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Review

Genomics, Proteomics and Nutrition : Applications to Obesity Research

Sumithra Urs, Ph.D.[1], Young-Ran Heo, Ph.D.[2], Suyeon Kim, M.S., Jung-Han Kim, Ph.D., Brynn H. Jones, Ph.D.[#] and Naima Moustaid Moussa, Ph.D.[§]

[1]SU and [2]YRH equally contributed to this paper

Department of Nutrition and Agricultural Experiment Station. University of Tennessee; Knoxville, TN, 37996, USA

[#]Oak Ridge National Laboratory, Life Sciences Division(BHJ), Oak Ridge, TN 37831

[§]University of Tennessee, Department of Nutrition; 1215 W. Cumberland Avenue, JHB 229. Knoxville, TN, 37996-1900, USA

Obesity is a major public health problem in western countries. Genetic and environmental factors, separately or in combination are major determinants of fat mass. Both central effectors (primarily hypothalamus) and peripheral tissues (such as adipose tissue) are implicated in the pathogenesis of obesity. A significant number of studies have documented potential contribution of adipose tissue -via its newly discovered secretory function - to the pathogenesis of obesity and co-morbid conditions including cardiovascular disease, diabetes and hypertension. Applications of analytical techniques such as genomics and proteomics have enabled better understanding of biological sciences in general and have only being applied recently to nutritional sciences including obesity research. Here, we review the recent progress in adipose tissue functional genomics and proteomics, and the importance of these studies in energy metabolism and obesity research.

Obesity, an epidemic problem:

Obesity is a major public health problem in western countries and over 61% of adult Americans are overweight or obese (US Department of Public Health Service, 2002). A major contributor to the epidemic of obesity is the current environment characterized by increased availability of high caloric foods and decreased physical activity (Flegal et al., 1998, Popkin et al. 1998). Several studies have demonstrated that genetic susceptibility contributes to obesity in some populations (Chagnon et al. 2000). Obesity research has primarily focused on the role of the hypothalamus in neuroendocrine regulation of food intake (Flier and Maratos-Flier 1998). However, a growing number of studies support a potential contribution of adipose tissue - via its newly discovered secretory function - to the pathogenesis of obesity and co-morbid conditions including cardiovascular disease, diabetes and hypertension (Mohamed-Ali et al., 1998, Kim and Moustaid-Moussa, 2000).

Adipose tissue, an endocrine tissue:

Adipose tissue has been long considered as inactive tissue, primarily responsible for storing lipids in times of food plenty and releasing them when energy is restricted. Research in the past decade and half has demonstrated that adipose tissue plays an important role in energy regulation via endocrine, paracrine and autocrine signals (reviewed in Kim and Moustaid-Moussa, 2001). These functions enable adipocytes to influence metabolic activity of adipose tissue as well as other tissues including the brain, liver and muscle. As shown in fig 1,

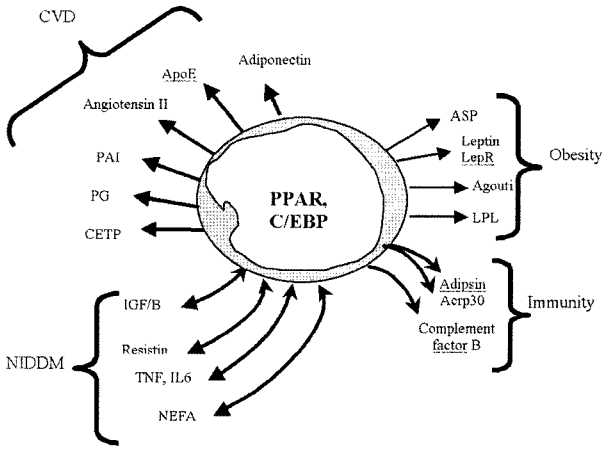

Fig 1. Dynamic view of the adipocyte, showing signals emanating from white adipose tissue. ApoE, Apoprotein E; PA, Plasminogen activator inhibitor; PG, prostaglandin; CETP, Cholesterol ester transfer protein; IGF-I, insulin- like growth factor I; TNF- , tumor necrosis factor-; IL-6, interleukin-6; NEFA, Non-esterified fatty acid; ASP, acylation-stimulating protein; LPL, lipoprotein lipase, acrp 30 : Adipocyte complement related Protein 30Kda.

