biomedicine at the turn of the century

JESÚS AVILA & JOSÉ M. MATO

In the last century, descriptive biology gave way to quantitative biology, while medicine based on simple visual diagnosis became a medicine of analysis and protocols. In both cases, what was sought was a new, more rigorous methodology offering reproducible data. Quantification and measurement were used in the quest to increase the scientific nature of biology and medicine. These criteria based on correct measurement led to the development of biochemistry, and later, molecular biology. At first, analyses were based on simple models, but these became increasingly more complex and could be extrapolated to help understand how human beings function. Thus, interpretations of growing complexity were carried out by studying simple processes, with few variables, and then studying how those processes interact. At the turn of the twentieth to twenty-first century, this became known as systems biology.

New discoveries in the transition

During the period of transition between the two centuries, scientists managed to decipher the information (or sequence) of the genomes of different organisms, including the human genome (Venter et al. 2001, 1304), carrying out a not—especially—rigorous description of the proteins expressed in those organisms, in terms of both the quantity and nature of those proteins.

Moreover, more was learned, at a fundamental level, about the basic elements of life. The central dogma of molecular biology indicates that the genome (DNA) is transcribed as RNA, one form of which—messenger RNA—is transposed, leading to the synthesis of proteins. Beyond the previously described messenger RNA, transfer RNA and ribosomic RNA, in recent years, a new type of RNA has been described: interference RNA, which acts to regulate genetic expression (Fire et al. 1998, 806).

Nevertheless, the second part of the genetic code, the key to how proteins are folded, remains unknown. This is important because there are various illnesses—called proteinopathies—that are based on the defective, nonfunctional folding of a protein. In many cases, this leads to the aberrant aggregation of those proteins.

Levels of study

Studies have been carried out at both molecular and cellular levels. While basic molecular processes are shared not only by the cells in a single organism, but also between one organism and another, the great variety of cells in superior organisms such as mammals has led to specific studies focused on different cell

types. Some types of cells proliferate in a determined cycle whose aberrations can lead to tumors, while others survive for a long time without dividing, in a differentiated state, such as neurons. There are cells that interact intimately among each other, like the ones that form epithelial tissue; while those that make up connective tissue surround themselves with an extra-cellular matrix. But in general, the process of differentiation begins with precursor cells that undergo various modifications, leading to mature cells. Those prolific precursor cells are in turn derived from more primitive cells called stem or mother cells. At the turn of this new century, an important advance has been made in the knowledge of those mother cells. Protocols have been developed to transform mother cells originating in human embryos into different cell types—muscle tissue, nerve tissue, and so on (Thompson et al. 1998, 1145)—suggesting that this process may be used for cellular regeneration therapy. Moreover, there is considerable discussion about the possibility that mother cells from certain adult tissues might be pluripotent, that is, that they might be able to transform into different types of tissue. This discussion continues today. More recently, a discovery of potential interest has been described. This discovery, reprogramming, consists in reversing the differentiation of mature cells from specific tissues, converting them into cells with the characteristics of embryonic mother cells. The expression of transcription factors, Oct4, Sox2, KLF4, and cMyc, converts—reprograms—differentiated fibroblasts into cells with characteristics similar to those of mother cells (Takahashi and Yamanaka 2006, 663). This reprogramming may occur naturally in the transition between epithelial and mesenquimal cells that takes place during development (Mani et al. 2008, 704). Afterwards, these cells with stem-cell characteristics may again differentiate themselves, becoming cell types other than the initial one. In rats, neurons obtained from reprogrammed fibroblasts can be transplanted into a mouse with the symptoms of Parkinson's disease, producing a functional recovery (Wernig et al. 2008, 5856).

New therapies

Beginning with the previously mentioned discovery of interference RNA (RNAi), and the characterization of mother cells, molecular therapy methods have been established that used RNAi to impede the expression of a protein that could be toxic for the organism; as well as cellular therapy methods that use precursor cells in regenerative processes. An alternative source of stem cells is blood from the umbilical cord (Kurtzberg et al. 1996, 157). These cells can be used for cellular therapy in illnesses such as leukemia or hemoglobinopathies, and banks have been set up to store such cells.

The regeneration of organs and tissues varies drastically depending on the nature of the cells that make up those tissues. It is know that, following a hepatomy, the liver regenerates itself, recovering its original size, just as cells change in the blood, skin and bones in a natural way. Artificially, in the case of skin cells, it has been possible to grow skin for regenerative purposes (O'Connor et al. 1981, 75), after cultivating keratinocyte and fibroblast cells. Similarly, the implantation of condriocytes has permitted the regeneration of cartilage (Brittberg et al. 1994, 889). The regeneration of other organs, such as the brain, is more difficult. The development of areas such as molecular biology, cellular biology and biotechnology has permitted better diagnosis of different diseases, and in some cases, the proposal of personalized treatment for patients. Clear differences can be observed among different human beings; different genders, races, hair colors, statures, body sizes, and so on. These differences are due in part to their genomes—DNA—and it has recently been observed that, while what are called polymorphisms of a single nucleotide give rise to small difference in the same gene in different individuals, the largest differences appear to be due to the existence of deletions and/or inversions of genetic material in an individual, as compared to the genetic material of another individual. This observation was considered by *Science* magazine as one of last year's most important scientific achievements (Kennedy 2007, 1833).

Publication of the results

It is important to point out the media's influence on the greater or lesser visibility of discoveries. As we mentioned above, at the end of each year, *Science* magazine announces its favorite discoveries. *Nature* does the same in the magazine, *Nature Methods,* where it chooses the methods of the year. This year, the main method of the year was new technology for faster and cheaper sequencing of genomes, including those of different human beings (Wold and Myers 2008, 19). It has been suggested that this was the technique used to sequence the genome of scientists, Watson and Venter.

Cell also publicizes discoveries published in its magazine. A few years ago, it celebrated its thirtieth anniversary with a special edition (Cell 30th Anniversary Supplement, January 23, 2004) featuring the most relevant works previously published in *Cell.* These included several that earned the Nobel Prize for their authors. One example is the work on the

mechanisms of decay of cytoplasm proteins that was carried out with a machine called the proteasoma (Rader and Daugherty 2008, 904). It is possible that some future Nobel Prize Laureates will be authors whose work was featured in that issue of *Cell.*

The most prevalent diseases

Moreover, molecular knowledge of the different types of cells found in different tissues can facilitate a greater knowledge of diseases. From a very general standpoint, we could say that there are four main types of tissues in an organism: muscle, nerve, epithelia, and connective tissue. The first is very related to cardiovascular problems, as the heart is a muscle. The second is associated with neurodegenerative diseases, the third with infectious processes, and both the third and fourth are associated with increased tumor formation. The solution for these four types of problems —cardiovascular, neurodegenerative, oncological, and infectious, are twenty-first century medicine's fundamental challenges. In this chapter, we will deal mainly with problems related to metabolic defects and neurodegenerative processes. For aspects relating to cardiovascular diseases, we would refer readers to a specific issue of *Nature* (volume 451, issue 7181), which covers this subject almost monographically. It includes discussion of possible new therapies for arteriosclerosis (Rader and Daugherty 2008, 904), possible treatment for thrombosis (Mackman 2008, 914) and the use of mother cells for heart disease (Segers and Lee 2008, 937).

