of these proteins are signalling molecules having various effects in other organs. Others have above all autocrine/paracrine roles within the adipose tissue. Some proteins (mainly inflammatory ones) are not only produced by the fat cells but also by the stroma cells in adipose tissue and participate in a low grade inflammatory reaction of adipose tissue that is observed in the obese state. Among the inflammatory proteins only interleukin-6 is proven to be secreted from adipose tissue into the circulation and it is possible, but not proven, that the increased release of interleukin-6, which is observed among the obese, could play a role for insulin resistance in liver and muscle.

Adiponectin is a protein that almost solely is produced by adipocytes. It has insulin-like effects and seems to protect against adverse effects of obesity, in particular in the liver. Although the adiponectin production of adiponectin is decreased in the obese state and in other insulin resistant disorders there is no proof yet in humans that adiponectin has a role in any metabolic complication associated with obesity. However, the putative receptors for adiponectin are isolated so it is possible that we in the near future will have a better knowledge about the pathophysiological role of adiponectin in obesity.

Leptin was the first discovered adipocyte specific hormone. This protein has major roles in energy homeostasis, appetite regulation and reproduction. It is, however, less clear whether leptin causes metabolic com-
plication to obesity or not. Indeed leptin production and circulating leptin are increased among the obese but the metabolic effect of this change is not known in man. Studies of animals have suggested that hyperleptinemia may cause increased lipid accumulation (i.e. lipotoxicity) in tissue outside of adipose tissue such the heart, skeletal muscle and the liver. Whether this also occurs in man remains to be proven. Recently other adipose specific proteins have been detected; as regard about metabolic complication to obesity, some of them are proven to be a false trait. For example, resistin is produced by rodent adipocytes and in an increased some of them are proven to be a false trait. For example, resistin is produced by rodent adipocytes and in an increased rate in obesity. In rodents this molecule causes insulin resistance. In man however, it is not produced by fat cells and seems to be of no or little concern for metabolic changes and/or insulin resistance among the obese. FFAs were discovered some 50 years ago and rapidly a relationship between obesity and circulating FFAs was established. FFA is still the strongest causative link between metabolic complications and obesity, which is discussed in some detail below.

**FFA LEVELS IN OBESITY**

It is well established that circulating FFA levels are increased in obesity and that this is associated with decreased overall insulin sensitivity. The relationship is particularly strong among upper body obese. A recent example from my own laboratory is shown in figures 1 and 2 examining the relationship between upper body obesity, in vivo insulin sensitivity (measured as HOMA-index) and fasting serum FFA levels. Among about 2,500 healthy subjects the serum FFAs (measured as log HOMA index) in 2,438 healthy subjects. See legend to figure 1 for further details.

**HOW ARE FFAS CAUSING METABOLIC COMPICATIONS IN OBESITY?**

As reviewed, elevated circulating levels of FFA lead to a number of abnormalities in non-adipose tissues. In skeletal muscle FFAs are competing with glucose as substrate for oxidation according to the so called Randle’s cycle. High availability of FFAs impairs glucose handling of the skeletal muscle and results in hyperglycaemia. A high uptake of FFAs by the muscle may lead to intracellular accumulation of triglycerides which causes insulin resistance. FFAs may also directly interfere with insulin signalling in muscle cells.

As regards the liver, FFAs have several effects. They impair insulin metabolism and may cause hyperinsulinemia. They inhibit insulin action and may cause hepatic insulin resistance. They stimulate gluconeogenesis and may cause increased glucose output from the liver and, finally, they are substrates for VLDL triglyceride synthesis by the liver and

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Fig. 1. Relationship between upper body fat (measured as waist) and fasting levels of serum free fatty acids (s-FFA) in 2,527 healthy subjects. Results of linear regression analysis.

Fig. 2. Relationship between fasting s-FFA and insulin resistance (measured as log HOMA index) in 2,438 healthy subjects. See legend to figure 1 for further details.
therefore may cause dyslipidemia. Finally, FFAs regulate insulin production by the pancreas and a high FFA levels may induce impaired insulin secretion and, thus, cause diabetes.

