



Analyzing the Impact of Long-Lasting Changes in Energy Homeostasis and Nutrient Sensing on Nutritional Programming of Hypothalamus in Rats

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Author's contribution

This work was carried out in collaboration between both authors. Authors SMHA and FK designed the study, performed the statistical analysis, wrote the protocol and interpreted and prepared the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJMAH/2020/v18i1230283

Editor(s):

(1) Dr. Ashish Anand, GV Montgomery Veteran Affairs Medical Center, USA.

Reviewers:

(1) Liaudat Ana Cecilia, Universidad Nacional de Río Cuarto, Argentine.

(2) Abhishek Soni, Pandit Bhagwat Dayal Sharma University of Health Sciences (Pt BDS PGIMS), India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/65228>

Received 01 December 2020

Accepted 03 February 2021

Published 13 February 2021

Original Research Article

ABSTRACT

Background: The diseases due to nutritional deficiencies or imbalanced diet can be due to different factors. These factors can be biological or environmental. The study indicated that there will be increase risks of acquiring diseases as a result of irregular intake of diet. These risks include the increased risk of diseases such as liver abnormality, hypertension, lipids deposition, and other diseases.

Methodology: The databases used for the collection of secondary information include Science Direct, PubMed, and Google Scholar. Besides, the keywords used for the searching of relevant research articles include “*experimental rats, nutritional deficiencies. Hypothalamus, mechanisms studies, gene ontology, metabolic syndromes*”. There were the use of female rats (n=8) and the weight was ranged between 200 to 250 grams. The experimental rats were placed in a cycle of 12 h light/dark. An adequate amount of water and food was provided for about one week to the rats before starting the experiment. Enzymatic procedures were used for the analysis of cholesterol, fatty acids, and triglycerides. Glucose concentrations in the blood samples were also assessed using the glucose meter. The insulin levels were also measured by using the assay kits. The hybridisation, scanning, and normalization of the data were also done. The extracted RNA from the

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hypothalamus was transcribed by using the “*Superscript II RNaseH- Reverse-Transcriptase*”. The t-test was also performed using statistical software. The groups of genes and their altered expressions were studied after the malnutrition conditions. The results obtained through PCR were expressed in the form of average and standard deviation values [1]. The mRNA expression levels in the samples of the hypothalamus were also assessed. The data were compared using the student test. The significance levels were measured at $p < 0.05$.

Findings: The findings of the study also indicated that the transcription of co-regulators is also link with the nutritional and temporal cues to the metabolism process by means of their association with circadian clock. The findings revealed that the hypothalamic circuits and its nutritional programming plays significant role in the regulation of energy homeostasis which is a major factor of obesity development in association with malnutrition in early life development.

Conclusion: Conclusively, it has been identified that the deficiency of nutrients during developmental period such as prenatal and postnatal is linked with the enhanced risk of different types of diseases in childhood as well as in adulthood.

Keywords: Gene ontology; hypertension; neuropeptides; nutritional programming (NP); hypothalamus.

1. INTRODUCTION

The deficiency of nutrients during the developmental period such as prenatal and postnatal is linked with the enhanced risk of different types of diseases in childhood as well as in adulthood. The risks of several diseases such as hypertension, obesity, and other chronic issues will be increased in the case of deficiency of nutrients [2]. It was observed that there are increased issues due to the problems of nutritional deficiencies that cause to increase the abnormalities within the metabolic system [3]. The issues within the metabolic system increase the abnormalities that enhance the chances of diseases such as obesity, upset of glucose levels within the body, high blood pressure, heart diseases, or stroke. The study indicated that there was also an increased risk of fats deposition and a cholesterol level that results in the intake of high cholesterol or high sugar diet. The disturbed lifestyle of a person increased the likelihood of obesity and metabolic syndromes [4].

It was noticed that children at the younger ages most likely to develop the risks of acquiring diseases if nutrients are deficient in the diet provided to them [5]. As a result, children develop obesity that can cause several complications in adulthood. It was documented that children lacking in nutrients most likely to develop the risks associated with acquiring cardiovascular diseases or hypertension. Therefore, it was observed that nutritional requirements must be fulfilled in childhood which will help in maintaining the homeostasis of the body and prevent the risks of diseases in the long run [6]. The research studies carried out on

animals showed that nutritional deficiencies and intake of high cholesterol diet increased the risks of several physiological issues that increase the abnormalities of the metabolic system [7]. The studies also indicated that the intake of an imbalanced diet during the pregnancy also imposed adverse impacts on the off-springs [8]. It was documented that the mechanism involved in the programming during the pre-natal and post-natal period for the regulation and maintenance of homeostasis is not well understood [8]. These mechanisms must be known for exploring the causes of changes in the metabolic processes due to the intake of a high-calorie diet or ingestion of a diet with a deficiency in nutrients. Besides, the research studies associated with the understanding of the molecular mechanism are also lacking [9].