For aspects relating to cancer, we would recommend that readers consult *Nature* (volume 441, issue 7092), which includes the supplement, *Insight: signaling in cancer,* with interesting articles including one by Benson JD et al, about the validation of new targets for testing anti-tumor compounds.

Before closing this introduction, we would like to briefly mention the use of animal models for studying illness, and some improvements in medical diagnosis from a general viewpoint.

Animal models

In many cases, efforts have been made to reproduce some pathological aspects of various diseases by using animal models that minify all or some of the disease's characteristics. Those models include worms, mice, and flies. Fundamentally, studies that employ mice as models use them as targets for drug testing, as a first step towards their posterior clinical use. The use of mice models was awarded the Nobel Prize for Medicine in 2007.

Improvements in medical diagnosis

Despite the advantages of models, it is very important to know specifically what happens in a diseased human body. In that sense, knowledge of medical diagnosis has grown considerably. Such knowledge can be obtained by analyzing the components of fluids such as blood, plasma, urine, and cephalorachidian liquid. But the greatest advance may well have been the development of new imaging technique, such as functional-image magnetic resonance, which offers excellent information about parts of the human body that are difficult to analyze, including the brain (Kerr and Denk 2008, 195).

As we mentioned above, in the present text, we will focus mainly on certain aspects of neurodegenerative processes and metabolic problems.

Alzheimer's disease

As an example of a neurodegenerative disease, let us start with Alzheimer's disease, a senile dementia characterized initially by memory loss, followed by great disorientation and dementia by the patient as the disease progresses (Alzheimer 1907, 146).

Aging is the greatest risk factor for suffering Alzheimer's disease. From the age of 65 on, its prevalence doubles every five years. Almost 2.5% of 65-year-olds suffer it, while the incidence is between 40 and 50% among persons over the age of 85.

Different problems arise around the fundamental ones associated with this disease—the patient's loss of memory, understanding, and will. As a consequence of this deterioration, the patient requires care by others, generally the closest relatives, who may well be those most harmed by the disease. Those are the fundamental problems, but they are accompanied by others in the social and economic spheres.

World Heath Organization data from 2001 (Vas 2001) estimated that Alzheimer's disease affected 18 million people on the planet at that time. Approximate data for the year 2006 raise that figure to 26 million people. It has been estimated that the number may triple by the year 2050, given the increased longevity of human beings today, and the fact that the greatest risk factor is aging. This was published on the web page of the Alzheimer's Association in 2007 under the title, *Alzheimer's Disease Facts and Figures* (http://www.alz.org/alzheimers_disease_facts_figures.asp). In that sense, it has also been suggested that, among persons aged one-hundred or more, the probability of suffering senile dementia could be greater than 50%. In other words, neurodegeneration could be considered a normal process occurring at very advanced ages.

From an economic standpoint, in the United States it has been calculated that caring for 5.6 million

patients could have a minimum cost of one hundred thousand million dollars (Glenner and Wong 1984, 1131; Masters et al. 1985, 4245). Moreover, data from 2006 indicates expenditure of 4,600 million dollars on palliative drugs, which are the only ones available at this time, as no cure has yet been found.

So this is a chronic illness, and it is devastating, with great human, social, and economic cost. Moreover, it is becoming increasingly prevalent, due to the population's increasing average age.

From 1907—when A. Alzheimer discovered the first case of this illness—to the nineteen eighties, knowledge of Alzheimer's disease was derived fundamentally from the anomalous behavior of its sufferers and from histopathological studies of their autopsies. Autopsies of patient's brains indicate an uncommon abundance of two types of aberrant structures: senile plaques and neurofibrillary tangles. Patient autopsies also reveal considerable neuronal death, especially in the hippocampus, the limbic zone, and the cerebral cortex.

In the nineteen eighties, researchers began studying what happens in Alzheimer patients' brains that leads to the formation of senile plaques and neurofibrillary tangles. They discovered that the main component of senile plaques was a peptide (Glenner and Wong 1984, 1131) and they obtained its sequence of amino acids. Since those plaques took the form of amyloid aggregates, they named its peptide *beta amyloid.* This peptide was later found to be a fragment of a protein (Masters et al. 1985, 4245), which was named Amyloid Precursor Protein (APP). This protein is obliquely present in different cell types, but the amyloid beta-peptide only produces aberrant aggregates in the nervous system. Studying the characteristics of the amyloid peptide, researchers concluded that the cuts occurring in APP to produce it were not the ones that usually occur in this protein in non-pathological circumstances. In normal cases, APP, which is located in the plasmatic membrane of cells, remains united to the membrane, or becomes disconnected when a protease called secretease alpha is cut. After the cut, the larger fragment is secreted into the extra-cellular medium. But in pathological conditions (Price et al. 1998, 461), APP is not cut by secretease alpha, but rather by another protease called secretease, and then by another protease, called secretease. When APP is cut by secretease alpha and secretese, it generates the amyloid peptide that leads to different types of aggregates, the largest of which are senile plaques. While the cut produced by beta secretease is precise, occurring between two specific amino acids, the cut produced by secretease γ is imprecise, falling in a particular region of the APP. This lack of precision generates amyloid beta-peptides of different sizes, the most usual of which contain forty $A\beta_{40}$ and forty-two $A\beta_{42}$ residues. The latter of these has a greater capacity to aggregate than the former, so $A\beta_{42}$ is considered the amyloid peptide with the greatest toxic potential. Once amyloid beta-peptides have formed, they can be broken down by proteases such as neprelysin or the enzyme that breaks down insulin, called IDE. By breaking them down, those proteases prevent the aberrant aggregation of amyloid beta-peptides, which can be toxic. That toxicity has been observed when the peptide is added to a culture of neuronal cells. The toxicity of aggregate amyloid beta-peptides may be due to the fact that they facilitate the entrance of calcium into the cellular cytoplasm and/or act as the antagonist in some neuronal signaling paths. Moreover, it has been suggested that they act on microglial cells, facilitating the secretion by those cells of cytokines and other factors, which would provoke an inflammatory process that could end in neuronal death.