**CONCLUSIONS**

There is strong evidence suggesting that molecules produced by adipose tissue are major factors behind the metabolic alterations among the obese. Although the release of several adipose tissue-derived proteins such as adiponectin, interleukin-6 and (maybe) leptin is influenced by obesity and such changes may alter insulin action and metabolic events in liver and muscle, there is not yet enough human data available to suggest a cause effect relationship. Elevated FFAs, which is a hallmark in obesity, seems, however, to be a causative link between metabolic complications and obesity. In the resting fasting state, there is increased delivery of FFAs from fat cells to the blood stream due to enhanced rate of basal lipolysis in all adipose regions among the obese, which at least in part is caused by local overproduction of tumour necrosis factor alpha within the adipose depots. During hormone stimulation, such as after exercise (catecholamines) and after meal ingestion (insulin) more FFAs are released from visceral fat than from subcutaneous fat due to regional differences in hormone action on lipolysis. This elevates “portal” FFAs and has selective effects on the liver. Since the regional variations in lipolysis are most prominent among upper body obese subjects, the depot variations might link between central obesity stronger to metabolic complications than peripheral obesity. It could be possible in the future to develop new therapeutic agents that modify lipolysis by adipose tissue and thereby improve the metabolic status among the obese.

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Nuevas aproximaciones al síndrome metabólico

Experiencia DRECE

MIGUEL A. RUBIO, EN REPRESENTACIÓN DEL GRUPO DRECE


En el estudio DRECE participan 52 centros de salud distribuidos homogéneamente por áreas rurales y urbanas de todo el territorio nacional, de acuerdo con un modelo sectorial de 8 grandes áreas propuesto por el Ministerio de Agricultura, Pesca y Alimentación.

RESULTADOS DEL DRECE-1

Realizado entre 1991 y 1992, se pudo conocer los diferentes factores de riesgo cardiovascular de la población objeto del estudio, así como los hábitos alimentarios en ese momento. En la tabla 1 se pueden ver algunos de los resultados en los que ya se apreciaban prevalencias elevadas de los principales factores de riesgo.

En cuanto a los hábitos alimentarios, se ponía de manifiesto una disminución del aporte de hidratos de carbono (un 40% de la energía) a favor de un mayor consumo de grasas (un 40-43% de la energía), sobre todo a expensas de las grasas saturadas (un 13-15%) procedentes del consumo de derivados lácteos y cárnicos, bollería y aperitivos. Se demostró que la ingestión de grasa saturada y colesterol se correlacionaba con las concentraciones de lípidos (positivamente para el colesterol de las lipoproteínas de baja densidad [cLDL] y los triglicéridos y negativamente para el colesterol de las lipoproteínas de alta densidad [cHDL]) en las regiones con mayor consumo de estos ácidos grasos (Levante, Andalucía, Canarias), áreas poblacionales que, por otro lado, se corresponden con las de mayor tasa de enfermedades cardiovasculares en España.

RESULTADOS DEL DRECE-2

Este segundo análisis de la cohorte DRECE se realizó 5 años después de la primera observación (1996-1997). En esta ocasión se seleccionó a todos los sujetos con factores de riesgo cardiovascular y a una muestra representativa de 600 individuos, apareados por edad y sexo, sin evidencia de factores de riesgo cardiovascular. Los resultados encontrados no mostraron apenas cambios respecto a 1992, entre otros motivos porque se

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tuvo especial esmero en no intervenir en la población objeto del estudio. Sólo los sujetos con factores de riesgo cardiovascular habían mejorado ligeramente sus hábitos alimentarios reduciendo significativamente el porcentaje de grasa saturada y colesterol. En este segundo estudio se analizó la prevalencia de síndrome metabólico y de obesidad abdominal en relación con los factores de riesgo cardiovascular. La prevalencia general de síndrome metabólico fue del 23,7% de la población, muy similar a otros resultados obtenidos en Canarias o Estados Unidos (un 22-26%). La obesidad abdominal y el síndrome metabólico tienen relación con un incremento significativo de la proteína C reactiva, lo que indica un mayor riesgo cardiovascular en este colectivo de sujetos.

Por último, se analizó la incidencia de eventos cardiovasculares incluyendo mortalidad por esa causa, y se observó que los sujetos tipificados como en riesgo cardiovascular presentaban una odds ratio de 3,96 de tener un episodio cardiovascular respecto a los individuos sin riesgo. El análisis de regresión permitió deducir que las concentraciones de triglicéridos, pero no otras variables clásicas, tienen relación muy significativa con el riesgo de contraer una enfermedad cardiovascular.

PERSPECTIVAS FUTURAS

Se ha comenzado a realizar un tercer corte transversal de la misma cohorte (DRECE-3), con un seguimiento de 14 años, lo que nos permitirá analizar con más detenimiento no sólo la evolución de los principales factores de riesgo y los hábitos alimentarios de la muestra sino, sobre todo, disponer de información relativa a morbilidad y mortalidad en esta población; sin duda, nos ayudará a explicar mejor las tendencias en esta enfermedad en un área geográfica como la nuestra.