§ To whom correspondence should be addressed.

adipocyte-derived factors reported so far include leptin, adipsin, acylation stimulation protein, agouti, angiotensin II, prostaglandins, Adipocyte complement related Protein 30Kda (Acrp30), resistin, Tumor necrosis factor (TNF-α), macrophage migration inhibitory factor, secreted protein acidic and rich in cystein (SPARC), and PPARγ angiopoetin related (PGAR)/fasting-induced adipose factor (FIAF) (reviewed in Gregoire, 2001). Most of these factors secreted from adipose tissue act in an autocrine/paracrine manner to regulate adipocyte metabo lism and upon secretion into the bloodstream, act as endocrine signals at multiple distant sites to regulate energy homeostasis (Mohamed-Ali et al.1998, Flier and Maratos-Flier 1998). Thus, adipose cells play a more dynamic role than previously recognized in physiological mechanisms including the autoregulation of adipocyte growth and development as well as regulation of whole body homeostasis. This important role of adipose tissue in energy metabolism and the intricate pathways leading to proper adipocyte function necessitates comprehensive methods for studying obesity.

Review of Genomics and Proteomics Technologies:

Applications of innovative analytical techniques have enabled better understanding of biological sciences in general and have only being applied recently to nutritional sciences including obesity research. Here, we will review the recent progress in these technologies with emphasis on adipose tissue functional genomics and proteomics.

Nutritional scientists use molecular biological approaches to identify genes that are important for specific cellular functions that are expressed under specific conditions. Once these genes are identified, several strategies exist for determining their functions and impact and subsequently their potential use for interventions. Therefore, Nutrition is a science of integration where studies at the cellular and molecular levels can be further tested in animal studies and together with investigations of functional and genetic properties of foods and nutrients, these approaches provide better understanding of optimal nutritional and pharmacological interventions in humans. The drive to understand basic biological mechanisms of nutrient-gene interactions has led to two distinct, yet related, approaches in the study of molecular biology : genomics and proteomics and the introduction of An additional approach, metabolomics. Genomics is the study and identification of an organism's genetic makeup; proteomics is the study of protein expression and protein interaction within cells; and metabolomics is the study of changes in intracellular metabolites.

The recent publication of the DNA sequence of genomes form several organisms including humans open new challenges for geneticists, physiologists as well as nutritionists to ascribe functions to these genes and to determine how these functions are altered by disease and nutritional status. Current knowledge indicates that mutations causing diseases are rare and future research may aid in identifying people with genetic predispositions to a specific disease that is modifiable with the lifestyle changes. Some genes may be active or inactive depending on the environment, especially the diet and thus the nutrient-gene interactions studies may provide useful information on how to modulate gene function.

The development of large scale and efficient gene analysis techniques such as microarrays (Schena, 2000) has provided and will continue providing information on genes associated with a specific nutrient function or with a specific disease. Further analysis of these genes, including the generation of animal models where gene expression can be manipulated will provide key information on their function and response to nutrients and other environmental stimuli. In concert with changes in gene and protein expression (genomics and proteomics, Nakayama, 2002), metabolomics yields valuable information as to how changes in gene and protein expression can be used to discriminate the regulation of metabolic pathways, using high throughput screening techniques. These techniques can be used to identify gene clusters and uncharacterized genes.