It has also been suggested that, in order for the amyloid peptide's toxic effect to take place in neurons, the latter would require the presence of the tau protein (see below). The main component of neurofibrillary tangles is a protein called *tau* (Grundke-Iqbal et al. 1986, 4913) associated with microtubules. Modified by hyperphosphorylation, this protein is present in what are called paired helicoidal filaments (PHF), whose aggregates are neurofibrillary tangles. Using the isolated tau protein, it was possible to determine that it, alone, was sufficient to produce aggregates similar to PHFs (Montejo De Garcini, Serrano, and Avila 1986, 790). Moreover, it has been reported that different kinase proteins can modify—phosphorilate—the tau protein, and that the one that modifies the most residues of the tau protein is the kinase known as GSK3 (Avila et al. 2004, 361). It has been suggested that both phosphorilated tau protein and aggregates made out of tau protein can be toxic for those cells in which they are present.

More recently, it has been suggested that, following neuronal death, intracellular tau moves to the extra-cellular medium, and that this extra-cellular tau may be toxic for nearby neurons, thus contributing to the propagation of the pathology (Gómez-Ramos et al. 2008, 673).

Alzheimer's disease has been divided into two types—family Alzheimer's disease, of genetic origin; and Alzheimer's disease of sporadic origin. The former is very rare. Possibly, less than one percent of the total of Alzheimer's disease cases are of family origin, so the most common type of this illness is the sporadic one.

Nevertheless, knowledge of the mechanism of family Alzheimer's disease offers important clues about this illness in general. That is significant because, if the process by which plaques and tangles are formed were known, it might be possible to design therapies to combat the disease.

Family cases of Alzheimer's disease are due to mutations in three different genes that codify three proteins: APP, the precursor protein of the amyloid peptide; presenilin 1 (PS-1) and presenilin 2 (PS-2). Mutations in APP, which induce the development of the illness, facilitate its cutting by beta and gamma secreteases and inhibit its cutting by secretease alpha. In all such cases, the formation of the amyloid beta-peptide is facilitated (Price et al. 1998, 461).

Because mutations in APP, PS-1, and PS-2 almost always lead to increased production of the amyloid beta-peptide, it was suggested in the "amyloid cascade hypothesis" (Hardy and Selkoe 2002, 353), that the first step in the development of the apparition of the disease was the presence of a specific amount of the amyloid peptide. One it aggregated, it could trigger posterior pathological processes, including hyperphosphorylation and the aggregation of the tau protein. Nevertheless, anatomical-pathological analyses of the development of this disease did not confirm that hypothesis. Apparently, what most correlates to this disease's pathological process is the pathology related to the tau protein, and not that related to the amyloid protein (Braak and Braak 1991, 239; Arriagada et al. 1992, 631). Therefore, analyses were carried out to see if the result of mutations in APP, PS-1, and PS-2 could converge in the modification of any other protein. One possible protein could be the kinase protein, GSK3, because mutations of APP leading to the apparition of amyloid beta-peptide facilitate the activation of GSK3's kinase activity. This is because the amyloid beta-peptide acts as an antagonist for signal paths, insulin, or WNT, leading to an inactivation of GSK3 (Avila et al. 2004, 361). On the other hand, mutations in PS-1 or PS-2 that lead to an increase in the amount of amyloid peptide can have the same consequences as those indicated for mutated APP, while those mutations in PS-1/PS-2 that do not lead to an increase in amyloid beta-peptide may augment GSK3 activity in other ways (Baki et al. 2004, 2586).

Given the confluence of APP, PS-1, and PS 2 mutations in the effect of activating GSK3, a transgenic mouse model was developed that overexpressed kinase in those parts of the hippocampus and cortex most affected by Alzheimer's disease (Lucas et al. 2001, 27). This mouse reproduced some aspects of the tau pathology and also showed memory deficits (Hernández et al. 2002, 1529). It has therefore been used as a target for testing drugs that could inhibit kinase activity and could therefore repair the cognitive deficit. In those genetically modified mice, the most evident lesion is the degeneration of the dentate gyrus (Engel et al. 2006, 1258), which also occurs with Alzheimer patients and may be responsible for the observed memory loss in both the animal model and Alzheimer patients.

Clinically, patients initially have a growing loss of memory, and a slight cognitive deterioration, which have been related to lesions in the region of the hippocampus where the dentate gyrus is located. Later, the pathology extends to the limbic zone of the temporal lobe, and even later, to the frontal cortex, leading to problems of memory consolidation, behavior, and language. Even later, neuronal death can be observed in the parietal cortex, which can lead to visual-spatial problems or problems of disorientation, for example, in the use of utensils, or incapacity to make decisions, which involves both the parietal and frontal cortexes. All of the problems related to disorientation are clinically called dementia. So this disease can be divided in a very general way into two large stages: an initial one characterized by memory loss, and a posterior one in which dementia appears. The two problems are of the utmost importance, but the second one requires greater attention by those persons caring for the patients. In the transition between the twentieth and twenty-first centuries, there has been a great advance in basic-level knowledge of this disease, but there is still not a good therapeutic application of that knowledge to combat it.

Until now, possible therapies have included palliative drugs, rather than curative or modifying ones. Those drugs rather timidly slow the disease's development, but tragically, it still develops in the end. The ones approved by the United States Food and Drug Administration (FDA) are: Tacrine, Donepezil, Galantamine, Rivastigmine, and Memantine. Annual sales of Donepezil alone are close to one-thousand million dollars, while sales of Memantine, the most recent to enter the market, are close to five-hundred million dollars (Mount and Downton 2006, 780; Stephen Salloway 2008, 65). The first four are inhibitors of the acetilcholinesterase enzyme (Stephen Salloway 2008, 65). This enzyme breaks down the neurotransmitter, acetilcholine, which is needed for perfect neuronal transmission. In Alzheimer's disease, there is preferential damage to cholinergic neurons, which use acetylcholine as a neurotransmitter. Those drugs are therefore used to attempt to maintain high

levels of the neurotransmitter, although the first one, Tacrine, was taken off the list because of its toxicity. Memantine is an antagonist to the receptor of another neurotransmittor, glutamate. It has been observed that, among elderly patients, there is an excessive activation of a type of glutamate receptors called NMDA. That activation can be toxic, damaging neurons. In order to protect the neurons of those elderly people, they are given Memantine, which is an antagonist to NMDA receptors (Parsons, Danysz, and Quack 1999, 735; Reisberg et al. 2003, 1333).

Those are the current drugs. Below, we will briefly discuss some diagnostic methods, and some possible future drugs.

