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Browning the fat may reset the metabolome through rebooting PPARδ network of genes.

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Abstract

Obesity and its associated metabolic disorders have been the spot light of modern life and contemporary research. The quality of fat and its distribution in the fabric of adipose through age and development can influence the overall metabolism in the body. The imbalance in brown fat ratio compared to white fat in the body could be the consequence or the driving force of metabolism associated diseases. In either case, the brown fat is healthier and likely factor in diverting many metabolic disturbances. This review explores current research emphasizing and elucidating the positive role of brown fat as a possible candidate for enrichment and amelioration in obesity associated metabolic diseases. Furthermore, the review tries to gather some specific information on constellation of candidate genes and molecules that play clear role in browning the fat. With supportive research and educated deductions, this study highlights PPARδ network of genes interactions with TGF-β and TNF-α as major performers in metabolic harmony. The cross talk mediators between such major players are done through many smaller molecules and proteins such as FOXC2 and PRDM16 transcriptional regulators and thermogenic genes, UCP1 and PGC-1α regulates thermogenesis and BAT development. It is concluded that the central metabolome genes of PPARδ, TNF-α and TGF-β are interconnected and rebooting the PPARδ central network of genes will rebalance the storage of fats and its consumption.

Keywords

 Brown adipose tissue, TGF-β and TNF-α, PPARδ, PRDM16, UCP1, thermogenesis, adipocytokines.

Introduction

Over the past 20 years, obesity has become pandemic with substantial medical and financial consequences. The World Health Organization (WHO) global estimates show that the worldwide prevalence of obesity more than doubled between 1980 and 2014 [1]. It is a critical global issue that requires a comprehensive, international intervention strategy. According to an analysis by Mckinsey Global Institute, the global economic

impact of obesity amounts to roughly $2 trillion annually, or 2.8 percent of global GDP-nearly equivalent to the global impact of smoking or of armed violence, war, and terrorism [2].

 Moreover; many of the 21st century's complex diseases stem directly and/or indirectly from obesity leading to an abatement of non-communicable diseases (NCDs) such as heart disease, cancer, cardiovascular diseases and Type II diabetes to name a few [3].

Since past decades, researches show that obesity is the key player in the progression of metabolic disorders [4-6]. This constellation of clinical disorders is characterized by metabolic disturbances, described in many cases as metabolic syndrome [6].

Adipose tissue has attracted increasing interest in the last decade, with stress on its function in obesity and the associated clinical disorders. It is now unequivocally known that adipose tissue is an active endocrine organ, releasing adipokines and free fatty acids (FFA) influencing various tissue as the brain, liver, and muscle affecting numerous metabolic processes including regulation of food intake, energy balance, and insulin sensitivity [7, 8].

 The newly revealed, multifunction nature of adipose in metabolism homeostasis has elevated its importance as an organ and has added additional layers of complexity to metabolic disorders. Diet, genetics and life style combined with energy intake and expenditure levels directly influence the adipose depot size and consequently its patho-physiologic activities [9-12]. Individual genotype and the associated occurrence and severity of metabolic complications of obesity are highly variable [12-14]. Some extremely overweight patients remain healthy with healthy insulin levels and no detectable cardiovascular diseases. [15-18]. Conversely, some individuals that are overweight develop insulin resistance and type II diabetes later [19, 20]. Gene-environment interactions usually explain this variance between individuals [18, 21]. However, studying the effects of these interactions on the endocrinological function of adipose depot is a complicated task that is not clearly understood.

Adding to the intricate nature of adipose is the seemingly opposed role of the two different types of adipose tissues. One hand white adipose tissue is specialized in energy storage whereas the brown adipose tissue specializes in thermogenic expenditure of energy [22].

Recently, some research has been devoted in describing the nature of and changes in adipose depot size in a more discriminatory fashion. The novel discovery that brown adipose tissue does not completely diminish with age and can be found in endothermic animals, including humans in relatively smaller amounts and especially in the lower neck and supraclavicular region has reinvigorated the interest of many scientists in the role of adipose [23]. Of special interest to many researchers and in view of the obesity epidemic,

it is the positive correlation of the amount of brown adipose versus white adipose in lean individuals which is the crux of the matter [23, 24].