The research studies indicated that certain abnormalities such as appetite control and regulation of energy levels within the nervous system are also involved. In addition, it was noticed that the lacking of nutrients in the early ages resulted in the production of the off-springs that will be unhealthy and showed abnormal growth and development [10]. It was found that the intake of a high-fat diet also includes an abnormal diet. In another study, the analysis of meal patterns indicated that abnormality in the metabolic system of rats was due to the intake of more meals and decrease eating latency [11]. The studies showed that the intake of less food or an imbalanced diet during the pregnancy led to the production of offspring that may have abnormalities in the hypothalamus due to the dysregulation of different neuropeptides in the food items. There are also anomalies observed in decreasing the number of neurons.

It was observed that there was a dysregulation in the levels of insulin, serotonin as well as leptin in the offspring of rats [12]. There was an issue observed in the dysfunctioning of the hypothalamus that is responsible for the maintenance of homeostasis of the different body systems as well as along with the intake of food that plays an important role in the regulation of the developmental system [13]. The programming was found to be disturbed due to the unavailability of proper nutrients in the diet. Despite the severity of issues in metabolic programming due to nutritional deficiencies, there is a lack of studies addressing the issues and studying molecular mechanisms that might help in the understanding of the physiological and behavioural alterations that occur due to lack of availability of proper nutrients in the prenatal period of development.

Therefore, the present study was conducted to understand the molecular pathways that helped in the understanding of physiological and behavioural changes caused due to undernutrition in the prenatal period of development using the rats as experimental animals. DNA chips and techniques of quantitative PCR were employed to examine the alterations within the hypothalamus transcriptome. The alterations were studied that occurred due to the intake of the diet with nutritional deficiencies in the pregnancy period and afterward. In addition, there was an evaluation of data associated with gene ontology to analyse the genes responsible for the transduction of cell signals. The understanding of the maintenance of metabolic processes and transcription of the genes was also done.

2. RESEARCH METHODS

2.1 Data Collection

The present study employed the qualitative approach to collect the secondary data for the evaluation and understanding of the effects and alterations in the maintenance of energy levels and programming of nutrients in the hypothalamus using the experimental rats. The sources used to collect the secondary data include the published research articles and books [14]. The relevant data and information associated with achieving the study objectives were used. The studies that had been done by the previous researchers on the rats for the assessment of the impacts of alterations of the

mechanisms associated with the nutritional programming (NP) of the hypothalamus in rats were included.

2.2 Inclusion and Exclusion Criteria of the Study

There were inclusion and exclusion criteria of the study for the selection of articles. The research studies that had been focused on analysing the impacts of nutritional deficiencies on the functions of the hypothalamus particularly associated with the alterations in the nutritional programming in rats were included. The research articles included in the present study employed the experimental rats weighed between 200 to 250 grams.

2.3 Materials and Methods

The methodology and techniques adopted in this study were previously used in the research article of Orozco-Solis et al in 2010 [15] to experiment on rats and mentioned as follows.

2.3.1 Animals used in the experiment

The experiment was designed according to the guidelines of the "European Communities Council" [16]. There was the use of a model of metabolic programming comprised of protein's restriction within the period of lactation and gestation. There were the use of female rats (n=8) and the weight was ranged between 200 to 250 grams. The experimental rats were placed in a cycle of 12 h light/dark. An adequate amount of water and food was provided for about one week to the rats before starting the experiment. The female rats were mated to the males (3 months age). After the successful mating of male rats with female rats, the pregnant female rats were kept at a separate place. The special feed was provided to the control rats consists of a protein diet of 20 gram/100 grams.