As biomarkers for this disease, it is possible to determine levels of the tau protein with different levels of phosphorilylation, and of the amyloid beta-peptide in cephalorachidian fluid. More recently, the levels of as many as 18 components of plasma have been determined as possible indicators of the disease (Ray et al. 2007, 1359), but the diagnostic methods that have probably received the most attention are those that employ imaging techniques such as the PET (Blennow and Zetterberg 2006, 753) and functional magnetic resonance (Logothetis 2008, 869). With these techniques, it is possible to follow the expansion of the ventricles following the neuronal death that affects Alzheimer patients. Of these two techniques, functional magnetic resonance seems to be the most advantageous. It measures hemodynamic changes in different parts of the brain, is non-invasive and has good time-space resolution that can show results correlated with a specific activity being carried out by an individual (Logothetis 2008, 869).

The new drugs are already, or very close to being clinically tested and may be modifiers (Stephen Salloway 2008, 65) rather than palliatives for this disease. In other words, these possible future drugs have a mechanism based on the different observations of this disease's pathology that have been carried out at a basic level. Some are being developed to reduce levels of amyloid beta-peptide, including Bapineuzumab, which involves the development of specific antibodies—vaccines—against the amyloid peptide. Inhibitors of beta and gamma secretease are also being developed, some of which modulate secretease in an effort to reduce the A_{1-42}/A_{1-40} relation. Some, such as Flurizan and Tarenflumbol, also show a possible anti-inflammatory effect. Still, recent news offers negative data for Flurizan. There are other compounds, such as Clioquinol, that prevent beta amyloid aggregation, or polyphenol extracts that may also prevent the oligomerization of the amyloid beta-peptide (Wang et al. 2008, 6388; Stephen Salloway 2008, 65). Others may maintain high levels of those enzymes, such as IDE, the enzyme that breaks down insulin, which can break down the amyloid peptide. One of these compounds is Rosiglitazone, an agonist of PPAR (Pedersen et al. 2006, 265). There has also been a search for inhibitors that do not link directly to secretease but rather to its substrate, impeding the proteolithic cut of the enzyme in that substrate, but not in others (Kukar et al. 2008, 925).

Moreover, it has been shown that the toxic effect of the excessive activity of NMDA receptors could increase levels of amyloid beta-peptide (Harkany et al. 2000, 2735) and of the tau protein (Amadoro et al. 2006, 2892). Therefore, there is a search for antagonists to NMDA receptors, other than Memantine. One of these is Dimebon. Another type of study has been the search for antioxidants that could act as neuroprotectors, but it has not shown significant results.

Finally, regarding pathology related to the tau protein, there is a search for specific inhibitors of kinases like GSK3, that primarily modify that tau protein. It has recently been observed that methyl blue, an antiaggregant of the tau protein, might have a therapeutic effect. Those are just a few examples of the tremendous effort being made to prevent this terrible disease.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is the most frequent cause of liver disease in the Western world and is thus a world health problem. It affects around 20 million people in the United States and a similar number in the European Union (Adams and Lindor 2007; Ahmed and Byrne 2007). NAFLD is frequent in patients with obesity, diabetes, hyperlipidemia, and hypertension (Abdelmalek and Diehl 2007). Increasing obesity in Westernized countries justifies growing interest in the study of NAFLD. Approximately 50% of obese individuals have NAFLD (Angulo 2007). NAFLD is a clinical-pathological term used to describe a broad range of situations running from a simple accumulation of fat on the liver (non-alcoholic steatosis) to non-alcoholic steatohepatitis (NASH, an accumulation of fat with inflammation, necrosis, and fibrosis). NAFLD is generally an asymptomatic disease, although in a minority of NAFLD patients, it leads to the development of cirrhosis, liver failure, and hepatocellular carcinoma (HCC). Approximately 10% of patients with NAFLD develop NASH, and of these, between 10–20% develop cirrhosis and HCC. Excessive alcohol consumption also produces fatty liver and, as

happens with NAFLD, the liver disease can also lead to steatohepatitis, cirrhosis, and HCC. Nevertheless, it is important to emphasize that NAFLD and alcohol-induced liver disease are two different diseases. NASH is also different than other forms of hepatitis caused by various viral infections, such as hepatitis B and C. While clinical diagnosis for NAFLD is based on increases of transaminases in the blood, on body mass index (BMI is calculated by dividing a person's weight in kilos by the square of their height in meters. Values of between 18.5 and 25 are considered normal), on accumulations of fat on the liver visible through sonograms or magnetic resonance, and on the presence of other factors, such as obesity, diabetes, hypertension, and hyperlipidemia. Confirmation of the presence of NASH and the degree of fibrosis and necrosis requires a liver biopsy.

Despite the fact that NAFLD is a world health problem, it is not known why it leads to NASH in some individuals and not in others. There are various hypotheses. The leading one states that two *hits* are necessary. The first hit is the accumulation of fat on the liver, but the nature of the second hit is unknown, though studies carried out in the last few years on genetically modified animals have offered new clues as to the second hit. Nor do we know why some NASH patients progress to cirrhosis and HCC and others don't.

About 25% of the adult population of the United States is obese and another 55% is overweight (Sturm 2002). While those numbers are somewhat better in Europe's adult population, obesity in the European Union is also a public health problem. Obesity is caused fundamentally by an excessive ingestion of calories relative to energy consumption. On an individual level, obesity can be more complex, involving genetic factors that regulate metabolism and lipid storage, the brain's control of eating and exercise habits, and other unknown factors. It is certain that some individuals have genetic factors that have a decisive influence on their tendency to accumulate fat, or that favor the sensation of hunger rather than satiation. For such individuals, weight control is very difficult, but for the majority of the population, genetic factors are less decisive and can be more easily compensated by changes in eating habits. Any obese individual will lose weight if he or she is obliged to maintain a low-calorie diet in combination with exercise. We know this to be true because patients subjected to a gastric by-pass (a surgical intervention to drastically reduce the size of the stomach from around one liter to only 30-60 milliliters) markedly reduce the amount of fat in their adipose tissue.

For most individuals, adipose tissue is nothing but an inert mass of fat, but since the mid nineteen nineties, we know that, biologically, it is very active tissue. In 1994, Friedman and his collaborators identified the leptin hormone, discovering that its lack was the cause of the extreme obesity in a mutant mouse called *obese* (Zhang et al. 1994). Those mice are enormous. While a normal mouse weights around 30 grams, *obese* mice can weigh as much as 90 grams. They have high levels of lipids in their blood and develop fatty livers. Leptin is synthesized in adipose tissue. In normal animals, the amount of leptin in the blood is proportional to the amount of adipose tissue. That is the mechanism used by adipose tissue to inform the brain that enough food has been ingested. *Obese* animals have a mutation in the leptin gene that leads them to synthesize a hormone that is not biologically active. Thus, their brains do not receive an adequate signal to stop eating. Unfortunately, in most obese individuals, the concentration of leptin is abnormally high, rather than low. But the few obese patients that have a genetic leptin deficiency respond well to treatment with this hormone, reducing the accumulation of body fat.

