The role of genetics in obesity essay

Design

AbstractionFleshiness is a complex disease caused through the interaction of environmental and familial factors. Epigenetic mechanisms and particularly the DNA methylation form, reflect this interaction. Methylation mechanism has been recognised as cardinal regulator of cistron written text. The 5-hydroxytryptamine receptor 2C (5-HT $_{2C}$ Roentgen) has an indispensable function in the ordinance of appetency and organic structure weight. This survey investigates a possible relation between methylation at the booster part of 5-HT $_{2C}$ R cistron and fleshiness steps in male topics.

Fifteen Greek male voluntaries were studied. The participants were divided in two groups, the corpulent survey group and the normal control group. Fleshiness estimated through the measuring of organic structure mass index (BMI) , organic structure weight and tallness, and waist perimeter. The 5-HT $_{2C}$ R booster methylation position was studied on genomic DNA, extracted from cheek epithelial cells. Methylation degrees were analysed and quantified by COBRA method. The entire physical activity degrees (METs) , and the ingestion of specific nutrient and drink were examined as fleshiness hazard factors.

Absence of methylation was detected at both CpG islands. The methylation degrees were non exemplifying differences between corpulent and non-obese control samples. In decision, this survey showed that methylation degrees at booster part of 5-HT2CR cistron are non related with fleshiness, within male topics.

Consequently, methylation forms at 5-HT2CR booster can non be evaluated as an epigenetic marker of fleshiness. *Key words* : 5-HT $_{2C}$ receptor, 5-

hydroxytryptamine, fleshiness, appetite, methylation, COBRA, bisulfite alteration. Table of ContentssChapter 1: Introduction1Chapter 2: Material and Methods32. 1 Subjects32. 2 Sample Collection and DNA extraction42.

3 Polymerase Chain Reaction Amplification52. 4 DNA methylation analysis by COBRA52. 5 Fleshiness measurings7Chapter 3: Consequences 9Chapter 4: Discussion 124.

1 Promoter Methylation124. 2 Methylation and Obesity134. 3 5HT $_{2C}$ R and Obesity144. 4 5-HT $_{2C}$ R booster methylation and Fleshiness154. 6 Methylation analysis164. 7 Research restrictions184. 8 Conclusion and Future research18Chapter 5: Appendixs 23Chapter 1: IntroductionFleshiness is often referred as an epidemic. The quickly increasing prevalence of fleshiness, during the last decennaries in developed states and more late in several developing states, comes to corroborate the epidemic proportions of the disease *(Waterland, et al.*

, 2008) . Fleshiness is a chronic disease that characterised by surplus of organic structure fat, which is different from merely surplus organic structure weight. Excess organic structure fat increases the hazard for a figure of diseases, such as diabetes mellitus (type II) , cardiovascular upsets or even certain types of malignant neoplastic disease *(Zhao, Goldberg and Vaccarino, 2013)* . Surveies on fleshiness suggest that familial and environmental factors interact to do fleshiness. This interaction is incused on the epigenetic forms.

Epigenetic mechanisms, and particularly DNA methylation, are related with the pathogenesis of fleshiness *(Zhao, Goldberg and Vaccarino, 2013)* .

Deoxyribonucleic acid methylation form occurs when a methyl group binds to a C at a cytosine-phosphate-guanine (CpG) site3. Methylated CpG sites can be found in a Deoxyribonucleic acid sequence either as individual sites or as a row of CpGs known as CpG Island (CpGI) . The CpGIs are normally located at booster part and seldom at cistron organic structure *(new wave Eijk, et al. , 2012)* .

The methylation of CpGIs at booster appears negative correlativity to the cistron look, which means that high degrees of methylation at booster reduces cistron look *(Jjingo, et al. , 2012)* . Storage of inordinate organic structure fat consequences from the instability between nutrient consumption and energy outgo *(Zhao, Goldberg and Vaccarino, 2013)* . Serotonin (5hydroxytryptamine – 5HT) is a neurotransmitter involved in legion of psychophysiological procedures, such as the ordinance of appetency and energy balance *(Yuan, et al.*

, 2000) . Surveies on the mechanisms of serotonergic system through which regulate feeding behaviors have distinguished the cardinal function of 5HT $_{2c}$ 5-hydroxytryptamine receptor (5HT $_{2C}$ R) subtype. It has been observed that mice with 5HT $_{2c}$ R cistron lack become hyperphagic and corpulent *(Heisler, Chu and Tecott, 1998)* . Furthermore, several polymorphisms at the booster part of 5HT $_{2C}$ R cistron are related with fleshiness in human *(Reynolds, Zhang and Zhang, 2003)* . The purpose of this survey was to prove the hypothesis that DNA methylation at promoter part of 5HT $_{2C}$ R is associated with fleshiness. The current hypothesis was tested through the comparing of corpulent and non-obese males. Corpulent and normal persons

were defined from the steps of organic structure mass index (BMI) , organic structure weight and waist perimeter (WC) .