In addition, the protein diet of 8 gram/ 100 grams was provided to the experimental rats during the period of pregnancy and afterward. The diet provided to the experimental rats was brought from the company in Netherland. In the control and experimental group (low protein diet), the male rats were fed with the laboratory prepared food. After completing the period of half a year, the animals were slaughtered. During the process of slaughtering, special care was taken related to the time of slaughtering that was 9 am to 10 am. This was to prevent the biasness that

can occur due to the circadian alterations for the expression of genes [1]. The blood samples were used for the level of insulin analysis. Different metabolite contents such as glucose, cholesterol, fatty acids, and triglycerides were measured. The fats were also dissected separately from the rats for the examination of the weight of body fats [15].

2.3.2 Analysis of metabolites and hormones

Enzymatic procedures were used for the analysis of cholesterol, fatty acids, and triglycerides. Glucose concentrations in the blood samples were also assessed using the glucose meter. The insulin levels were also measured by using the assay kits [3].

2.3.3 DNA extraction and quantitative PCR (Real-time)

RNA samples were extracted from the hypothalamus and the determination of RNA in terms of quality and quantity was measured. The hybridisation, scanning, and normalization of the data were also done. The extracted RNA from the hypothalamus was transcribed by using the "Superscript II RNaseH- Reverse-Transcriptase". The diluted DNA samples were used for the amplification of PCR. The primers were sequenced for the process of amplification [3].

2.3.4 Analysis of data

The program of gene clusters was used to understand the differences in the control and experimental rats (low protein diet). The hierarchy associated with the genes and biological samples were also examined. The t-test was also performed using statistical software. The groups of genes and their altered expressions were studied after the malnutrition conditions. The results obtained through PCR were expressed in the form of average and standard deviation values [1]. The mRNA expression levels in the samples of the hypothalamus were also assessed. The data were compared using the student test. The significance levels were measured at $p < 0.05$.

3. RESULTS

3.1 Metabolic Process Regulated by Genes in Hypothalamus

The process of nutritional programming in the hypothalamus is based on the several genes that regulated the metabolic process controlled by the

hypothalamus. According to a study conducted by Blouet and Schwartz in 2010, the expressions of genes that can be changed by the restriction of perinatal protein are usually involved in the metabolic processes regulation [17]. This study used 180 days old rats' experimental sample fed either a low *protein diet* (80g/kg) or a *control* (200 g/kg) through lactation and pregnancy. The bio-informatic analysis of the study indicated that the restriction of perinatal protein alters the expression of two gene clusters that are responsible for regulating common cellular mechanisms. The first cluster of gene contains several gate keeper genes that are associated with the regulation of nutrient sensing and insulin signalling. On the other hand, the second cluster of gene contains a functional network of co-regulators and nuclear receptors of transcription responsible for the use and detection of lipid nutrients as fuel.

In addition to this, the findings of the study also indicated that the transcription of co-regulators is also link with the nutritional and temporal cues to the metabolism process by means of their association with circadian clock. The findings revealed that the hypothalamic circuits and its nutritional programming plays significant role in the regulation of energy homeostasis which is a major factor of obesity development in association with malnutrition in early life development. The role of hypothalamus in the nutritional programming can also be evaluated through the findings some studies [3], which indicated that the down-regulation of genes that regulate metabolism processes results in the long-lasting changes in energy homeostasis and nutrient sensing which may affect the nutritional programming of hypothalamus. A study of Vaisermanin (2014) further emphasised that the insufficient diet or malnutrition in early life development can permanently alter the homeostatic pathways and function and structure of specific organs, which can also affect the programming of individual's longevity and health status [18].

3.2 Role of Cell Signalling Pathways in Nutrient Sensing and Energy Homeostasis in Hypothalamus

The findings of a study of Sleeth et al. in 2010 indicated that the energy homeostasis and nutrient sensing are controlled by the hypothalamus where energy intake and nutrient sensing are processed [19]. Intake of protein may influence the regulation of energy

homeostasis and nutrient sensing indirectly through triggering the peripheral signal pathways. On the other hand, protein directly influences nutritional programming in the hypothalamus by means of altering the levels of free amino-acids in the brain. In the support, the findings of a study indicated that perinatal under-nutrition LP rats showed two fold increased expression of galanin receptor 1, glutamate receptor, and cannabinoid receptor than controls. These receptors were found to be involved in the regulation of energy homeostasis and food intake. The findings of the present study indicated that the down regulation of metabolic genes with a *p-value* of 0.02 and 0.04 resulted in the altered expression of neurotransmitter receptors responsible for the regulation of metabolism during early life development in rats.