While the discovery of leptin did not lead to the curing of obesity, it did mark a permanent change in how we think about the physiopathology of obesity. Since the discovery of leptin, researchers have discovered other hormones and cytokines (proteins whose main activity is the control of inflammation) originating in adipose tissue that regulates appetite and/or lipid metabolism. These include adiponectine (a hormone synthesized by adipose tissue that favors the oxidation of fatty acids and the metabolism of glucose and whose levels in the blood are inversely proportional to BMI); resistine (a hormone synthesized by adipose tissue and related to inflammation and diabetes), and tumor necrosis factor alpha (TNF-alpha, a cytokine involved in the regulation of cell death, differentiation and proliferation that plays an important role in the etiology of diverse diseases, including diabetes). In other words, adipose tissue is much more than a mere fat deposit. It also plays a fundamental role in appetite control and the metabolism of lipids and carbohydrates.

Signals that encourage us to eat must be balanced with the brain's appetite control center so that the latter initiates the desire to eat when a negative energy balance occurs. Studies carried out over the last fifteen years with genetically modified mice have made it possible to identify obesity genes. Most genetically modified animals that develop obesity do so because they eat more. Their extreme obesity is

caused by mutations that affect their eating habits. It is the increased ingestion of food, and not the capacity to metabolize fats, that causes obesity. In other words, most obesity genes regulate appetite, not lipid metabolism. For example, an obese mutant mouse called *diabetic* has a leptin receptor deficiency. This mouse's problem is that the lack of a biologically active leptin receptor impedes leptin from informing the brain cells that it is time to stop eating. Another obese mouse, known as *yellow mouse,* has a mutation that affects the route of pro-opiomelanocortine (POMC), which is important for appetite reduction. The mutation of those obesity genes produces an accumulation of fat in those mice that is independent of the existence of other genetic or environmental factors. Generally speaking, the situation in humans is more complex, and obesity rarely occurs as a consequence of the mutation of a single gene. In humans, there is usually more than one gene involved in the development of obesity, and the environment generally has an important role in the body's fat accumulation. In other words, on an individual level, eating habits are the main cause of obesity, although an individual's genetic characteristics can also play an important role in that behavior.

Fatty acids are the fat components of triglyceride molecules. Triglycerides are the main component of the fat in adipose tissue and in food. Fatty acids thus come from food, but can also be synthesized from carbohydrates, mostly in the liver. Fatty acids are an important energy reserve, not only because they have a greater caloric density per gram than sugars or proteins, but also because they are hydrophobic. Rather than attracting water, they repel it, and can thus be stored in the body in a more compact fashion than carbohydrates or proteins, which do attract water. Thus, while the caloric density of fat is 9 kcal per gram, that of sugars and proteins is around 4 kcal per gram. Moreover, while fats do not accumulate water, carbohydrates accumulate 2 grams of water per gram of sugar. In other words, there is approximately six times more energy stored up in a gram of fat than in a gram of sugar. This means that if the human body were to store energy in the form of carbohydrates, rather than as fat, an individual would need to store around 100 kilos of glucogen in order to have the energy equivalent of 15 kilos of fat, which is the approximate amount of fat on a non-obese adult human.

When triglycerides from food enter the stomach, the stomach acids, bile salts, and digestive enzymes known as lipases break down those triglycerides into their two components: fatty acids and glycerol. Lipases are synthesized by the pancreas and bile salts come from the liver through the gall bladder. Once freed from the triglycerides, fatty acids enter the cells that make up the intestinal walls and are again converted into triglycerides. There, along with cholesterol esters, phospholipids, and proteins, they create nanoparticles called kilomicrons. The latter are carried to the blood by the lymph system. In the blood, they come into contact with high-density lipoproteins, with which they exchange triglycerides for cholesterol esters. As kilomicrons pass through capillaries, adipose tissue, muscles, the heart, and other non-hepatic tissues, they lose their load of fatty acids as a result of the activity of the lipase lipoprotein enzyme. The fatty acids generated in that way are oxidized to produce energy needed by each of those tissues to fulfill its biological function, or they accumulate in the form of triglycerides. Finally, the kilomicrons remaining in the blood, almost totally freed of their load of triglycerides, are transported into liver cells to be metabolized.

Food intake also leads to the secretion of insulin. This secretion by the pancreas's beta cells stimulates the synthesis of glucogen in muscles and in the liver. In adipose tissue, insulin also stimulates the metabolism of glucose and the synthesis of glycerol, the molecule to which fatty acids link to form triglycerides. Also, in the liver, insulin suppresses gluconeogenesis (the synthesis of glucose and glucogen) and accelerates glucolysis (the metabolism of glucose), which increases the synthesis of fatty acids that accumulate in the form of triglycerides. If intake of fat and carbohydrates surpasses their consumption in a chronic way, the excess energy accumulates in adipose tissue and the blood carries it to the liver in the form of free fatty acids linked to albumin. Finally, those fatty acids accumulate in the liver in the form of triglycerides, producing NAFLD.

We have known for at least the last 500 years that when ducks and geese are overfed, they develop fatty livers. In 1570, Bartolomé Scappi, Pope Pius V's chef, published a cookbook titled *Opera,* in which he wrote that, "…the livers of domestic geese raised by the Jews reach the extreme size of 3 pounds." Overfeeding not only produces NAFLD in birds. In the laboratory, overfeeding rats and mice with a diet rich in fatty acids and carbohydrates to induce the generation of fatty livers continues to be a very widespread experimental method.

