CREATING A DIET INDUCED OBESITY ZEBRAFISH MODEL FOR ANALYSIS OF METABOLIC PROGRAMMING IN TUMORIGENESIS.

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ABSTRACT:

BACKGROUND AND AIM: Overweight is now more common than smoking as a modifiable risk factor for carcinogenesis, accounting for 40% of cancer cases reported in the United States. Using the framework of the hallmarks of cancer, we here outline how obesity may affect the carcinogenic hallmarks in somatic cells. To analyze the attenuation of adipocytes in zebrafish by feeding coffee and green tea infused food. **MATERIALS AND METHOD:** Zebrafish husbandry involved keeping the fish at a controlled temperature of 28 degrees Celsius with a light-dark cycle of 14 hours. Feeding protocol for zebrafish: Four distinct fish groups were examined, each receiving a unique diet. By providing standard food, the first group (n = 10) was constituted as a control group. By giving an excess of food to the second group (n = 10). Third group (n=10) was fed food that had been infused with coffee. Fourth group (n = 10) served meals laced with green tea. **RESULT:** Measurements of weight, length, and BMI showed that the overfed group's weight was noticeably higher than that of the regularly fed group. The weight of the fish significantly decreased in the group that received green tea and coffee treatments over feeding. **DISCUSSION:** Compared to the overfeeding group, the body weight and length parameters (BMI values) were comparatively lowered to the normal level by coffee and green tea. These investigations lead us to the conclusion that, when given to zebrafish before they are overfed, coffee and green tea significantly lower blood glucose and BMI in comparison to the overfed group. When the fish are fed coffee and green tea, the vital metabolic parameters are brought up to compare with the regular feeding group.

Keywords: Obesity model, tumorigenesis, zebrafish, induced diet, coffee, green tea, overfeed.

INTRODUCTION:

An increasing global health concern, obesity is brought on by shifting lifestyle patterns, such as nutritional consumption as a result of increased global development(Smolińska *et al.*, 2024). Blood glucose levels are said to be stabilized and obesity's outward appearance, particularly around the abdomen, is reduced by coffee and green tea. Through this study, the zebrafish will be used as a model to analyze adipocytic traits in metabolic diseases like obesity through food(Angom and Nakka, 2024; Zang *et al.*, 2024).

Forty percent of cancer cases reported in the United States are obesity-related, and obesity is now more common than smoking as a modifiable risk factor for carcinogenesis(Deevi *et al.*,

2024). Here, we describe how obesity may affect the carcinogenic hallmarks in somatic cells using the framework of the hallmarks of cancer(Zang *et al.*, 2024). We go through how obesity affects various processes in the body, including genome instability, tumor-promoting inflammation, inducing angiogenesis, activating invasion and metastasis, reprogramming energy metabolism, avoiding immune destruction, avoiding growth suppressors, resisting cell death, enabling replicative immortality, and avoiding apoptosis(Kuttikrishnan *et al.*, 2024).

In this developing topic, we outline the state of knowledge and debates at the moment and draw attention to a few areas that require more interdisciplinary study. For example, the relative contribution of the several obesity-related factors that may be causal is unknown for each form of cancer(Prabhuvenkatesh *et al.*, 2024). Whether one obesity-related factor consistently plays a predominant role, or if this varies between patients or even within a single patient over time, is still unknown, even within a single tumor type(Reshma *et al.*, 2023)(Bhandari, Bharani and Khurana, 2022). Elucidating the ways in which obesity impacts the hallmarks could result in innovative approaches to prevention and therapy for the rising number of obese individuals.

Cancer is caused by a sequence of progressive changes in normal cells that take place under selective pressure, leading to the neoplastic transformation(Bhandari, Bharani and Khurana, 2022). Any alteration has the potential to shift the balances in favor of cancer progression(Kakada *et al.*, 2024). In a multicellular organism, the development and multiplication of cells must be synchronized with the availability of adequate nutrients to facilitate the synthesis of macromolecules. As a result, signaling networks that convey nutritional status and regulate growth are tightly linked within cells and across the body. Because obesity is a condition of nutritional excess, it raises the risk of neoplastic transformation by persistently activating cellular growth factor signaling pathways(Morocho-Jaramillo *et al.*, 2024).

