# Histone H3K4me3 Distribution

Subjects: Biology

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Background: Different diets result in significantly different phenotypes through metabolic and genomic reprogramming. Epigenetic marks, identified in humans and mouse models through caloric restriction, a high-fat diet or the intake of specific bioactives, suggest that genomic reprogramming drives this metabolic reprogramming and mediates the effect of nutrition on health. Histone modifications encode the epigenetic signal, which adapts genome functions to environmental conditions, including diets, by tuning the structure and properties of chromatin. To date, the effect of different diets on the genome-wide distribution of critical histone marks has not been determined. Methods: Using chromatin immunoprecipitation sequencing, we investigated the distribution of the trimethylation of lysine 4 of histone H3 in the liver of mice fed for one year with five different diets, including: chow containing yellow corn powder as an extra source of plant bioactives or specifically enriched with cyanidin-3-O-Glucoside, high-fat-enriched obesogenic diets, and caloric-restricted pro-longevity diets. Conclusions: Comparison of the resulting histone mark profiles revealed that functional food containing cyanidin determines a broad effect.

Keywords: diets ; functional food ; anthocyanins ; epigenetics ; histone modifications ; mouse liver

## 1. Introduction

Diet is among the most effective environmental factors in inducing phenotypic changes. Caloric restriction (CR) and high-fat (HF) diets are significant examples: CR increases longevity and retards aging in a variety of organisms [1], whereas a long-term, high-fat-enriched diet induces well-known pathological traits in mammals [2][3]. Epigenetic mechanisms have been hypothesized to mediate the biological effects of diet, including regulation of the incidence of aging-associated diseases and life span [4][5].

Histone tail modifications are covalent reversible post-translational modifications that regulate chromatin structure and affect genome functions, particularly gene expression and DNA repair. Hundreds of histone modifications have been identified regulating chromatin states and genome functions [6]. The trimethylation of lysine 4 of histone H3 (H3K4me3) is an extensively investigated histone modification involved in the chromatin remodeling of processes associated with transcription and frequent on the promoters of actively transcribed genes in the mouse [7] and humans [8].

As observed for diets based on the restriction or variation in macronutrient composition, preclinical studies suggest that the dietary intake of polyphenols impacts the onset and progression of several diseases through epigenetic mechanisms, including targeting histone modifier enzymes [9]. Cyanidin-3-*O*-glucoside (C3G) is the most abundant among the anthocyanins, a class of plant flavonoids present in the human diet. C3G is abundant in fresh fruits, such as grapes, berries, blood oranges, peaches, and apples, and derived beverages or pigmented cereals, such as rice and corn with the typical purple color [10][11].

Several preclinical studies have established that C3G dietary intake induces healthy effects such as: anti-obesity and anti-inflammatory [12][13][14], anti-cancer [15], and cardio [16] and neuroprotective [17][18]. At the biochemical level, C3G and its metabolites are shown to target a variety of regulatory pathways within cells: AMPK-mTOR-S6K [19], Cox2 [20], NF-kB [21], Caspases/PARP [22], Stat/Vegf [23], and Sirtuin 1 [24]. In laboratory mice, the transcription profiles of skeletal muscle [25], liver [26], fat [27], retina [28], and heart cells [29] were consistently found to be largely influenced by the dietary intake of C3G.

Despite the abundance of biological effects attributed to polyphenols and their similarities with the effect of caloric restriction or high-fat diets, a comparative study of the effects of different diets on the distribution of histone marks is not yet available.

## 2. Current Insights

Nutritional epigenetics is a growing field of investigation focused on the deeper interaction between diet and the genome. According to the “nutraepigenomics” hypothesis, variation in macro- and micronutrient intake can imprint diet-specific epigenetic signatures that can ultimately affect tissue function and even be transmitted to progeny [30]. In particular, the dietary intake of plant bioactives, such as: genistein, epigallocatechin-3-gallate, curcumin, resveratrol, indole-3-carbinol, and phenylisothiocyanate, was found to impact the histone code [31].

The current study aimed to disclose the effect of dietary intake of C3G on the H3K4me3 epigenetic signal in the liver and compare such a C3G-induced H3K4me3 profile with low-calorie and high-fat diets profiles. For this, we compared the H3K4me3 genome-wide distribution induced by standard (SD), low-calorie (CR), and obesogenic diets (HF) with two functional foods: one with a higher content of flavonoids, except C3G from yellow corn (YD), and the other enriched in C3G from purple corn (RD).

The content of C3G in human foods ranges from a few to hundreds of milligrams/100 g (http://phenol-explorer.eu). This is effective to achieve a plasmatic concentration of 0.1–0.5 ng C3G/mL enduring for hours after a meal [32]. However, C3G is catabolized in the gut, leading to a number of bioactive phenolic metabolites, such as protocatechuic, hippuric, vanillic, and ferulic acids, which are absorbed together with undegraded C3G and may impact phenotype [33]. Using the same isogenic anthocyanin-enriched plant materials described here, providing approximately 12–36 mg/kg of body weight/day of C3G to mice, we have previously demonstrated that dietary intake of C3G from corn reduced myocardial injury in ischemia/reperfusion induced by doxorubicin [16][34]. Interestingly, the purple corn diet was also found to induce a long-lasting reprogramming of adipose tissue macrophages toward the anti-inflammatory phenotype, even when cells were isolated from their physiological microenvironment, suggesting the potent epigenetic effect of C3G dietary intake [14].

The results reported here establish that, after ten months of treatment of mice with different diets, C3G and the corn matrix remodel H3K4me3 in the liver chromatin. Analysis of the H3K4me3 peak distributions in the different diets shows significant differences. The YD and RD profiles segregate from the others, and annotated peaks associate with genes belonging to some specific metabolic processes. A distinct effect of the HF diet appears with an increase in the average signal on the TSSs of the most expressed genes, indicating an overall transcriptional activation of chromatin in liver cells by fat intake.

Focusing on differential sites, comparisons of the H3K4me3 signals within the promoter regions of the RD, YD, CR, and HF with respect to the SD reveal that the RD has an impact on a larger number of loci, affecting several signaling pathways, including the integrin-linked kinase signaling, which could be linked to the anti-inflammatory effect previously observed [14]. Intersecting all the comparisons, no genes were found marked in common with the different non-standard diets together. The straight comparison between the RD and YD to check the net effect of C3G identified only one gene. This resulted from a lower statistical power ascribed to the heterogeneity of YD profiles, which did not separate in the PCA from the RD profiles. Therefore, YD is also effective in regulating H3K4me3 distribution. However, the effect of C3G on H3K4me3 is highlighted by the overall GSEA using all the marked TSSs. Using this tool, several genes, in particular those related to amino acid metabolism, were found to be regulated by the RD vs. SD and the RD vs. YD. This effect of C3G was not shown by the CR and HF diets, suggesting a different action on H3K4me3 by C3G with respect to dietary changes in calories or macronutrient composition.

Methylation of histone tails is a fairly dynamic process and is maintained by histone-modifying enzymes, such as methyltransferases and demethylases. Notably, several polyphenols were found to inhibit lysine-specific demethylase-1 that regulates histone methylation, removing mono or dimethyl groups from methylated proteins, specifically H3K4 [35]. It is possible that C3G or its metabolites may directly affect histone-modifying enzymes.

In conclusion, the findings presented here demonstrate the effective role of dietary C3G consumption in regulating H3K4 trimethylation in the mouse liver, particularly within promoter regions.

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