BIBLIOGRAFÍA GENERAL

On the trail to arrest the progression of the metabolic syndrome

JOSE F. CARO


The metabolic syndrome is a cluster of metabolic anomalies that represent one of the major unmet medical needs. The metabolic anomalies include among others, diabetes, obesity, hypertension and atherosclerosis. They represent the phenotype of survival genes in an environment of plenitude.

There are two major goals for treatment: a) secondary prevention — in those patients identified with the metabolic syndrome, reductions of the risks for atherosclerotic disease is the primary goal, and b) primary prevention — in those populations at risk for the development of the metabolic syndrome lifestyle interventions, dietary changes, physical activity and possibly pharmacotherapy are the primary goals.

SECONDARY PREVENTION

The current recommendations of the American Heart Association/National Heart, Lung, and Blood Institute are summarized in table 1. There is general agreement on those recommendations by the American Diabetes Association, the European Association of the Study of Diabetes2, the International Diabetes Federation3, and the practicing physicians at large.

The role of statins targeting LDL cholesterol in the reduction of CVD risk, morbidity and mortality is now well established. These studies were recently reviewed4. The role of fenofibrate targeting HDL cholesterol was recently published5. The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study assess the effect of fenofibrate on cardiovascular events in patients with diabetes. Fenofibrate did not significantly reduce the risk of the primary outcome of coronary events5, but it did reduce secondary outcomes. The role of pioglitazone on the secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study was recently reported6. Pioglitazone did not reduce the primary composite endpoint but it did reduce secondary outcomes6. In summary, the results from these well-executed trials5,6 do not warrant yet a general recommendation different from that in table 1.

PRIMARY PREVENTION

There are not primary prevention studies on the metabolic syndrome. However, there are many prevention studies on diabetes. A significant percent of subjects with pre-diabetes, impaired glucose tolerance or impaired fasting glucose have a phenotype consistent with that of the metabolic syndrome. Therefore learning from these studies could be applied to the larger and more heterogeneous population of patients with the me-
**TABLE 1. Therapeutic goals and recommendations for clinical management of metabolic syndrome**