Since the discovery of the Polymerase Chain Reaction (PCR) method, many years passed before the strengths and weaknesses of this technique were recognized and more powerful (quantitative, fast and sensitive) applications, such as real-time PCR, were developed and refined. Some of these techniques include advanced PCR like RT-PCR, real time quantitative PCR and Microarray in the genomic front while 2-D gel electrophoresis and mass spectrometry are being used for proteomics.

Genomics and adipose tissue:

Initially, nutrient-gene interactions were approached using Northern blot analysis and RT-PCR. Northern blot consists of separating the RNA on a gel and transferring the RNA to a membrane. The membrane is hybridized with the radioactive probe of interest and visualized by autoradiographic method. Whereas RT-PCR is a sensitive technique for mRNA detection and quantitation. The technique consists of two parts : synthesis of cDNA from RNA by reverse transcription (RT) and amplification of a specific cDNA by polymerase chain reaction (PCR). These approaches were limited to studies of each gene individually. Quantitative and Real time PCR is the most advanced technique for RNA quantification which is similar to RT-PCR but combines the use of a fluorescently labeled probe specific to the fragment of interest. This is a quick and efficient method for quantification of RNA (fig 2). Given that genes do not function independently rather interactively (i.e. metabolic path-

ways), it was necessary to develop novel approaches for large scale analysis of gene and protein expression.

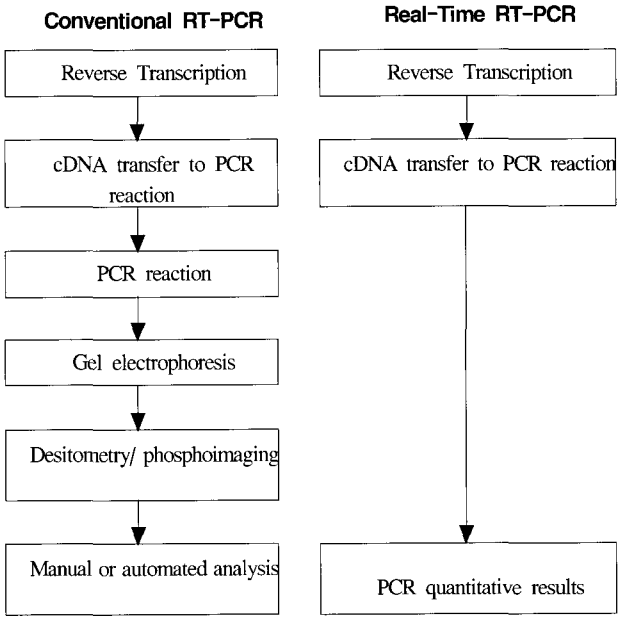

Fig 2. Schematic representation of conventional and real time RT-PCR

Microarray technology represents a cutting-edge technology that promises extraordinary advances in the study of health and disease. Microarray technology provides scientists the tools to scan simultaneously the array expressed genes in a cell and glean myriad information about cellular function. In the face of such power and precision, it may appear as if hypotheses are superfluous to the pursuit of research. However, one of the powerful aspects of the microarray technology is that it may provide crucial information about research direction based on specific sets of genes and pathways that are regulated by a specific treatment (Firestein and Pisetsky 2002).

Microarrays facilitate the simultaneous quantitation of thousands of mRNAs and provide a comprehensive assessment of expression levels. Two board types of microarrays are commonly used, cDNA microarray and oligonucleotide arrays. The use of cDNA micro array involves the spotting of 3″expressed sequence tags or known genes on glass slides that are subsequently probed with fluorescently labeled cDNAs (frequently Cyt 3 and Cy 5 are used) from experimental samples (Duggan et al. 1999). Fig 3 outlines the major steps of microarray analysis. Oligonucleotide arrays are produced by combinational creation of short oligonucleotides complementary to expressed genes (Lipshutz et al. 1999). RNA from tissue or cells are labeled with fluorescent dyes and hybridized to chips or slides printed with EST″s or oligonucleotides. A detailed understanding of microarray chip technology has recently been reviewed in a book edited by Schena, 2000 (outlined in fig 4). The interaction is visualized by scanning the slides at appropriate wavelength depending on the excitation of the dye used and recording the intensity of fluorescence. Several commercial slides and chips are now available for both cDNA and oligonucleotide slides.eg. Affymetrix, Clonetech. and other companies.