This important observation led to the hypothesis that the amount and distribution of different types of adipose tissue may have unique pathophysiological effects that could modulate different metabolic activities [25- 27]. Enrichment of brown fat may reset and reformulate the small molecule metabolites found in the human body.

This review plans to explore, in a comprehensive fashion, the roles of various types of adipose tissues in metabolic homeostasis based on their genomic interactions with environmental factors. The work will focus on studies delineating the changes and conversions in white and brown adipose tissue and the correlations with complications arising from obesity. In addition; we would like to explore the role of peroxisome proliferator-activated receptor γ (PPARδ) network of genes which could be a major player in or a consequence to setting the dynamic nature of types of adipose tissues. The contemporary analysis of current important research in this topic will also highlight additional molecular signals and cross- talk mediators between the two types of adipose tissues that can be used as potential molecular therapeutic targets in obesity- related metabolic disorders.

Role of adipose tissue in metabolic homeostasis

Controlled and prioritized synthesis, degradation, mobilization and transformation of carbohydrates, lipids, and proteins for efficient energy production across tissues and organs are the crux of metabolic homeostasis. This complex interchange between adipose, liver, muscles and other endocrine glands and tissues is an intricate dance in the organismic bid to achieve proper energy balance in an ever changing environment.

The major regulators of these metabolic cascades ensuring harmonized crosstalk are hormonal commands and enzymes catalysts. Due to its adipokine production, adipose tissue is now considered to be one of the major players in the regulation of homeostasis. In the past 20 years, many researchers showed beyond doubt that white adipose tissue, while being a fat depot, is also an active, multifunctional endocrine organ with intense crosstalk signaling with other peripheral tissues modulating many of their metabolic activities and pathophysiological processes [28-32]
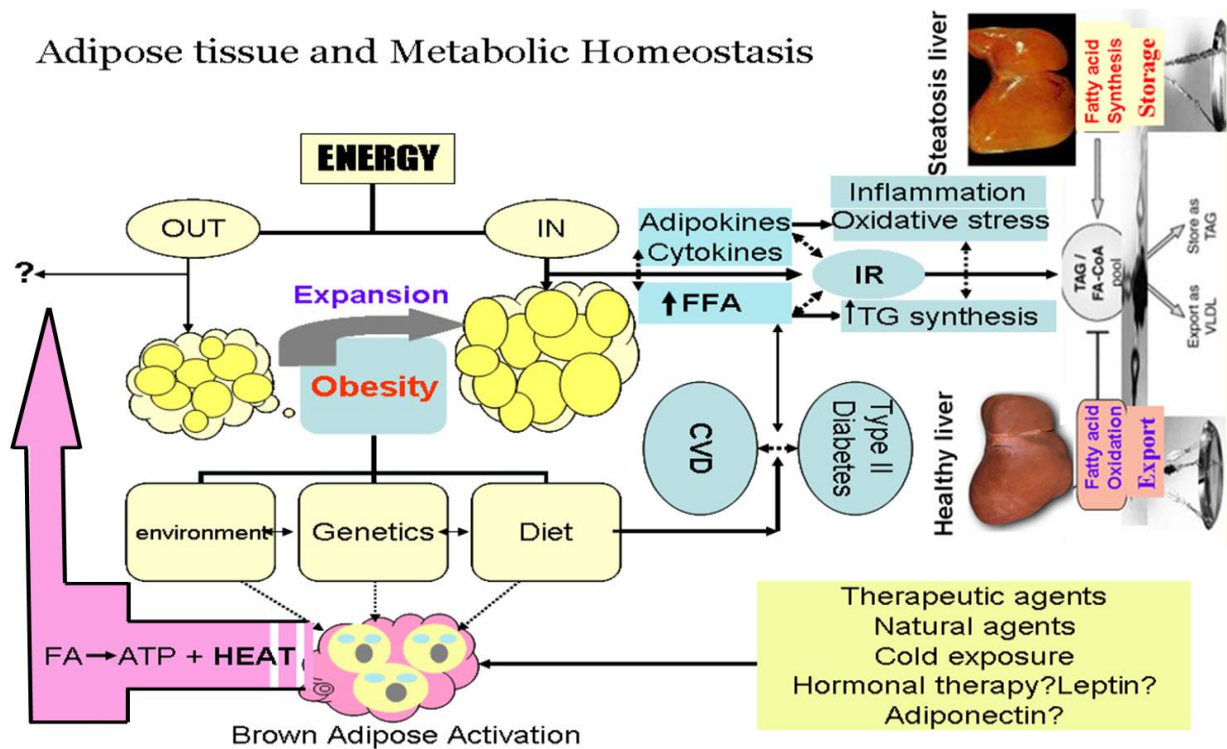
Fig.1: Schematic diagram showing the general role of adipose tissue and its expansion on Metabolic Homeostasis (Drawn by hand).