Research carried out over the last ten years with genetically modified mice has been fundamental in showing that the deactivation of certain enzymes needed for hepatic synthesis of fatty acids and

triglycerides—acetil coenzyme A carboxilase, diacilglycerol aciltranferase, elongase of long-chain fatty acids, glycerol 3-phosphate mitochondrial aciltransferase, and stearoil-coenzyme A desaturase—prevents the formation of fatty acids induced by a diet rich in fat and carbohydrates (Postic and Girard 2008). These data suggest that the decrease in hepatic synthesis of triglycerides may possibly be an important therapeutic target for the treatment of NAFLD. Nevertheless, it is important to emphasize that the accumulation of triglycerides in the liver is not necessarily toxic. Instead, it may be a way of protecting the liver from toxicity caused by free fatty acids—fatty acids not linked to glycerol molecules to form triglycerides. For example, in *obese* mice, the inhibition of triglyceride synthesis improves steatosis but worsens liver damage (necrosis, inflammation, and fibrosis) (Yamaguchi et al. 2007). If free fatty acids are not oxidized to produce energy, they are metabolized by the microsomal system called cytochrome P450 2E1 (CYP2E1 is particularly active in the liver. Not only does it metabolize exogenous substances such as alcohol, drugs, and pro-carcinogens, it also participates in the metabolism of cholesterol, bile acids, and fatty acids). The metabolism of fatty acids by CYP2E1 generates cytotoxic substances, such as those that react to oxygen (ROS) and peroxidized lipids, that produce hepatic inflammation and necrosis.

In the liver, triglyceride molecules accumulate in the cytoplasm of hepatocytes, forming small drops of lipids. Those drops of lipids are not a simple accumulation of triglycerides—like the drops that form when oil is mixed with water—they are organules whose creation requires the presence of certain specific proteins. One of these proteins is called ADFP. Mice lacking ADFP do not develop NAFLD when overfed with a fat-rich diet (Chang et al. 2006). While ADFP may be a therapeutic target for treating NAFLD, it is not yet known whether the inhibition of the accumulation of triglycerides in obese animals through the inhibition of ADFP may increase liver damage. Another experimental approach, which has been used to prevent NAFLD, is to block the activity of certain transcription factors (proteins that link DNA and regulate the expression of specific genes) that control the synthesis of lipids. One of those transcription factors, called SREBP-1c, mediates the effect of insulin on the expression of those enzymes that regulate the synthesis of fatty acids. Steatosis improves in *obese* mice that are deficient in SREBP-1c (Yahagi et al. 2002), but it is not yet know whether the inhibition of SREBP-1c can increase liver damage in the long run. In sum, even though the inhibition of the synthesis of triglycerides, or of their accumulation in the form of vesicles in the liver, are theoretically good therapeutic approaches to the prevention of NAFLD, it is important to recall that those procedures are not free of possible side effects that might produce liver damage. Therefore, it is not clear that they can have any clinical application.

Surprisingly, malnutrition can also provoke fatty liver. It is not entirely known how this happens, although studies carried out in recent years with genetically modified animals offer new data about the importance of certain nutrients in the development of NAFLD.

In 1930, Banting and Best, the discoverers of insulin, observed that diabetic dogs treated with insulin developed fatty livers, and that this situation could be corrected by administering choline—a micro-nutrient that is a precursor to the synthesis of methionine. Some years later, Best, du Vigneaud, and other research groups observed that when mice or rats are fed a diet lacking methionine and choline, in just a few weeks they also develop steatosis that leads to NASH and, in some animals, even HCC, if the diet is maintained. Those animals fed with a diet lacking methionine and choline not only are not obese; in general, they weigh less than mice fed with a normal diet. Those experiments not only related steatosis to diabetes, they also provided the very first evidence of the importance of a group of micro-nutrients known as methyl-group donors (choline, methionine, betaine, and folic acid) in the prevention of steatosis (Mato et al. 2008).

In mammals, including humans, methionine is an essential amino acid, that is, it cannot be synthesized by the body and must be taken in through food. When methionine is administered to a person orally, the blood levels of this amino acid increase transitorily, returning to their basal levels in two or three hours. The speed with which a person returns to basal levels of methionine after ingesting it is an indicator of the metabolism of this amino acid in the body. In cirrhotic patients, the metabolism of methionine is markedly slower than in individuals with normal hepatic functions. The first step in the metabolism of methionine is its conversion into S-adenosilmethionine (SAMe), a molecule discovered by Giulio Cantoni in 1953 (Cantoni 1975). SAMe has a special place in biology due to its capacity to modify other molecules and their biological activity by adding a methyl group (a methyl group is a carbon atom linked to three hydrogen atoms). Those molecules include DNA, proteins, and phospholipids. This reaction, known by the general name of methylatin, can prevent the expression of certain genes. In other

words, it can cause the same result as a genetic mutation, even though its mechanism is not genetic, but instead, epigenetic.

SAMe synthesis is markedly reduced in the livers of cirrhotic patients (Duce et al. 1988) and treatment with SAMe increases the survival of patients with alcoholic cirrhosis (Mato et al. 1999), which confirms the important role that an alteration of the methionine metabolism has in the progression of liver disease. Consequently, mice with deficient hepatic synthesis of SAMe, though normal sized and not obese, develop steatosis, NASH, and HCC (Lu et al. 2001). In mice with deficiencies of the enzyme glycine N-methyltransferase—the main enzyme that metabolizes SAMe in the liver—the hepatic concentration of SAMe is around 40 times higher than in normal mice (Martínez Chantar et al. 2008). Surprisingly, even though those "super-SAMe" mice are normal-sized and not obese, they also develop steatosis, NASH, and HCC. Such results indicate that both a deficiency and an excess of SAMe in the liver induce NAFLD, and even the apparition of HCC, in the absence of obesity. This brings out the importance of the metabolism of methyl groups in the regulation of the hepatic function and complicates the therapeutic use of this molecule. CUP2E1 liver activity is increased in patients with NASH, diabetics, and individuals who have fasted for long periods of time. It is also increased in patients with alcoholic steatohepatitis, a disease very similar to NASH. Moreover, hepatic CYP2E1 activity is increased in animals that have been fed a diet deficient in methionine and choline, and in mice with deficient hepatic synthesis of SAMe. These and other results have shown the importance of oxidative stress generated by the peroxidation of lipids via CYP2E1 in the pathogenesis of NASH, that is, in the progression from steatosis to NASH. Surprisingly, a CYP2E1 deficiency in mice did not prevent the development of NASH induced by a diet lacking in methionine and choline, nor did it prevent the peroxidation of lipids, which indicates the existence of an alternative system of lipid peroxidation that acts in the absence of CYP2E1 (Leclercq et al. 2000). Those authors also observed that in CYP2E1-deficient mice treated with a diet lacking in methionine and choline, the hepatic expression of CYP4A10 and CYP4A14 is induced, and that these two enzymes are responsible for the lipid peroxidation and generation of ROS in those animals. CYP4A10 and CYP4A14 belong to the family of microsomal enzymes known by the generic name of CYP 450, of which CYP2E1 is also a member. This means that other members of the CYP 450 family that are not very active in normal conditions can substitute for CYP2E1 in the peroxidation of lipids when the activity of that enzyme is inhibited or mutated. That is what happens with "super-SAMe" mice. SAMe is an inhibitor of the hepatic expression of CYP2E1 and, as a result, its expression is inhibited in "super-SAMe" mice even when they have developed NAFLD. In those mice, as in CYP2E1-deficient animals fed with a diet lacking methionine and choline, the expression of CYP4A10 and CYP4A14 is stimulated and catalyzes the peroxidation of lipids and the formation of ROS.