<table>
<thead>
<tr>
<th>Therapeutic target and goals of therapy</th>
<th>Therapeutic recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lifestyle factors</strong></td>
<td></td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>Consistently prevent CVD and prevent (or treat) of type 2 diabetes mellitus</td>
</tr>
<tr>
<td>Reduce body weight by 7% to 10% during year 1 of therapy. Continue weight loss thereafter to extent possible with goal to ultimately achieve desirable weight (BMI &lt; 25)</td>
<td></td>
</tr>
<tr>
<td><strong>Physical inactivity</strong></td>
<td></td>
</tr>
<tr>
<td>Regular moderate-intensity physical activity; at least 30 min of continuous or intermittent (and preferably ≥ 60 min) 5 days/week, but preferably daily</td>
<td>In patients with established CVD, assess risk with detailed physical activity history and/or an exercise test, to guide prescription. Encourage 30 to 60 min of moderate-intensity aerobic activity: brisk walking, preferably daily, supplemented by increase in daily lifestyle activities (e.g., pedometer step tracking, walking breaks at work, gardening, household). Longer exercise times can be achieved by accumulating exercise throughout day. Encourage resistance training 2 days/week. Advise medically supervised programs for high-risk patients (e.g., recent acute coronary syndrome or revascularization, CHF)</td>
</tr>
<tr>
<td><strong>Atherogenic diet</strong></td>
<td>Recommendations: saturated fat ≤ 7% of total calories; reduce trans fat; dietary cholesterol ≤ 200 mg/dl; total fat 25% to 35% of total calories. Most dietary fat should be unsaturated; simple sugars should be limited</td>
</tr>
<tr>
<td>Reduced intake of saturated fat, trans fat, cholesterol</td>
<td></td>
</tr>
<tr>
<td><strong>Metabolic risk factors</strong></td>
<td>Shorter-term prevention of CVD or treatment of type 2 diabetes mellitus</td>
</tr>
<tr>
<td>Atherogenic dyslipidemia</td>
<td>Elevated LDL-C. Elevated non-HDL-C</td>
</tr>
<tr>
<td>Primary target: elevated LDL-C. Secondary target: elevated non-HDL-C (optional: &lt; 100 mg/dl [2.6 mmol/l] for very high-risk patients), Moderately high-risk patients: &lt; 160 mg/dl (4.1 mmol/l); therapeutic option: &lt; 130 mg/dl (3.4 mmol/l), Moderate-risk patients: &lt; 160 mg/dl (4.1 mmol/l), Lower-risk patients: &lt; 190 mg/dl (4.9 mmol/l)</td>
<td>First option to achieve non-HDL-C goal: Intensify LDL-lowering therapy. Second option to achieve non-HDL-C goal: Add fibrate (preferably fenofibrate) or niacin acid if non-HDL-C remains relatively high after LDL-lowering drug therapy. Give preference to adding fibrate or niacin acid in high-risk patients. Give preference to avoiding addition of fibrate or niacin acid in moderately high-risk or moderate-risk patients. All patients: If TG is 500 mg/dl, initiate fibrate or niacin acid (before LDL-lowering therapy; treat non-HDL-C to goal after TG-lowering therapy)</td>
</tr>
<tr>
<td>Tertiary target: reduced HDL-C</td>
<td>Reduced HDL-C</td>
</tr>
<tr>
<td>No specific goal: Raise HDL-C to extent possible with standard therapies for atherogenic dyslipidemia</td>
<td>Maximize lifestyle therapies: weight reduction and increased physical activity. Consider adding fibrate or niacin acid after LDL-C-lowering drug therapy as outlined for elevated non-HDL-C</td>
</tr>
<tr>
<td>Elevated BP</td>
<td>For BP ≥ 120/80 mmHg: Initiate or maintain lifestyle modification in all patients with metabolic syndrome: weight control, increased physical activity, alcohol moderation, sodium reduction, and emphasis on increased consumption of fresh fruits, vegetables, and low-fat dairy products. For BP ≥ 140/90 mmHg (or ≥ 130/80 mmHg for individuals with chronic kidney disease or diabetes): As tolerated, add BP medication as needed to achieve goal BP</td>
</tr>
<tr>
<td>Reduce BP to at least achieve BP of &lt; 140/90 mmHg (or &lt; 130/80 mmHg if diabetes present), Reduce BP further to extent possible through lifestyle changes.</td>
<td></td>
</tr>
<tr>
<td>Elevated glucose</td>
<td>For IFG, encourage weight reduction and increased physical activity. For type 2 diabetes mellitus, lifestyle therapy, and pharmacotherapy, if necessary, should be used to achieve near-normal HbA1c (&lt; 7%). Modify other risk factors and behaviors (e.g., abdominal obesity, physical inactivity, elevated BP, lipid abnormalities)</td>
</tr>
<tr>
<td>For IFG, delay progression to type 2 diabetes mellitus. For diabetes, hemoglobin A1c &lt; 7%</td>
<td></td>
</tr>
<tr>
<td>Prothrombotic state</td>
<td>High-risk patients: Initiate and continue low-dose aspirin therapy; in patients with ASCVD, consider clopidogrel if aspirin is contraindicated. Moderately high-risk patients: Consider low-dose aspirin prophylaxis</td>
</tr>
<tr>
<td>Reduce thrombotic and fibrinolytic risk factors</td>
<td></td>
</tr>
<tr>
<td>Proinflammatory state</td>
<td>Recommendations: no specific therapies beyond lifestyle therapies</td>
</tr>
</tbody>
</table>


1 High-risk patients are those with established ASCVD, diabetes, or 10-year risk for coronary heart disease > 20%. For cerebrovascular disease, high-risk condition includes TIA or stroke of carotid origin or > 50% carotid stenosis.

2 Very high-risk patients are those who are likely to have major CVD events in next few years, and diagnosis depends on clinical assessment. Factors that may confer very high risk include recent acute coronary syndromes, and established coronary heart disease + any of following: multiple major risk factors (especially diabetes), severe and poorly controlled risk factors (especially continued cigarette smoking), and metabolic syndrome.

3 Moderately high-risk patients are those with 10-year risk for coronary heart disease 10% to 20%. Factors that favor therapeutic option of non-HDL-C < 100 mg/dl are those that raise individuals to upper range of moderately high risk: multiple major risk factors, severe and poorly controlled risk factors (especially continued cigarette smoking), metabolic syndrome, and documented advanced subclinical atherosclerotic disease (e.g., coronary calcium or carotid intima-medial thickness > 75th percentile for age and sex).

4 Moderate-risk patients are those with 2+ major risk factors and 10-year risk < 10%.

5 Lower-risk patients are those with 0 or 1 major risk factor and 10-year risk < 10%.

From: Grundy et al.1
The metabolic syndrome. Several studies have demonstrated that weight loss and lifestyle interventions reduced the incidence of type 2 diabetes in patients at risk. One of the first pilot studies demonstrating this benefit used bariatric surgery as a way to achieve long lasting and significant weight loss.