These processes include the metabolism and mobilization of fatty acids in adipose and liver tissues influenced via the production of hundreds of adipocytokines such as C-Reactive Protein, Tumor Necrosis Factor-α, Interleukin-6, Interleukin-9, Interleukin-18, adiponectin and leptin. The increase of white adipose tissue in obesity disrupts and amplifies many of the polytrophic effects of adipocytokines and alters the distribution of lipids in the body tissues. This increases the disregulation of adipokines which stimulates the rise to many different metabolic disorders ranging from insulin resistance, fatty liver disease, inflammation, endothelial dysfunction to cardiovascular malfunctions [7, 33-36]. The exerting pleiotropic effects spread at both the local and the systemic level [28, 29].

Fig.1 shows a schematic diagram of adipose tissue and its metabolic homeostasis. It has been suggested that adipocyte number, which stays constant in both lean and obese individuals, is the major factor for the entire fat mass in adults [37]. Conversely, the increased lipid gain is contained in adipose tissue through their expansion by hypertrophy of present adipocytes and/or more differentiation of pre-adipocytes. However, the

aptitude of adipose tissue to expand in accommodating changes in energy availability is variable between individuals and limited overall. [38].

The exact molecular and cellular mechanisms of how diet can induce obesity and the expansion of adipose effects on insulin sensitivity and glucose intolerance are under investigation by numerous researchers. Hoffman and colleagues showed that dietary fat intake promotes adipocyte up-take of triglyceride-rich lipoproteins (TGRL) through the Adipocyte LDL receptor-related protein-1 which is considered to be an indirect regulator of adipocyte energy homeostasis. They further proposed that the functional disruption of LDL receptor-related protein-1 leads to reduced lipid transport, increased insulin sensitivity, and muscular energy expenditure [39]. It is plausible that the differential expression of LDL receptor-related protein-1 in adipocytes could be one of the genetic underlying bases for obesity in certain individuals. Over-expression of LDL receptor-related protein-1 could be the initiating factor for increased lipid storage in mature adipocytes, leading to increased adipose tissue expandability and consequently affecting the maintenance of metabolic homeostasis.

Contrary to white adipose tissue (WAT), brown adipose tissue (BAT) is rich in mitochondria and is highly metabolically active. This making it important for both basal and inducible energy expenditure. Brown adipose tissue has a major role in thermogenesis. It is the main reservoir in which the tissue-specific uncoupling protein 1 (UCP1), a mitochondrial anion carrier protein, which separates oxidative phosphorylation from ATP synthesis resulting in the uncoupled energy dissipating as heat [26]. This evolutionary pre-determined characteristic of brown adipose tissue is of major importance for inducing thermogenesis in shivering animals and their survival in different stages and environmental conditions.

