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# Gene transcripts associated with BMI in the motor cortex and caudate nucleus of calorie restricted rhesus monkeys

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ABSTRACT

Obesity affects over 500 million people worldwide, and has far reaching negative health effects. Given that high body mass index (BMI) and insulin resistance are associated with alterations in many regions of brain and that physical activity can decrease obesity, we hypothesized that in Rhesus monkeys (*Macaca mulatta*) fed a high fat diet and who subsequently received reduced calories BMI would be associated with a unique gene expression signature in motor regions of the brain implicated in neurodegenerative disorders. In the motor cortex with increased BMI we saw the upregulation of genes involved in apoptosis, altered gene expression in metabolic pathways, and the downregulation of pERK1/2 (MAPK1), a protein involved in cellular survival. In the caudate nucleus with increased BMI we saw the upregulation of known obesity related genes (the insulin receptor (INSR) and the glucagon-like peptide-2 receptor (GLP2R)), apoptosis related genes, and altered expression of genes involved in various metabolic processes. These studies suggest that the effects of high BMI on the brain transcriptome persist regardless of two months of calorie restriction. We hypothesize that active lifestyles with low BMIs together create a brain homeostasis more conducive to brain resiliency and neuronal survival.


## 1. Introduction

Since 1980 the number of people worldwide considered obese has doubled with over 1.5 billion people now considered overweight (body mass index, BMI, 25 or higher) and 500 million people considered obese (BMI 30 or higher) [1]. The negative effects of obesity on health are far reaching. As weight increases to levels defined as overweight and obese, the risks for the following conditions also increase: coronary heart disease, type 2 diabetes, cancers, hypertension, dyslipidemia, stroke, liver and gallbladder disease, sleep apnea, osteoarthritis, and gynecological problems [2–4]. According to the American Medical Association the best ways to decrease the risk of obesity are to eat a diet low in fat and to increase physical activity levels [5]. Indeed, modern sedentary life is associated with increased risks for obesity, as well as cardiovascular disease, type II diabetes [6], and depression [7,8].

In the hypothalamus of the central nervous system (CNS) both insulin and leptin control body weight and feeding behavior. Leptin, a hormone derived from fat circulating in the blood, informs the hypothalamus via JAK/STAT pathway about the energy status of peripheral tissues [9], and reduces the expression of neuropeptides (neuropeptide Y (NPY), agouti-related peptide (AgRP), and melanocyte stimulating hormone (MSH also known as POMC)) that stimulate feeding [9–11]. Insulin, another circulating hormone, serves as a feedback signal from the periphery to reduce appetite and body weight in the hypothalamus [12] and serves to cause peripheral tissues to take glucose up from the blood and store it as glycogen [13].

Furthermore, insulin receptors (receptor tyrosine kinases) located throughout the brain signal through both the PI3K/AKT and ERK1/2 survival signaling pathways [14]. In obesity resistance to leptin and insulin impairs proper signaling about energy status that results in increased body weight and feeding behaviors. The AKT and ERK1/2 signaling pathways are also downstream of many neurotrophic factor signaling pathways and activation of these pathways leads to neuroprotection in several regions of the brain [15,16]. Thus, insulin resistance, may lead to decreased activation of AKT and ERK1/2, increased apoptosis, and decreased survival of neurons. This could explain why type II diabetes (insulin resistance) is often linked to Alzheimer's disease, a neurodegenerative disorder, and could be even further detrimental to the total health of the brain [17].

Insulin resistance that results in impaired signaling also affects other regions of the brain [18]. The effects of insulin on cortical

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activity are negatively related to the amount of body fat and the degree of peripheral insulin resistance [19]. Furthermore, decreased brain volumes have been seen in the frontal lobe, the temporal lobe, the anterior cingulate, the hippocampus, and the basal ganglia with high BMI [20]. Using correlations of BMI with brain volumes the same group showed that overweight and obese individual's brains age prematurely [21]. Other studies also link higher BMI with smaller regional brain volumes [21–24]. These findings provide evidence for cerebral insulin resistance in overweight humans.

Given that high BMI is associated with insulin resistance, that insulin resistance is associated with specific brain alterations, and that physical activity can decrease obesity, we hypothesized that we could find genes associated with BMI in motor regions of the brain. We investigated transcriptome changes associated with BMI in the motor cortex and caudate nucleus of 14 Rhesus monkeys with DNA microarrays. We found a distinct BMI associated gene expression signature in both the motor cortex and the caudate nucleus, and decreased protein phosphorylation of a survival kinase, pERK1/2, in the motor cortex. Our studies indicate that obesity is also associated with molecular changes in motor regions of the brain that these changes include alterations in metabolic and apoptotic related genes and that increases in BMI are associated with decreases in the phosphorylation of a survival kinase.

The same cohort of monkeys was used in a previous calorie restriction study, as numerous studies indicate that calorie restriction, like exercise, is neuroprotective. To mimic the typical American diet Rhesus monkeys were fed a diet high in fat for the last 3 years of their life. They also underwent one month of a 30% calorie reduction followed by one month of a 60% calorie reduction at the end of the study [25]. This is typical of a dieting person, and increases the production of BDNF and activates the AKT and ERK1/2 signaling and survival pathways in the brain [26]. While calorie restriction could theoretically represent a potential confound in our experiments as it may overcome some of the effects of a high fat diet and insulin resistance [27], this was not the case: decreased pERK1/2, increased CASP9 & FOXO3 mRNA, and an enrichment of apoptosis and metabolism related genes, all indicate decreased neuronal survival, suggesting that calorie restriction does not overcome the transcriptional signature of high BMIs in the brain.