While RNA preparation and microarray experiments per se are simple and straightforward; the amount of data obtained and the required extensive analysis is a major issue with these technologies. Biologists are now teamed with computer scientists whose primary goal is to develop appropriate softwares to analyze these data. Development of microarray technology along with the genome sequence analysis has led to a development of a new discipline called bioinformatics/ computational biology to address the data mining issues.

Identification and screening of differentially expressed genes with Microarrays must be confirmed using more traditional techniques like Northern blots or RT-PCR or real time PCR for conclusive evidence of the expression of the gene. These techniques rely on mRNA identification in the specific tissues. Further to substantiate the expression of the genes, the proteins are detected and identified to confirm the hypothesis of the identified gene/protein since there is often a discrepancy observed between the relative abundance of mRNA present in a tissue and the amount of protein produced.

The identification of expressed sequences in the human genome and development of DNA array-based technologies have opened the possibility to simultaneously measure the expression of thousands of genes. As discussed by Brown and Botstein (1999) in a recent review, "analysis of gene expression by DNA array allows discovery of things we neither knew nor expected". Thus, in addition to hypothesis-driven research, the technology allows non-hypothesis-driven exploration of gene expression patterns.

Lately with the advancement of this technology, more proteins and gene products are being identified as potential factors affecting obesity and related disorders. Usefulness of this method in adipose tissue biology was demonstrated by the screening of approximately 18,000 genes for expression in human adipose tissue. Recently, Gabrielsson et al. (1999) reported gene expression in human adipose tissue using DNA array. Approximately 50% of identified genes had previously not been reported to be expressed in adipose tissue by UniGene or MEDLINE searches. The genes expressed at high level in adipose tissue were tentatively classified into categories depending on their putative functions according to the classification used by Human gene Anatomy project. The