3.3 Impact of Changes in Energy Homeostasis and Nutrient Sensing on NP in Hypothalamus

In addition to this, a study of Vucetic et al. in 2010 [20] indicated that the perinatal protein restriction is responsible for increased expression level of genes in hypothalamus involved in the signal transduction pathway for the regulation of insulin. The findings of this study are in line with the findings of Guo et al. in 2019 [21] which indicated that the up-regulation of cell-signaling pathway such as *P13K signalling pathway* in skeletal muscle of mice altered the nutritional programming in hypothalamus which consecutively resulted in diet-sensitive obesity, hyperphagia, and leptin resistance. In contrast, the findings of a study of Sequea, D.A., Sharma, Arias, and Carteein 2013 [22] indicated that the increased activity and expression of GSK3 β in the skeletal muscles of rats resulted in the developed of type 2 diabetes. It indicates that the increased metabolic disturbances and food intake observed in nutritional programmed rat models are induced by the disrupted signalling of insulin in the hypothalamus. It has also been observed in the findings of the study of Orozco-Solis et al. in 2011 [23] that the regulation of circadian clock can also be permanently changed by the restriction of perinatal protein. Circadian clock activity is based on the energy metabolism and temporal availability of nutrients. The findings of this study indicated that the regulation of *CLOCK/Bmal1 complex* is based on the lipid and glucose biosynthesis. Due to the perinatal protein restriction, the *CLOCK/Bmal1 complex* induced metabolic dysfunction and circadian

clock disruption. It indicates that the protein restriction or malnutrition during early life development may result in the susceptibility to insulin resistance and obesity. The findings of the included studies revealed that the changes in nutrient sensing and energy homeostasis are linked directly to the nutritional programming alternations in the hypothalamus with the significance value of $p < 0.05$.

4. DISCUSSION

The findings of the studies included in this study indicated that nutritional deficiency or malnutrition during perinatal development was highly correlated with an elevated risk of diabetes, obesity, and hypertension development in adulthood. It was also been observed that the nutritional programming in the hypothalamus can be affected by the long-lasting alterations of energy homeostasis and nutrient sensing during perinatal development [24]. In support, Bouret [25] stated that the hypothalamus plays an essential role in the integration of endocrine, nutritional, and neuronal cues. The nutritional programming in the hypothalamus can be influenced through perinatal protein restriction which may result in long-lasting changes in the several metabolic pathways and remain persistent throughout adulthood [26]. On the other hand, Lillycrop and Burdge [27] indicated that nutrient restriction during early life may result in transcriptional alterations in the hypothalamus. The findings of the previous studies revealed that calorie or protein restriction during suckling or gestation is associated with an altered expression of genes in the hypothalamus, including orexigenic and anorexigenic genes [23]. It asserts that malnutrition during perinatal development affects the hypothalamic circuits, which is responsible for regulating energy expenditure and nutrient sensing [28]. The alterations in hypothalamic circuits may result in long-term metabolic changes such as insulin resistance and obesity development [29].

5. CONCLUSION

The study indicated that there will be increase risks of acquiring diseases as a result of irregular intake of diet. These risks include the increased risk of diseases such as liver abnormality, hypertension, lipids deposition, and other diseases. It has also been analysed in the present study that the energy homeostasis and nutrient sensing controlled by the hypothalamus can be affected by the perinatal protein

restriction which may result in the long-lasting changes in the several metabolic pathways and remain persistent throughout the adulthood. It has also been in this study that the malnutrition during perinatal development affects the hypothalamic circuits, which is responsible for regulating energy expenditure and nutrient sensing.

The study also indicated that the calorie or protein restriction during suckling or gestation is associated with an altered expression of genes in hypothalamus, including orexigenic and anorexigenic genes. It has also been analysed that the regulation of circadian clock can also be permanently changed by the restriction of perinatal protein. On the whole, it has been identified that the hypothalamic circuits and its nutritional programming plays significant role in the regulation of energy homeostasis which is a major factor of obesity development in association with malnutrition in early life development. The role of hypothalamus in the nutritional programming indicated that the down-regulation of genes that regulate metabolism processes results in the long-lasting changes in energy homeostasis and nutrient sensing and can also affect the nutritional programming in hypothalamus during perinatal development.

CONSENT

It's not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:
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