In addition to the physical activities and the right diet, activating brown adipose tissue can be another natural instrument balancing energy intake and energy consumption by modulating energy expenditure. It has been shown that the brown adipose tissue activity is negatively correlated with obesity and being overweight [40]. Theoretically, augmenting the brown fat stores and activity can be achieved by frequent exposures to cold temperatures, which ultimately cause an increase in oxidative phosphorylation, energy expenditure and consequently, convert more of the lipid stores into heat [40]. Thermogenesis, mediated by more expression of the tissue-specific uncoupling protein 1 (UCP1), modifies sensitivity to insulin and susceptibility to weight gain [24]. On the other hand, the role of classical beta-adrenergic pathways (including cAMP production and lipolysis) in regulation of BAT metabolism in response to cold temperatures is well documented. Norepinephrine (NE), released from sympathetic nerve terminals, can activate the tissue via binding to adrenergic receptors. This sympathetic stimulation leads to the release and accumulation of fatty acids from the hydrolysis of triglyceride in white adipose, in addition to decreasing accumulation of free fatty acids in brown adipocytes

due to rapid oxidation in the mitochondria. High expressions of brown adipose tissue specific (UCP1) can channel more of the ATP produced from fatty acid oxidation into heat. For example, diet-induced obese rats when treated with β3-adrenoceptor agonist showed hypertrophy of brown adipocytes in both brown and white adipose tissue and resulted in the reversal of their obesity [41]. Such compounds that selectively stimulate brown adipocyte β-adrenoceptors may be considered as a potential thermogenic anti-obesity agents.

In a number of reports, melatonin has been shown to increase thermogenesis as well as differentiation of brown fat through its antagonist effect on reacting oxygen species (ROS) and suppressing Phosphatidylcholine transfer protein "Pctp" expression [42- 44]. Quantity and quality of diet also influences both white and brown fat store depots and activity. For example, low protein diets result in the activation of brown adipose tissue leading to metabolome-regulatory thermogenesis under hypothalamic control through a leptin-dependent recruitment of brown adipose tissue [26]. For example, rats showed reduced amount of white adipose tissue when scallop shell powder was included in diet which induced the expression of uncoupling protein-1 [45]. Narvez and colleagues demonstrated that the presence of uncoupling protein-1 in white adipose tissue is associated to lean phenotype and resistance to diet-induced obesity [46].

Adipose genomics

Genomic expression and status description of tissues are dynamic and complex processes which are influenced by numerous internal biological activities and external factors. The detection of genomic transcript expression levels in different types of tissues has become more feasible than ever due to the revolutionary changed in high-throughput technology. These technologies have so far contributed in describing the genome, transcriptome and proteome in many complex human diseases, including obesity. Differential gene expression analysis of adipose tissue reflects its broad function in metabolism and signaling. One of the first expression profiling studies done by Gabrielsson and colleagues revealed that about 19.8% of genes expressed in the adipose tissue of non-obese individuals were related to cell signaling/communication and about 16.2% were related to metabolism [48]. The histological and pathophysiological changes of adipose in obesity such as the expansion of adipose, altered adipocytokines levels, irregularities in adipocyte proliferation and differentiation, and imbalance between lipolysis and lipogenesis are influenced by differential gene expression. For example, down-regulation of growth factors and up-regulation of MAPKs in the adipose tissue of obese subjects limited their adipocyte proliferation and differentiation [48]. Many of the clinical complications of obesity that are associated with immuno-inflammatory processes and altered adipocytokines signaling have been substantiated both at the gene expression and serum levels [28, 49, 50]. In several in vivo studies, stimulated inflammation of human

adipose modulated the expression of multiple adipocytokine and chemokine genes thought to be secreted by adipocytes and monocytes involved in the recruitment of lymphocytes, adhesion molecules and antioxidants [51]. Examples of this differential cytokine expression can be seen in numerous studies including the decrease of anti-inflammatory cytokine IL-10 in metabolic syndrome patients [52]; hypo-adiponectinemia in obesity and type 2 diabetes and its strong link with high systolic blood pressure [53, 54]; and insulin sensitivity in peripheral tissues which are all influenced by the level of many adipose derived factors such as TNF-α, interleukin 6, resistin, adiponectin, and leptin [34]. In addition, reduction in serum adiponectin and rise in serum resistin levels has been shown in obese patients with insulin resistance. In addition, such patients show low level of adiponectin gene expression in the adipose tissue, which as a result, may make such patients susceptible to the progressive form of NAFLD or NASH [55]. Moreover, the up regulation of inflammatory cytokines is differentially regulated in the adipose tissue of obese patients and obesity associated stages of NAFLD. For example, it has been shown that the adipose of NASH patients over expresses a number of genes encoding mostly secreted pro-inflammatory proteins and cytokines [56].