The combined bisulfite limitation analysis (COBRA) was used to observe and quantify DNA methylation. COBRA is a quantitative method based on the bisulfite intervention and limitation enzyme digestion of DNA. The bisulfite intervention of Deoxyribonucleic acid followed by PCR elaboration, converts unmethylated C to thymine (C-U-T) , while methylated C is retained as C *(Xiong and Laird, 1997)* . The transition or the keeping of C in CpGIs expresses possible methylation, which can be revealed with limitation enzyme digestion. Chapter 2: Materials and Methods *2. 1 Subjects* As already mentioned before, 15 male voluntaries were used as topics. Participants represent a random sample of Grecian males aged between 28 to 63 old ages with a mean of 43 old ages. 5HT $_{2C}$ R cistron has been mapped at X chromosome (q24) *(Song, Gu and Schanen, 1999)* .

Given that males contain a individual transcript of X chromosome, the usage of male persons as capable makes it easier the 5HT $_{2C}$ R cistron survey. All voluntaries were recruited in Greece. The pick of the peculiar ethnicity was discussed and decided with the research supervisor. The cultural background of the topics was estimated as a confounding parametric quantity. The frequence of familial and epigenetic discrepancies shows fluctuation across different population *(Wallace, Zai, Brandl and Muller, 2011)* .

Deoxyribonucleic acid methylation form may non look important fluctuation between European populations. However, the survey on a specific European cultural group can still supply interesting scientific informations. From the 15

participants the 8 were corpulent (BMI ? 30kg/m ^{2}) and the remainder, 7 work forces, were non-obese (BMI 18. 5-24.

9 kg/m ^{2}) . A written engagement consent signifier was obtained from every voluntary (Appendix I) . Current survey has been taken moralss blessing from the University Research Ethics Committee (UREC) .

2. 2 Sample Collection and DNA extraction Buccal (cheek) cell samples were collected from 15 grownup males with Catch-All ^{Thulium} Sample Collection Swabs (Epicentre, USA) . One swab was used for each voluntary. Quick Extract ^{Thulium} Deoxyribonucleic acid Extraction Solution 1. 0 (Epicentre, USA) was used for DNA extraction. The related protocols were provided by the maker (Appendix II) . *2. 3 Polymerase Chain CR Amplification* A specific part of 201bp from the booster part of 5HT $_{2C}$ R cistron (GeneBank accession figure U49516) was isolated and amplified by PCR (Table 1) .

PCRs of 20? l volumes were used that incorporating x1 GoTaq ® Master Mix (Promega, USA) , 8-45 ng/? l mark DNA and 0. 2? M primers. The set of primers and conditions were used for PCR shown inTable 1. *2. 4 DNA methylation analysis by COBRA* Two CpGIs nowadays at the selected sequence of 5HT $_{2C}$ R booster (Table 2) were selected for scrutiny. Methylation degree of these CpGIs was determined utilizing combined bisulfite limitation analysis.

The EZ DNA Methylation-Gold Kit ^{Thulium} (ZymoResearch, USA) was used for the bisulfite intervention of the mark sequence. The recommended

procedure protocol was given by the maker (Appendix III) . After the bisulfite alteration (Ci? U) Nested PCR was used in order to finish the transition procedure (Ui? T) and magnify the merchandise. The Nested PCR is characterized by the usage of two consecutive sets of primers in two consecutive PCRs (Table 2) .

The engagement of the nested primers, external set and internal set, increase the specificity and output on PCR elaboration. Nested PCR was set up at the same conditions as the criterion PCR, as described below. Then, the modified Nested PCR merchandises were treated with the limitation enzyme BstUI (CGE‡CG) (Table 2) . The keeping of limitation site for BstUI at CpGIs expresses possible methylation, which can be revealed with limitation enzyme digestion. Methylated CpGs were digested by BstUI, while unmethylated CpG sites remained untrimmed. In order, to quantify methylation degree at the mark sequence, both the bisulfite treated amplicons and the unmodified amplicons from the standard PCR were treated with BstUI enzyme. Cytosines transition was verified with a 2nd limitation digestion by HaeIII (GGE‡CC) (Table 2) .

HaeIII digests the mark sequence merely if the unmethylated Cs transition is unsuccessful. Digestion merchandises were analysed at 2 % agarose gel cataphoresis. A 100 bp molecular marker (New England BioLabs ® Inc. , USA) was used at the gel cataphoresis. *2. 5 Fleshiness measurings* Body weight (kgs) , tallness (meters) and waist perimeter (centimeters) were measured for each voluntary.