About 60% loss of excess body weight in patients with clinically severe obesity (> 45 kg excess body weight) prevented the progression of impaired glucose tolerance to diabetes by > 30-fold. The incidence of type 2 diabetes (patients/years) was 1.682 in the experimental group (incidence rate = 0.15) and 6/127 in the control group (incidence rate = 4.75). The incidence rate (100 person-years) of the control group was similar to other observation studies pre-diabetes.

This study conducted from 1980 to 1991 used a patient population and a method of weight loss that was neither representative of the majority of obese subjects nor of the optimal treatment modality. Therefore, it had to be established that less obese individuals with pre-diabetes were able to prevent or delay their conversion to type 2 diabetes by weight loss using less invasive therapeutic procedures. More recently, evidence from three large randomized controlled and longer-term trials examining the impact of lifestyle changes on the progression of pre-diabetes to type 2 diabetes has been reported. These trials have been recently reviewed and they are summarized in Table 2.

Thus, lifestyle interventions are the recommended approaches for the prevention of type 2 diabetes and therefore also the metabolic syndrome. Pharmacotherapy with metformin, thiazolidinediones, alpha-glycosidase inhibitors, and others, recently reviewed, also prevent type 2 diabetes, but in general to a lesser extent than lifestyle interventions. Thus, pharmacotherapy may well become part of the secondary approach for prevention of the metabolic syndrome just as it may be for type 2 diabetes.

CONCLUSIONS

It is clear that the continuing globalization and the adoption of the “western” lifestyle across the world will most certainly cause more widespread conflict between these “thrifty genes” and the environment. Thus, effective prevention will only be achieved by a multi-factorial approach, including lifestyle interventions, socio-economical interventions, and pharmacological interventions. For the present, it is essential that research effort is directed to understand the etiology and mechanism(s) of the metabolic syndrome. This information will be used to refine the current working diagnostics criteria of the syndrome and targeted therapy for the myriad of diseases that will prove to include the metabolic syndrome.

REFERENCES

Nuevas aproximaciones al síndrome metabólico

Síndrome metabólico y riesgo cardiovascular

RAFAEL CARMENA


CONCEPTO Y DEFINICIÓN

La combinación de factores de riesgo cardiovascular de origen endógeno en un mismo individuo fue descrita por Marañón y Kylin a comienzos del pasado siglo y alcanzó especial relieve con la publicación por Reaven en 1988 del llamado síndrome X. Desde esa fecha aumentó exponencialmente el número de publicaciones dedicadas a ese síndrome conocido como síndrome X, síndrome de resistencia a la insulina o cuarteto de la muerte, hasta la más utilizada denominación actual de síndrome metabólico (SM).

El SM se define como la agrupación en un mismo individuo de factores de riesgo cardiovascular de origen endógeno y confiere un riesgo elevado de contraer diabetes tipo 2 y enfermedades cardiovasculares, notablemente cardiopatía isquémica (CHD). Su prevalencia es alta en las sociedades industriales y su utilidad para identificar fácilmente en una población a sujetos de alto riesgo está bien demostrada.

Los principales alteraciones o factores de riesgo habitualmente incluidos en el SM son: obesidad abdominal (expresada por el perímetro de la cintura), hipertensión arterial, alteraciones del metabolismo de los hidratos de carbono, anomalías lipoproteínicas (hipertrigliceridemia, colesterol de las lipoproteínas de alta densidad [cHDL] bajo y aumento de los ácidos grasos libres, las partículas de lipoproteínas de baja densidad [LDL] pequeñas y densas y de las lipoproteínas portadoras de apolipoproteína B) y microalbuminuria. Más recientemente se han ido añadiendo otros componentes, relacionados con la inflamación, la disfunción endotelial, el estado protrombótico, la steatosis hepática, etc., que describiremos en otro apartado. Conviene aclarar que el perímetro de la cintura puede utilizarse como un marcador indirecto pero fiable del contenido de grasa intraabdominal, ya que se correlaciona significativamente con dicho depósito cuantificado por tomografía computarizada (TC).

Se piensa que las alteraciones descritas tienen una base fisiopatológica común, la resistencia periférica a la insulina (RI), acompañada generalmente de hiperinsulinismo compensador. La RI y el hiperinsulinismo en ayunas tienen relación independiente con la dislipemia, la hipertensión arterial, la disfuncción endotelial y otras manifestaciones del síndrome. La RI está condicionada por factores genéticos y ambientales, como una dieta hipercalesérica rica en grasa saturada, obesidad, tabaquismo y sedentarismo. La relación entre RI y SM no es la de una simple agrupación, sino que se trata de una verdadera conexión causa-efecto y explica, entre otras, la dislipemia, la disglucemia y la hipertensión. La elevación de los ácidos grasos libres en sangre es una de las consecuencias fisiopatológicas más importantes de la RI, a la que a su vez potencian. La elevación
de citocinas, como el factor de necrosis tumoral alfa (TNFα) y otras, contribuye también a potenciar la RI y que se desarrolle un estado inflamatorio crónico, objeto de otras presentaciones. Otros componentes del SM, como la estatoxisis hepática no alcohólica, el estadio protrombótico, la disfunción endotelial, las elevaciones de leptina y ferritina, etc., son también objeto de otras presentaciones.