Except in extremely wealthy cultures or in experimental settings, food availability is not constant in nature, therefore calorie intake is rarely consistent. Plants and other eukaryotes store energy as complex carbohydrates and lipids(Benchoula *et al.*, 2024; Green *et al.*, 2024). The maintenance of health and reproductive potential, as well as the maintenance of physiological homeostasis, depend on energy storage, which supports evolution's primary driver, the survival of the species. But because of a more sedentary lifestyle, people have been consuming more calories consistently over the past few decades, while their energy expenditure has decreased as well. This

net increase in calorie intake combined with having access to an endless supply of foods high in energy leads to a metabolic imbalance, which is accompanied by adipose tissue hypertrophy and hyperplasia and, ultimately, obesity, which is linked to fat. It is evident that the impact of obesity on cancer risk varies depending on the organ, and the tumor-promoting pathways it elicits are gender- and tissue-specific(Wang *et al.*, 2018).

MATERIALS AND METHOD:

In zebrafish husbandry, the fish were kept in a light-dark cycle of 14 hours at a regulated temperature of 28 degrees Celsius. Zebrafish feeding protocol: Four different fish groups were looked at, and each was fed a different diet. The first group (n = 10) was established as a control group by giving them standard food. by providing the second group (n = 10) with an abundance of food. The third group (n=10) was given coffee-infused overfeed. Green tea laced overfeed were served to the fourth group (n = 10). Food was prepared by taking 20:1 parts of fish food and coffee or green tea. Stored in a dry place. Feeds were given in regular intervals for all the four groups. Activities were monitored. Few parameters were evaluated like blood glucose level, height and weight being taken under consideration. (Figure 1,2)

Figure 1: Showing fish feed with green tea(top left), fish feed with coffee(top right), powdered(lower left), pasted and dried and stored in containers(lower right).

Figure 2: Showing grouping of fishes in tanks. Each tank comprises ten zebra fishes.

RESULTS:

Among the recorded values, group1 (control), group3 (coffee+overfeed), group4 (green tea+overfeed) showed nearly similar values while group 2 (overfeed) showed elevated blood glucose rate. Next category of weight and height showed increased value in group 2(overfeed). Representing excessive growth and development evident in the overfeeding group of zebrafishes. The weight of the overfed group was remarkably higher as compared to the normally fed group. In coffee and green tea treated over feeding groups, there was a significant decrease in the weight of the fishes. (Figure 4,5,6)

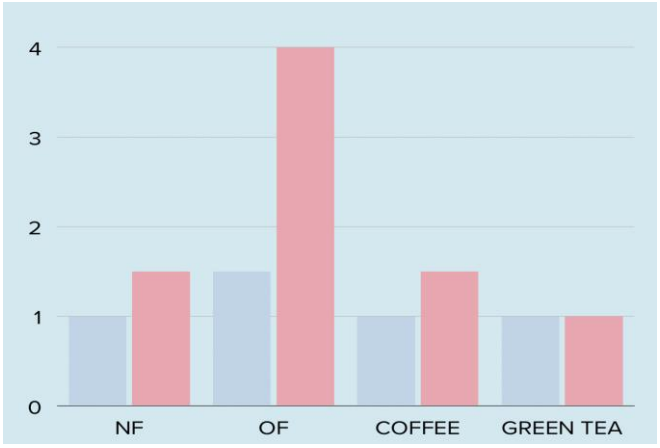

Figure 4: Showing blood glucose level(mg/dl) in four groups. NF-normal feeding, OF-overfeeding, coffee-coffee+overfeeding, green tea-green tea+overfeeding.

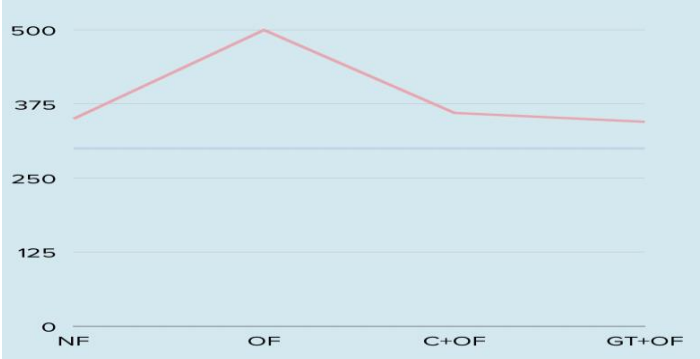

Figure 5: Representing weight in mg. NF-normal feeding, OF-overfeeding, C+OF-coffee+overfeeding, GT+OF-green tea+overfeeding.