An important conclusion of these studies is that therapeutic approaches targeting a single enzyme from the CYP 450 microsomal system are not efficient in preventing the generation of ROS and the peroxidation of lipids, and thus fail to block the initiation and progression of NASH. One of the main characteristics of biology is the redundancy of biochemical routes that control essential biological functions, such as cellular proliferation or defense against external cytotoxic agents. The evolutionary advantages to having developed a complex system such as CYP 450, which contains tens of enzymes whose mission is to protect the liver from the cytotoxic action of innumerable xenobiotics, is obvious. On the other hand, the redundancy of enzymes from the CYP 450 complex is a disadvantage when seeking to neutralize that system in order to avoid its side effects, such as the progression of NASH in individuals with steatosis.

Bibliography

Abdelmalek, M. F. and A. M. Diehl. "Nonalcoholic fatty liver disease as a complication of insulin resistance." *Journal Med. Clin. North Am* 91, November 2007, 1125.

Adams, L. A. and K. D. Lindor. "Nonalcoholic fatty liver disease." *Journal Ann. Epidemiol* 17, November 2007, 863.

Ahmed, M. H. and C. D. Byrne. "Modulation of sterol regulatory element binding proteins (SREBPs) as potencial treatments for non-alcoholic fatty liver disease (NAFLD)." *Journal Drug Discovery Today* 12, September 2007, 740.

Alzheimer. A. *Psych. genchtl Med* 64, 1907, 146.

Amadoro, G., M. T. Ciotti, M. Costanzi, V. Cestari, P. Calissano, and N. Canu. "NMDA receptor mediates tau-induced neurotoxicity by calpain and ERK/MAPK activation." *JournalProc Natl Acad Sci USA* 103, February 2006, 2892.

Angulo P. "Obesity and nonalcoholic fatty liver disease." *Journal Nutr. Rev.* 65, June 2007: S57.

Arriagada, P. V., J. H. Growdon, E. T. Hedley-Whyte, and B. T. Hyman. "Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease." *Journal Neurology* 42, March 1992, 631.

Avila, J., J. J. Lucas, M. Pérez, and F. Hernández. "Role of tau protein in both physiological and pathological conditions." *Journal Physiol Rev* 84, April 2004, 361.

Baki, L., J. Shioi, P. Wen, Z. Shao, A. Schwarzman, M. Gama-Sosa, R. Neve, and N. K. Robakis. "PS1 activates PI3K thus inhibiting GSK-3 activity and tau overphosphorylation: effects of FAD mutations." *Journal Embo* J 23, July 2004, 2586.

Blennow, K. and H. Zetterberg. "Pinpointing plaques with PIB." *Journal Nat Med* 12, July 2006, 753.

Braak, H. and E. Braak. "Neuropathological stageing of Alzheimer-related changes." *Journal Acta Neuropathol* 82, 1991, 239.

Brittberg, M., A. Lindahl, A. Nilsson, C. Ohlsson, O. Isaksson, and L. Peterson. "Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation." *Journal N Engl J Med* 331, October 1994, 889.

Cantoni GL "Biochemical methylations: selected aspects." *Journal Ann. Rev. Biochem* 1975. 45: 285-306.

Chang, BH-J., L. Li, A. Paul, S. Taniguchi, V. Nannegari, W. C. Herid, and L. Chan. "Protection against fatty liver but normal adipogenesis in mice lacking adipose differentiation-related protein." *Journal Mol. Cell. Biol.* 26, February 2006, 1063.

Duce, A. M., P. Ortiz , C. Cabrero , and J. M. Mato . "S-Adenosy-L-methionine synthetase and phospholipd methytransferase are inhibited in human cirrhosis." *Journal Hepatology* 8, January-February 1988, 1530.

Engel, T., J. J. Lucas, P. Gómez-Ramos, M. A. Morán, J. Avila, and F. Hernández. "Cooexpression of FTDP-17 tau and GSK-3beta in transgenic mice induce tau polymerization and neurodegeneration." *Journal Neurobiol Aging* 27, September 2006, 1258.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. "Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans." *Journal Nature* 391, February 1998, 806.

Glenner, G. G. and C. W. Wong. "Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein." *Journal Biochem Biophys Res Commun* 122, August 1984, 1131.

Gómez-Ramos, A., M. Díaz-Hernández, A. Rubio, M. T. Miras-Portugal, and J. Avila. "Extracellular tau promotes intracellular calcium increase through M1 and M3 muscarinic receptors in neuronal cells." *Journal Mol Cell Neurosci* 37, April 2008, 673.

Grundke-Iqbal, I., K. Iqbal, Y. C. Tung, M. Quinlan, H. M. Wisniewski, and L. I. Binder. "Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology." *Journal Proc Natl Acad Sci USA* 83, July 1986, 4913.

Hardy, J. and D. J. Selkoe. "The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics." *Journal Science 297*, July 2002, 353.

Harkany, T., I. Abraham, W. Timmerman, G. Laskay, B. Toth, M. Sasvari, C. Konya, et al. "Beta-amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis." *Journal Eur J Neurosci* 12, August 2000, 2735.

Hernández, F., J. Borrell, C. Guaza, J. Avila, and J. J. Lucas. "Spatial learning deficit in transgenic mice that conditionally over-express GSK-3beta in the brain but do not form tau filaments." *Journal J Neurochem* 83, December 2002, 1529.

Kennedy, D. "Breakthrough of the year." *Journal Science* 318, December 2007, 1833.

Kerr, J. N. and W. Denk. "Imaging in vivo: watching the brain in action." *Journal Nat Rev Neurosci* 9, March 2008, 195.

Kukar, T. L., T. B. Ladd, M. A. Bann, P. C. Fraering, R. Narlawar, G. M. Maharvi, B. Healy, et al. "Substrate-targeting gamma-secretase modulators." *Journal Nature* 453, June 2008, 925.

Kurtzberg, J., M. Laughlin, M. L. Graham, C. Smith, J. F. Olson, E. C. Halperin, G. Ciocci, et al. "Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients." *Journal N Engl J Med* 335, July 1996, 157.

Leclercq, I. A., G. C. Farell, J. Field , D.R. Bell, F. J. González , and G. H. Robertson. "CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis." Journal J. Clin. Invest. 105, April 2000, 1067.

Logothetis, N. K. "What we can do and what we cannot do with fMRI." Journal Nature 453, June 2008, 869.