**CRITERIOS DIAGNÓSTICOS**

Un problema persistente al abordar el estudio del SM es el de los criterios para diagnosticarlo, y hay cerca de una docena de ellos propuestos por grupos de expertos o sociedades científicas. En las tablas 1 a 3 resumimos los 3 criterios más aceptados en la actualidad.

La Organización Mundial de la Salud (OMS) publicó en 1999 unos criterios para definir el SM y considera la IR como factor *sine qua non*. Estos criterios exigen que haya una tolerancia anormal a la glucosa o diabetes mellitus o IR (valorada con el modelo HOMA). Como es obvio, esta definición tiende a identificar a sujetos que ya tienen una alteración de la regulación del metabolismo de la glucosa y alto riesgo de contraer diabetes tipo 2. Es cierto que aproximadamente el 80% de los diabéticos tipo 2 cumplen criterios diagnósticos de SM, pero al menos un tercio de los sujetos con SM conservan una tolerancia normal a la glucosa. Por ello, las normas del Grupo Europeo para el estudio de la Resistencia a la Insulina (EGIR) para el diagnóstico del SM incluyen la IR y excluyen la diabetes, con la razonable justificación de que una posible complicación del síndrome no debe formar parte de sus criterios diagnósticos.

El National Cholesterol Educational Program Adult Treatment Program III (NCEP ATP-III), en Estados Unidos, publicó otras normas que dan prioridad a la presencia de obesidad abdominal y requieren que concurran 3 de los siguientes 5 determinantes: aumento de la circunferencia de la cintura, triglicéridos elevados, cHDL bajo, presión arterial elevada y glucemia en ayunas alterada. Por su pragmatismo y fácil aplicación, han recibido la aceptación mayoritaria y han sido actualizados recientemente. Finalmente, en 2005, la International Diabetes Federation (IDF) ha introducido modificaciones a los criterios ATP-III para dar especial importancia a la obesidad abdominal y cambiar los puntos de corte del perímetro de la cintura según las diferentes etnias (tabla 2).

El criterio diagnóstico utilizado influye en las estimaciones de prevalencia del SM en una población. En España, la prevalencia de SM en población adulta oscila entre el 18 y el 30%, dependiendo de la edad de la población y de los criterios usados para su diagnóstico. En todos los estudios epidemiológicos se observa una clara tendencia al aumento de la prevalencia con la edad de la población.

Los criterios NCEP ATP-III e IDF identifican sobre todo a sujetos con alto riesgo cardiovascular, mientras que los de la OMS identifican a sujetos con riesgo de diabetes mellitus tipo 2. Usando los criterios ATP-III, que tienen un umbral diagnóstico más bajo, se identifica a un número más alto de sujetos con SM que con los criterios de la OMS. Con los criterios de IDF de 2005, con puntos de corte para perímetro de cintura más bajas, se puede identificar a más sujetos en algunas poblaciones. Sin embargo, en Estados Unidos, la mayoría de los varones con cintura > 94 cm y las mujeres con cintura > 88 cm cumplen también los criterios del ATP-III e igualmente se los diagnosticaría. Por ahora no disponemos de datos comparativos sobre la asociación del SM con el riesgo cardiovascular usando cada una de las definiciones.

**SÍNDROME METABÓLICO Y RIESGO CARDIOVASCULAR**

El SM y sus distintos componentes confieren un elevado riesgo cardiovascular, y se estima que éste duplica el hallado en sujetos sin SM de iguales edad y sexo. Debe quedar claro, sin embargo, que el SM no es un predictor de riesgo absoluto y que la estimación de éste exige el empleo de tablas o algoritmos que incluyan otros factores de riesgo cardiovascular (edad, sexo, tabaquismo, cLDL, antecedentes familiares, etc.). Por lo tanto, una vez establecido el diagnóstico de SM, procede calcular el riesgo absoluto o riesgo general, y en función de ello decidir si, además de la impecindible intervención con cambios del estilo de vida, hay que añadir fármacos. Las tablas y ecuaciones más utilizadas para el cálculo del riesgo absoluto son las de Framingham, PROCAM y otras mencionadas más adelante. Aunque los estadísticos que trabajan con datos epidemiológicos continúan discutiendo si el riesgo cardiovascular atribuido al SM supera al aportado por la suma de los de cada componente, la mayoría se inclina por lo primero. Es decir, el riesgo que acompaña al SM es fruto de un efecto multiplicativo, más que de una mera adición.