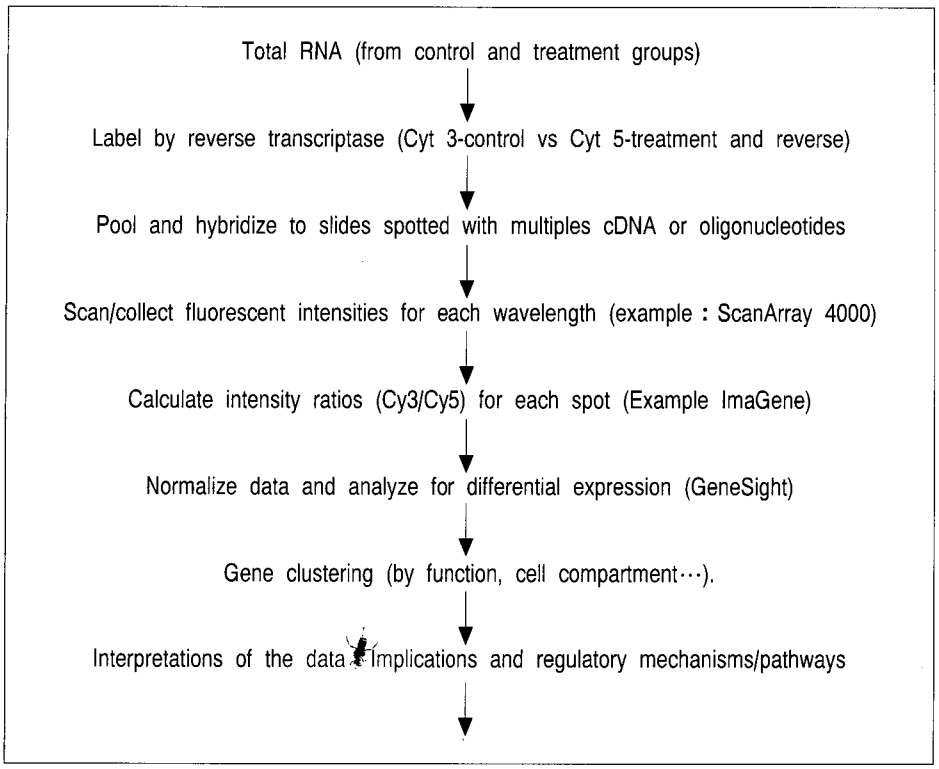

Fig 3. Schematic outline of microarray analysis

300 genes investigated were highly expressed in adipose tissue, however, for many of them, the function remains to be determined. For example, the gene with the highest ranking on the array was identified as sulfonylurea receptor (SUR) 2 which is in agreement with recent studies from our group documenting expression of SUR in human adipose tissue (Shi et al., 1999). Expression of this receptor in adipose tissue is significant as these sulfonlylureas are used in type II diabetics, who are also frequently obese. This demonstrates that exploration of gene expression can provide clues that merit further investigation. In addition, further use of DNA array-based monitoring of gene expression in adipocytes, in adipose tissue in response to different stimuli, and in adipose tissue obtained from lean and obese subjects is likely to provide important information on the regulation and regulatory role of adipose tissue in health and disease (Gabrielsson et al. 1999). Our preliminary microarray studies on human adipose tissue identified several lipogenic and adipogenic genes expressed in differentiated vs undifferentiated adipose tissue; most of these genes have also been reported in the mouse by Guo and Liao (2000), Boeuf et al. (2001) and Nadler et al. (2000).

Proteomics and adipose tissue:

Proteins are purified and separated by 2-D gel electrophoresis. MS (mass spectrometry) analysis is carried out on peptides obtained after enzymatic degradation of the gel-separated proteins. The sequence of peptides can be determined by interpreting the data resulting from fragmenting the peptides in tandem mass spectrometers. In this technique, one peptide species out of a mixture is selected in the first MS and is then dissociated by collision with an inert gas such as argon or nitrogen (Mann et al. 2001). The resulting fragments are separated in the second part of the tandem MS, producing the tandem mass spectrum or MS/MS spectrum. Liquid chromatography (LC) coupled to tandem mass spectrometry is called LC-MS/MS. It is a powerful technique for the analysis of peptides and proteins. This method combines efficient separations of biological materials and sensitive identification of the individual components by MS. Complicated mixtures of hundreds of proteins can be analyzed directly even when concentration levels of different proteins vary by orders of magnitude. LC-MS/MS can be used alone or in combination with 1-D or 2-D electrophoresis, immunoprecipitation, or other protein purification techniques (Mann et al. 2001).

Recent studies studies by Kratchmarova et al., 2002 used a one dimentional gene electrophoresis combined with tandem MS to identify proteins secreted by 3T3-L1 adipocytes or preadipocytes. Some of the proteins that increased during adipocyte differentiation and identified by these methods include several previously reported

adipocyte-specific peptides such as adipsin, adipocyte complement-related protein 30Kda (Acrp30) and fibronectin. In addition, this study also reported new proteins secreted by preadipocytes such as Pigment-Epithelium-erived factor (PEDF), a potent antiangiogenic protein and adipocyte-secreted proteins such as HCNP and NGAL and several adipocyte-secreted proteins such as resistin, SPARC, Cystatin C and others.

CONCLUSION

The development of a variety of genomics and proteomics technologies combined with our current knowledge of the human gene and other organisms sequence are valuable tools that nutritionists can successfully utilize to understand diet-gene interactions and identify possible targets for nutritional and as appropriate for drug therapies. Ultimately, these technologies can be utilized to target specific treatments to individuals or populations.

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