## 2. Results

### *2.1. Microarray data analysis reveals a BMI-associated gene expression pattern*

We hypothesized that BMI would be associated with a unique gene expression signature in the motor cortex and caudate nucleus that may be of importance to neurodegenerative disorders given observed alterations in the brain in insulin resistance. To discover other alterations we performed whole genome expression profiling of the motor cortex of 14 female, calorie restricted rhesus monkeys with a wide range of BMIs using both Affymetrix Rhesus Macaque Genome (RhG) and Human Genome (HG) microarrays (Fig. 1). The rhesus and human genomes share 93% sequence identity [28] and human microarrays successfully have been used to query rhesus monkey gene expression changes in the past [29]. Whereas the Affymetrix RhG microarrays provided us with the most specific hybridization information for rhesus monkeys, the Affymetrix HG microarrays contained more extensive and higher quality annotations.

Microarray data were log2 transformed, RMA-normalized [30,31] and linearly modeled with BMI. We focused on convergent findings between the RhG and HG microarrays, which enabled us to identify a strong and distinct BMI-associated gene expression signature in the motor cortex and caudate nucleus. Two types of data-mining approaches were employed. First, we identified the transcripts that were associated with BMI, followed by a molecular pathway analysis that revealed the most affected BMI-associated groups of interdependent genes.

We identified 27 transcripts that were associated with BMI in the motor cortex (17 positively correlated and 10 negatively correlated) and 51 transcripts that were associated with BMI in the caudate nucleus (41 positively correlated and 10 negatively correlated) (Supplementary Table 1 and 2). All altered transcripts in the motor cortex and in the caudate nucleus were changed in the same direction on both human and monkey microarrays, suggesting a high degree of concordance between the two data sets, and a very low false discovery rate in our experiment. Our Pearson Hierarchal Clustering [32] revealed a progression of gene expression levels from monkeys with high BMI to monkeys with to low BMI (Fig. 2). Furthermore, we validated increased expression of caspase 9 (CASP9) in the motor cortex and increased expression of forkhead box O3 (FOXO3) in the caudate nucleus with increasing BMI by qPCR (Fig. 3).

Next, we performed Gene Ontology (GO) Biological Process analysis (Supplementary Table 3) using the Gene Ontology Enrichment Analysis Software Toolkit (GOEAST) [33,34] and GSEA assessment using the Broad Institute's Gene Pattern Software [35] (Tables 1 and 2) to decipher which biological processes were involved with the BMI associated gene expression signature. GO Biological Process analysis searched our differentially expressed gene datasets (Supplementary Table 1 and 2) for significantly altered GO Biological Processes. While these analyses revealed no significant biological processes associated with significant gene expression changes in the motor cortex, in the caudate nucleus we identified a large number significantly altered biological processes that suggested alteration of gene networks related to metabolism, cell death, and cellular development (Supplementary Table 3).

We also assessed our data for the enrichment of functional gene pathways using Gene Set Enrichment Analysis (GSEA) [35] using the gene sets in the Molecular Signatures Database (MSigDBv.3.0) for the human (HG) arrays. We found 9 gene sets positively enriched with BMI in the motor cortex (Table 1), and 3 gene sets negatively enriched with BMI in the caudate nucleus (Table 2). Prominently, in the motor cortex the BMI-associated changes included VEGFA targets (genes upregulated in HUVEC endothelium cells at 30 min after VEGFA stimulation [36]) and AKT phosphorylation targets in the cytosol, while in the caudate nucleus significant gene sets included HDAC targets (genes whose transcription is altered by histone deacetylase inhibitors [37]).

### *2.2. The observed gene expression changes are putatively a result of altered ERK1/2 signaling*

Several of the BMI-dependent genes attracted further attention. Our data identified inversely correlated mRNA levels of forkhead box O3 (FOXO3) with BMI in the caudate nucleus. FOXO3 is activated via the growth factor pathway, and involved in apoptosis and neuronal survival [15]. Furthermore, as both AKT and ERK1/2 are effector signaling pathways of neurotrophic factors [15,16,26,38], we next investigated the association of the AKT and ERK protein phosphorylation state with BMI (Fig. 4) through Western analysis. While AKT levels and its phosphorylation state was unchanged, we identified a strong negative correlation of both ERK1/2 and pERK1/2 levels with BMI (pERK1/2/tubulin $r = -0.663$ with $p = 0.010$ and pERK1/2/ERK1/2 of $-0.595$ with $p = 0.025$) (Fig. 4). As pERK1/2 phosphorylates CREB, a transcription factor in the nucleus to reduce apoptosis [38] and activate transcription of neuronal survival genes [15], the decreased phosphorylation of ERK1/2 with increasing BMIs suggests that this might be a critical signaling pathway mediating some of the detrimental effects of BMI on the gene expression in the brain.

## 3. Discussion

In summary, we discovered a distinct gene expression signature associated with BMI after calorie restriction in the motor cortex and caudate nucleus. Our linear model allowed us to model BMI and