Many clinical complications of obesity arise due to the imbalance between lipolysis and lipogenesis. This imbalance is controlled and influenced by many genes and their signaling products. For example, the fat formation is inhibited in a dosage-sensitive manner by the Anti-obesity Adipose (Adp) ADP gene, which is conserved from worms to mammals [57]. On the other hand, ATGL, HSL, and mono-acylglycerol (MAG) lipase are known largely to regulate and sequentially catalyze the main lipolysis process in adipocytes [58- 61]. Recently, it has been shown that G0/G1 switch gene 2 (G0S2) that is highly expressed in adipocytes and adipose tissue explants, inhibits ATGL lipase activity and reduces ATGL-mediated lipolysis by specifically interacting and co-localizing with adipose triglyceride lipase (ATGL) [62]. Under expression of G0S2 may be the probable reason for ATGL high expression in adipose tissue of obese patients [63, 64].

A generalized theme found in the majority of such genomic and proteomic researches on the biology of adipose tissue in obesity and its related metabolic disorders points in one way or another to the involvement of the PPARγ, TGF-β, TNF-α and the cytoskeleton molecules and networks. Most of these master molecules have been central players in molecular and signaling pathways involved in growth, differentiation, maintenance and inflammation processes (See Fig. 2). The cross talks between the three master players, PPARγ, TGF-β and TNF-α and their network molecules, in any biological organizational level, are mediated by the cytoskeleton and the extracellular matrix.
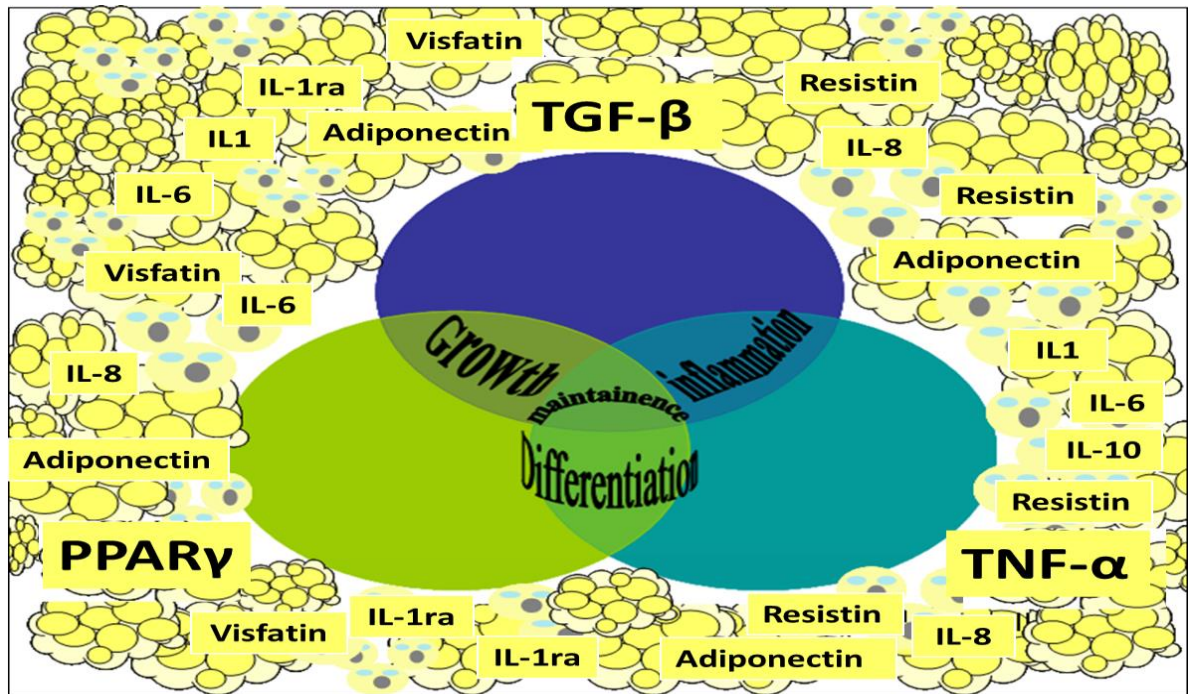
Fig 2: Diagram showing the central players in molecular and signaling pathways involved in growth, differentiation, maintenance and inflammation processes.(Drawn by hand).