Lu S. C., L. Álvarez, Z. Z. Huang, L. Chen, W. An, F. J. Corrales , M. A. Avila , et al. "Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation." *Journal Proc. Natl. Acad. Sci. USA* 98, May 2001, 5560.

Lucas, J. J., F. Hernández, P. Gómez-Ramos, M. A. Moran, R. Hen, and J. Avila. "Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice." *Journal Embo* J 20, January 2001, 27.

Mackman, N. "Triggers, targets and treatments for thrombosis." *Journal Nature* 451, February 2008, 914.

Mani, S. A., W. Guo, M. J. Liao, E. N. Eaton, A. Ayyanan, A. Y. Zhou, M. Brooks, et al. "The epithelial-mesenchymal transition generates cells with properties of stem cells." *Journal Cell* 133, May 2008, 704.

Martínez-Chantar M. L., M. Vázquez-Chantada, U. Ariz, N. Martínez, M. Varela, Z. Luka, A. Capdevila, et al. "Loss of glycine N-methyltransferase gene leads to steatosis and hepatocellular carcinoma in mice." *Journal Hepatology* 47, April 2008, 1191.

Masters, C. L., G. Simms, N. A. Weinman, G. Multhaup, B. L. McDonald and K. Beyreuther. "Amyloid plaque core protein in Alzheimer disease and Down syndrome." *Journal Proc Natl Acad Sci USA* 82, June 1985, 4245.

Mato J. M., J. Camara , J. Fernández de Paz, L. Caballería , S. Coll , A. Caballero , L. García-Buey, et al. "S-adenosylmethionine in alcoholic liver cirrosis: a randomized placebo-controlled, double-blind, multicenter clinical trial." *Journal J. Hepatol.* 30, June 1999,1081.

Mato, J. M., M.L. Martínez-Chantar, and S. C. Lu. "Methionine metabolism and liver disease." *Journal Annu. Rev. Nutr.* 28, August 2008, 273.

Montejo de Garcini, E., L. Serrano, and J. Avila. "Self assembly of microtubule associated protein tau into filaments resembling those found in Alzheimer disease." *Journal Biochem Biophys Res Commun* 141, December 1986, 790.

Mount, C. and C. Downton. "Alzheimer disease: progress or profit?" *Journal Nat Med* 12, July 2006, 780.

O´Connor, N. E., J. B. Mulliken, S. Banks-Schlegel, O. Kehinde, and H. Green. "Grafting of burns with cultured epithelium prepared from autologus epidermal cells." *Journal Lancet* 8211, January 1981, 75.

Parsons, C. G., W. Danysz, and G. Quack. "Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist--a review of preclinical data." *Journal Neuropharmacology* 38, June 1999, 735.

Pedersen, W. A., P. J. McMillan, J. J. Kulstad, J. B. Leverenz, S. Craft, and G. R. Haynatzki. "Rosiglitazone attenuates learning and memory deficits in Tg2576 Alzheimer mice." *Journal Exp Neurol* 199, June 2006, 265.

Postic, C. and J. Girard. "Contribution of de novo fatty acid síntesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice." *Journal J. Clin. Invest.* 118, March 2008, 829.

Price, D. L., R. E. Tanzi, D. R. Borchelt, and S. S. Sisodia. "Alzheimer's disease: genetic studies and transgenic models." *Journal Annu Rev Genet* 32, 1998, 461.

Rader, D. J. and A. Daugherty. "Translating molecular discoveries into new therapies for atherosclerosis." *Journal Nature* 451, February 2008, 904.

Ray, S., M. Britschgi, C. Herbert, Y. Takeda-Uchimura, A. Boxer, K. Blennow, L. F. Friedman, et al. "Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins." *Journal Nat Med* 13, November 2007, 1359.

Reisberg, B., R. Doody, A. Stoffler, F. Schmitt, S. Ferris, and H. J. Mobius. "Memantine in moderate-to-severe Alzheimer's disease." *Journal N Engl J Med* 348, April 2003, 1333.

Segers, V. F. and R. T. Lee. "Stem-cell therapy for cardiac disease." *Journal Nature* 451, February 2008, 937.

Stephen Salloway, J. M., F. W. Myron, and J. L. Cummings. "Disease-modifying therapies in Alzheimer´s disease." *Journal/Alzheimer´s & Dementia* 4, 2008, 65.

Sturm, R. "The effects of obesity, smoking and drinking on medical problems and costs." *Journal Health Affaire* 21, March-April 2002, 245.

Takahashi, K. and S. Yamanaka. "Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors." *Journal Cell* 126, August 2006, 663.

Thomson, J. A., J. Itskovitz-Eldor, S. S. Shapiro, M. A. Waknitz, J. J. Swiergiel, V. S. Marshall, and J. M. Jones. "Embryonic stem cell lines derived from human blastocysts." *Journal Science* 282, November 1998, 1145.

VAS, C. J. "Alzheimer´s disease: The brain killer." World Health Organization, 2001.

Venter, J. C., M. D. Adams, E. W. Myers, P. W. Li, R. J. Mural, G. G. Sutton, H. O. Smith et al. "The sequence of the human genome." *Journal Science* 291, February 2001, 1304.

Wang, J., L. Ho, W. Zhao, K. Ono, C. Rosensweig, L. Chen, N. Humala, D. B. Teplow, and G. M. Pasinetti. "Grape-derived polyphenolics prevent Abeta oligomerization and attenuate cognitive deterioration in a mouse model of Alzheimer's disease." *Journal J Neurosci* 28, June 2008, 6388.

Wernig, M., J. P. Zhao, J. Pruszak, E. Hedlund, D. Fu, F. Soldner, V. Broccoli et al. "Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease." *Journal Proc Natl Acad Sci USA* 105, April 2008, 5856.

Wold, B. and R. M. Myers. "Sequence census methods for functional genomics." *Journal Nat Methods* 5, January 2008, 19.

Yahagi, N., H. Shimano, A. H. Hasty, T. Matsuzaka, T. Ide , T. Yoshikawa, M. Amemiya-Kudo et al. "Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lep(ob/Lep(ob) mice." *Journal J. Biol. Chem.* 277, May 2002, 19353.

Yamaguchi, K., L. Yang, S. McCall, J. Huang, X.X. Yu, S .K. Pandey, S. Bhanot et al. "Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis." *Journal Hepatology* 45, June 2007, 1366.

Zhang, Y., M. Proenca, M. Maffei, M. Barone, L. Leopold, and J. M. Friedman. "Positional cloning of the mouse gene obese and its human homologue." *Journal Nature* 372 December 1, 1994, 425.