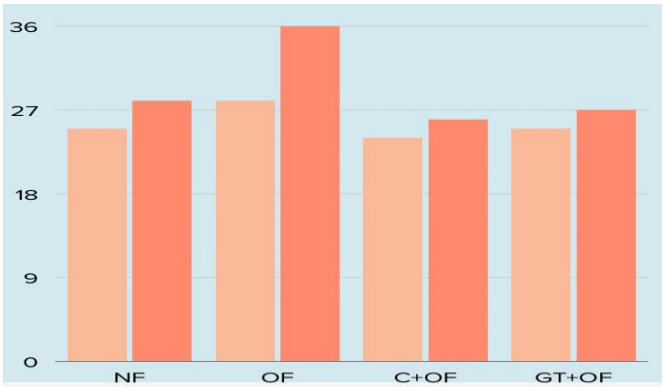

Figure 6: Showing height of zebrafish in millimeters recorded in week 1 and 2. NF-normal feed, OF- overfeeding, C+OF-coffee+overfeed, GT+OF-green tea+overfeed.

DISCUSSION:

Public awareness is being raised about obesity, a major global health issue. Over 650 million individuals worldwide are plagued by adult obesity, which nearly quadrupled in prevalence between 1975 and 2016, according to the World Health Organization (WHO)(Bhandari, Bharani and Khurana, 2022). It is uncertain, therefore, if obesity accelerates the growth of squamous cell carcinoma. In an earlier investigation, we discovered that individuals with oral squamous cell carcinoma (OSCC) at an early stage (T1/2N0M0) had a poor prognosis when they were obese. Further research is required to determine the underlying pathophysiological mechanisms and the link between obesity and epithelium carcinogenesis(Yang *et al.*, 2019).

Developing better early intervention measures to lower the risk of oral cancer may arise from an understanding of how obesity affects the onset and progression of OSCC. Many pathways have been revealed by studies of obesity to promote carcinogenesis; of these, immune response

dysregulation and a chronic inflammatory state are crucial. Two weeks we have performed a study on zebrafish, which gave us major results in blood glucose level, height and weight. Coffee and green tea even when overfeed shows evidence of inhibiting the adipose deposition and helps in static development.

Our data clearly show that both coffee and green tea significantly reduced blood glucose levels at the week 1 and week 2 endpoints. Additionally, compared to the overfeeding group, the body weight and length parameters (BMI values) were comparatively reduced to the normal level by green tea and coffee. These investigations lead us to the conclusion that, when given to zebrafish before they are overfed, coffee and green tea significantly lower blood glucose and BMI in comparison to the overfed group. When the fish are fed coffee and green tea, the vital metabolic parameters are brought up to par with the regular feeding group. Significant alterations were noted in the zebrafish model as of the advancement of weeks one and two. When compared to other groups, the group that consumes coffee and green tea is more active. Even when fish are overfed, blood glucose levels tend to drop. In the near future, more assessments will be conducted, including a histological investigation of the liver of zebrafish, quantification of gene expression, measurement of blood triglycerides, and analysis of liver damage.

CONCLUSION:

There have been significant alterations to the zebrafish model as of weeks one and two of the progression. When compared to other groups, the coffee and green tea overfeeding group is more active. Low blood glucose levels can occur even when fish are overfed.In the near future, additional assessments such as a histological examination of the liver of zebrafish, gene expression quantification, serum triglyceride measurement, and liver toxicity analysis will be conducted. Future research endeavors will encompass investigations utilizing additional phyto-therapeutic agents beyond green tea and coffee to ascertain their efficaciousness in mitigating the genotypic and phenotypic consequences of an unfavorable diet.

A poor diet is not the only way to cause obesity; research should also be done on other metabolic illnesses like diabetes.

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