It has been shown that up-regulation of genes related to the extracellular matrix (ECM) components, along with the integrin family, is one of the main suggested mechanisms in connecting local inflammatory phenomena in the alteration of WAT metabolic functions in obese subjects [65]. The importance of this finding is highlighted by the fact that the majority of adipokines released from adipose tissue, excluding adiponectin and leptin, originate from non-fat cells embedded in the extracellular matrix [66].

The cytokine TGF-β is involved in the coordination of many differentiation programme especially to suppress adipogenesis. Moreover, it plays a role in the maintenance of the adipocyte tissue mass in morbid obesity [56, 67].

For instance, genetically obese mice (i.e., ob/ob and db/db) showed an increase in TGF-β mRNA and protein in the adipose tissue in contrast to lean mice [68] and enhanced release of TGF-β was detected in obese human adipose tissue [69]. White and brown adipose tissue masses were greatly reduced due to the transgenic overexpression of TGF-β, leading to the failure of adipocytes to differentiate [70]. The rate of adipogenesis is regulated by endogenous TGF-β signaling through Smad6 and Smad7 negative regulators [71].

Functional analyses of 97 adipose specific genes linked to obesity were found to be centered on TGF-β [56]. In one such study, TNF-alpha was found to increase TGF-β mRNA expression in the adipose tissue of lean mice and stimulate TGF-β production in cultured adipocytes [68]. This increase of TGF-β levels may be due to the increased expression of the tumor necrosis factor [72], a cytokine that inhibits the adipocyte differentiation in vitro and mover it has also been shown to reverse the adipocyte phenotype [73, 74]. It was also reported that 18% of the genes that were differentially expressed in the visceral fat of obese NASH patients were influenced by TNFα and/or IL-6 [56]. These results were verified in morbidly obese patients with NASH by the measurement of the corresponding serum levels of TNFα [75].

The role of PPARγ in maintenance of fat topography, adipocyte phenotype, and in fat metabolism and storage has also been highlighted [76].

A wide range of secreted factors and inflammatory mediators from both adipocytes and macrophages are influenced by the activation of PPARγ. A few examples of this time of mediator include adiponectin, resistin, IL-6, TNFα, PAI-1, MCP-1 and angiotensinogen [76]. Moreover, PPARγ resulted in the reduction of NEFA supply in plasma [76].

The molecular mechanisms and signals by which PPARγ modulates adipose tissue and its related disorders are now believed to include a wide range of activators and suppressors.

Suh and colleagues proposed that the anti-adipogenic effect of the ADP gene is through the inhibition of PPAR activity by binding histones and HDAC3 [57].

An important co-activator of PPARγ, PGC-1α, plays a key role in regulating the development and metabolic functions of both white and brown adipose tissue. Many such co-activators and repressors, that have been shown to influence adipose tissues activities, work by fine-tuning PPARγ activity and adipocyte differentiation. For example, it has been shown that Retinoblastoma-Histone Deacetylase 3 Complex inhibits PPARγ and consequently, adipocyte differentiation [77]. Moreover, Rb and p107 promote pre-adipocyte differentiation preferentially into white versus brown fat through the repression of PGC-1α [78]. PGC-1α is essential for brown fat thermogenesis but not brown fat differentiation, and the PGC-1 co-activators play an absolutely essential but complementary function in differentiation-induced mitochondrial biogenesis [79].