En apoyo de esta postura, los resultados de estudios prospectivos llevados a cabo en Europa, como el Estudio Bruge, el Kuopio Ischemic Heart Disease Risk Study y el Estudio LIPID, no han podido identificar un efecto aditivo en el riesgo cardiovascular. Sin embargo, estos resultados son de muy reciente publicación y no se han podido replicar en otras poblaciones.

**TABLA 1. Criterios diagnósticos del síndrome metabólico**

<table>
<thead>
<tr>
<th>ATP-III 2001-2005</th>
<th>OMS 1999</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Para los varones</strong>: Obesidad abdominal: perímetro de la cintura &gt; 102 cm (varones) o &gt; 88 cm (mujeres). Hipertrigliceridemia ≥ 150 mg/dl (1,6 mmol/l) cHDL bajo</td>
<td>Al menos 1 de los siguientes: Diabetes mellitus tipo 2. Tolerancia anormal a la glucosa. Resistencia a la insulina (HOMA Q4)</td>
</tr>
<tr>
<td><strong>Para las mujeres</strong>: Obesidad abdominal: perímetro de la cintura &gt; 80 cm. cHDL bajo</td>
<td>Más al menos 2 de los siguientes: Hipertensión ≥ 140/90 mmHg. Obesidad (IMC ≥ 30). Hipertrigliceridemia ≥ 150 mg/dl o cHDL bajo (&lt; 35 mg/dl en varones y &lt; 40 mg/dl en mujeres) Microalbuminuria ≥ 20 µg/min</td>
</tr>
<tr>
<td><strong>Para el cálculo del riesgo cardiovascular</strong>: Glucemia basal ≥ 100 mg/dl (≥ 5,6 mmol/l)</td>
<td></td>
</tr>
</tbody>
</table>

**TABLA 2. Consenso de la IDF 2005. Definición de obesidad central**

<table>
<thead>
<tr>
<th>Más de 2 de los siguientes: Triglicéridos ≥ 1,7 mmol/l (150 mg/dl)* cHDL</th>
<th>Glucemia en ayunas ≥ 5,6 mmol/l (100 mg/dl)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varones &lt; 1 mmol/l (40 mg/dl)*</td>
<td>Microalbuminuria ≥ 20 µg/min</td>
</tr>
<tr>
<td>Mujeres &lt; 1,1 mmol/l (50 mg/dl)*</td>
<td></td>
</tr>
<tr>
<td>Presión arterial sistólica ≥ 130 mmHg* o Presión arterial diastólica ≥ 85 mmHg*</td>
<td></td>
</tr>
</tbody>
</table>

*O estar recibiendo tratamiento específico. Tomado de Alberti et al.
Las recomendaciones más aceptadas indican que si el riesgo cardiovascular estimado a 10 años es bajo, se debe comenzar con cambios en el estilo de vida; en cambio, si se considera elevado el riesgo (> 20%), se iniciará al mismo tiempo un tratamiento farmacológico de los diferentes componentes del síndrome.

TRATAMIENTO DEL SÍNDROME METABÓLICO

Dado que el SM es una constelación de distintas alteraciones interrelacionadas, su tratamiento exige un enfoque multifactorial de todos los factores de riesgo existentes, comenzando por los cambios de estilo de vida. Quede claro que no hay, por ahora, un tratamiento específico del SM, aparte del tratamiento intensivo de todos sus componentes. Además, la estrategia terapéutica estará precedida por una valoración del riesgo cardiovascular de cada sujeto. En la tabla 4 se recogen los objetivos terapéuticos según las recomendaciones publicadas recientemente.

Cambios de estilo de vida

La obesidad y el sedentarismo son los determinantes básicos de la mayoría de los componentes del SM y se debe tratarlos inicialmente y con intensidad. Una pérdida significativa de peso mejora todos los factores de riesgo relacionados con el SM y reduce también el riesgo de diabetes tipo 2. Se recomienda pautar una dieta hipocalórica (1.000-1.200 kcal/día) y baja (< 8%) en grasas saturadas y un programa personalizado de ejercicio aeróbico (idealmente, de 30 min al día) con el objetivo de una pérdida ponderal de un 7-10% en 1 año. La cirugía bariátrica puede ser una opción válida en casos de obesidad mórbida y SM, que llega a desaparecer en el 95% de los casos al año de la intervención.