Brown adipose tissue development is also regulated by several other transcriptional regulators that suppress and promote brown fat cell fate. FOXC2 and PRDM16 transcriptional regulators promote BAT development. However, brown fat cell fate is determined only by PRDM16 in a cell-autonomous manner. When PRDM16 is expressed in WAT precursors in culture or in vivo, it causes expression of complete BAT-selective genes. PRDM16 actions are partly mediated through PGC-1α and PGC-1β transcriptional co-regulators and do not require DNA binding. Both BAT and myogenic lineage arise from precursors that express myf5. Seale and colleagues reported that primary brown fat preadipocytes culture with knockdown PRDM16 gene showed skeletal myocytes

interspersed among fat cells. Conversely, the control culture, expressing PRDM16, showed normal adipogenic differentiation. Knockdown of PRDM16 resulted in complete ablation of brown fat cell-selective genes such as UCP1, CIDEA and elovl3/cig30. Additionally, the knockdown of PRDM16 also gave rise to an increased expression of myogenic genes. The ectopic expression of PRDM16 in C2C12 myoblast cell line resulted in failure to undergo myogenic differentiation due to the inhibition of myotube-specific genes. In addition, the PRDM16-expressing C2C12 myoblasts differentiated into adipocytes in the presence of adipogenic inducers compared to the control group which differentiated into skeletal myotubes. PRDM16 expression in C2C12 myoblasts caused an increase in mRNA levels of adipocyte specific genes, PPARγ and aP2/FABP4, and decrease in the levels of myogenic genes. Moreover, PRDM16 expression also increased the levels of brown fat cell specific genes, elovl3 and CIDEA; and thermogenic genes, UCP1 and PGC-1α [80].

In addition, it has been proposed that Liver X Receptor α reduces energy expenditure by acting as a transcriptional repressor for the UCP1 Gene in Brown adipose via the recruitment of the co-repressor RIP140 to an LXRα binding site, resulting in the removal of PPARγ [81]. Recent research in this field has revealed additional roles for PPARδ and its regulators in brown fat metabolism. It has been shown that PPARδ induces twist-1 expression and mediates the actions of PGC-1α in a negative-feedback regulatory mechanism [82]. Moreover, it has been shown that stimulating PRD1-BF1-RIZ1 homologous domain-containing-16 and peroxisome proliferator-activated receptor-γ co-activator-1-α induces brown fat cell differentiation through bone morphogenetic protein-7 [83]. Finally, the latest research findings linking FTO gene variants and adipocytes browning in human have recently been studied. Researchers have shown that they are mainly influenced via short circuit of small molecules of ARID5B, rs1421085, IRX3 and IRX5 as candidate involvements in a pathway of adipocyte thermogenesis. [84]. The indicative role of IRX3 or IRX5 is to increase lipid storage. More research for identifying and linking of all smaller molecules involved in the metobolome of brown adipose to white adipose should be emphasized. Formatting circuits using KEGG, PubChem, MetaCyc and ChEBI etc. to study of the structure and pathway viewing applets will with time elucidate the overall networks and complex circuits of the dynamic between brown and white adipose. A complex set of signature genes down regulated as stress and inflammation related genes, FOS, JUN, ETS, C/EBPB, C/EBPD; while other unique set are upregulated as homeobox transcription factors and extracellular matrix structural proteins.

In conclusion, the central metabolism genes of PPARδ, TNFα and TGF-β are influenced and interconnected with all of the above mentioned genes. Rebooting the PPARδ central network of genes would re-balance the storage of fat and its consumption. Future therapeutic applications can be more feasible upon identifying key genes or small

molecular networks as mentioned above. Comprehensive gene expression profiling under simulated metabolic health scenarios focusing on such networks is vital. Examples of approaches would include reactivation or enrichment of brown fat through different techniques followed by microarray expression profiling. This will enable target drug designing for intervention as well as a possible amelioration of obesity associated medical complications.

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