La abstención absoluta del hábito tabáquico y el consumo moderado de bebidas alcohólicas, sal, azúcares simples o hidratos de carbono con alto índice glucémico forman parte importante de este apartado.

Dos estudios prospectivos recientemente publicados respaldan la importancia de los cambios en el estilo de vida para la prevención del SM y de su evolución hacia la diabetes tipo 2. El 53% de los participantes en el Diabetes Prevention Program (DPP) fueron diagnosticados de SM al inicio del estudio. Respecto al grupo control, el tratamiento con un programa intensivo de ejercicio aeróbico y dieta hipocalórica en grasa saturada y ejercicio aeróbico permite prevenir la glaucosa y SM. Una pérdida de peso de aproximadamente 4,5 kg y la práctica habitual de ejercicio aeróbico proporciónaron una significativa reducción de SM y de la progresión a diabetes tipo 2 respecto a los resultados del tratamiento con metformina o placebo.

Por lo tanto, la combinación de dieta hipocalórica pobre en grasa saturada y ejercicio aeróbico permite prevenir la aparición del SM en un porcentaje significativo de los sujeto...
Tratamiento farmacológico de los factores de riesgo

Si las medidas expuestas no bastan para alcanzar los objetivos terapéuticos, como ocurre a menudo en sujetos de riesgo alto o muy alto, se añadirán fármacos. La prioridad para su empleo son las elevaciones del cLDL, presión arterial y glucemia.

El tratamiento coadyuvante de la obesidad con fármacos puede ser necesario en algunos casos. El empleo de orlistat, sibutramina o antagonistas de los receptores endocannabinoides tipo 1, como rimonabant, ha mostrado utilidad, si bien son necesarios estudios de confirmación más prolongados.

El tratamiento de la dislipidemia con estatinas en sujetos con SM se ha demostrado beneficioso reduciendo significativamente la cantidad de cLDL y apolipoproteína B y el riesgo cardiovascular. Los fábricos también se han mostrado capaces de reducir el riesgo en este grupo de sujetos y la combinación de estatinas con fenofibrato puede resultar especialmente eficaz en algunos casos. No se debe utilizar, en cambio, combinada con gemfibrozilo, por el mayor riesgo de miositis.

Se recomienda que el tratamiento de la hipertensión arterial en sujetos con SM se inicie indistintamente con inhibidores de la enzima de conversión de angiotensina o antagonistas de los receptores de angiotensina II. Un aspecto que destacar en el contexto del SM con tolerancia anormal a la glucosa es la aparente protección contra la aparición de diabetes ofrecida por estos fármacos, como han puesto de manifiesto recientes estudios. Como en el caso de la diabetes, una proporción importante de los hipertensos con SM requerirá el empleo de al menos 3 fármacos hipotensores para alcanzar los objetivos.

El tratamiento farmacológico de la tolerancia anormal a la glucosa o de la diabetes se ha de llevar a cabo con agentes orales (inhibidores de la alfa2 glucosidasa intestinal, insulinosecretagogos, metformina o glitazonas) o, en su caso, insulina. El recientemente publicado estudio PROACTIVE ha mostrado una reducción de las complicaciones cardiovasculares en los diabéticos tipo 2 de alto riesgo que, además de su tratamiento farmacológico (inhibidores de la enzima de conversión de angiotensina o antagonistas de los receptores de angiotensina II) o, en su caso, insulina. La combinación de estatinas con fenofibrato puede resultar especialmente eficaz en algunos casos. No se debe utilizar, en cambio, combinada con gemfibrozilo, pero el mayor riesgo de miositis.

COMENTARIOS FINALES

El SM se ha convertido recientemente en el centro de una controversia, más semántica que científica, sobre su verdadera utilidad y fisiopatología. Aun cuando algunas de las críticas vertidas no carecen de fundamento, pensamos que el concepto del SM ha funcionado como un paradigma útil en la práctica clínica para identificar a sujetos en riesgo de diabetes o complicaciones cardiovasculares por arteriopatía coronaria, y por ello se debe conservarlo. Además, el concepto integrador de SM sirve como testimonio de que el riesgo de enfermedad coronaria isquémica (CHD) es multifactorial y existe más allá de los valores de cLDL. Ciertamente, es imprescindible investigar más en sus mecanismos fisiopatológicos y valorar mejor el riesgo cardiovascular aportado por los distintos componentes del síndrome. Pero, en cualquier caso, hay más argumentos a favor de conservar el concepto los que se en contra, y el interés por su detección precoz para la prevención de las complicaciones antes aludidas está más que justificado.

BIBLIOGRAFÍA