Determination of fleshiness was made by ciphering the organic structure mass index (BMI kg/m ^{2}) . BMI consequences from the division of organic structure weight (kilogram) by the square of tallness (m) . The normal scope BMI fluctuates between 18. 5 and 24. 9 kg/m ^{2} , while corpulent persons have BMI ? 30 kg/m ^{2} . Current graduated tables of BMI were based on the recommended values of World Health Organisation (WHO, 2004) . Short version of IPAQ (International Physical Activity Questionnaire) (Appendix IV) was used to mensurate the physical exercising degree of each voluntary. Physical inaction seems to associate with increasing prevalence of fleshiness.

A Grecian version of short IPAQ (IPAQ-Gr) has been translated and developed by Papathanasiou et Al. (2009) . The IPAQ-Gr is available at IPAQ group web site (www. ipaq. ki. se) . The short version of IPAQ inquires about 3 peculiar types of activities: walking, moderate-intensity activities and vigorous-intensity activities. The continuance (in proceedingss) and frequence (yearss) of each activity during the last 7 yearss were recorded.

The mark of each type of exercising was defined in METs (Metabolic Equivalent of Task) . The metabolic equivalent is a physiological step specifying the energy rate of physical activities *(Patterson, 2005)* . The equation of *MET-value ten proceedingss (continuance) ten yearss (frequence) = MET-minutes/week* was used in order to cipher the mark of each activity type. The three types of activities referred in IPAQ have different MET value (walking: 3.

3 METs, moderate-intensity activities: 4. 0 METs and vigorous-intensity activities: 8. 0 METs) . The entire physical activity in MET-min/week for each voluntary was used in the analysis. Entire physical activity consequences from the amount of all three types of exercising in MET-min/week (walking+moderate+vigorous MET-min/week= entire MET-min/week) .

Entire MET-min/week mark of each participant was categorised as low, moderate and high. A short dietary questionnaire was used in order to specify a general facet of the dietetic wonts of the voluntaries (Appendix V) . This questionnaire was based on the Food Frequency Questionnaire (FFQ) that developed by Walter Willett at Harvard University (2007) . Current questionnaire asks about some declarative nutrient and imbibe classs (fruits, veggies, meat, soft drinks and alcoholic drinks) . These nutrient and imbibe classs were examined for a possible relation with fleshiness. Each voluntary was asked for possible happening of metabolic syndrome (MS) and related symptoms (Appendix V) . The MS is a composite of interrelated hazard factors for cardiovascular disease and diabetes that express the badness of the fleshiness *(Alberti, et al. , 2009)* . The hazard factors include cardinal fleshiness (WC & gt ; 102cm or BMI ? 30kg/m ^{2}) , dyslipidemia (high degree of triglycerides and low degree high-density lipoprotein cholesterin [HDL]) , increased blood force per unit area (130/85 mm Hg) and increased fasting blood sugar (? 100 mg/dl) *(Alberti, et al. , 2009)* . Individual that has diagnosed with any three or more of these hazard factors mean that suffers from the metabolic syndrome.

Chapter 3: ConsequencesThetable 3shows the demographic, clinical and laboratory features of the 15 males that were voluntarily participated at this experiment.

The corpulent group is distinguished by the average BMI of 36. 11kg/m ^{2} , while normal group has 23. 44kg/m ^{2} . The age of the corpulent topics ranged from 28 to 59years with a mean of 45. 50 old ages. On the other manus, age of thin voluntaries range between 27 and 63 old ages with a mean of 38.

57 old ages. Has been designed and optimised COBRA methylation check in order to analyze volunteers' methylation degree at promoter part of 5HT $_{2C}$ cistron. COBRA provides a quantitative step of methylation degree. Two CpGIs, at promoter part of 5HT $_{2C}$ cistron, were covered in the current experiment.

From the 15 samples merely the 7 (voluntaries 1, 2, 4, 11, 13, 14 and 15) of them eventually worked, 3 corpulent samples and 4 normal samples. The remainder 8 samples were ne'er shown PCR merchandise. Sing the 7 tested samples, no methylation form was detected in both examined groups. Thefigure 4 illustratesthe concluding experimental result incorporating agarose gel cataphoresis images. The undermentioned gel images show the size of PCR merchandises for normal (un-modified) and modified (bisulphite treated) Deoxyribonucleic acid samples, and the size of fragments that ensuing from possible digestion with BstUI enzyme. The normal undigested Deoxyribonucleic acid samples appear a individual set at 201 bp verifying the elaboration merchandise. The modified undigested samples show a

individual set near at 200 bp every bit good, but fractionally above because Nested PCR amplicons were sized at 209 bp. Then, the 2nd lane in each voluntary illustrates the fragments of normal DNA produced by BstUI digestion.

Particular enzyme has two acknowledgment sites (CGE‡CG x2) in the examined sequence bring forthing three fragments of 89, 84 and 28 bp. On the other manus, the Forth lane that contains the digestion merchandise of the bisulfite treated DNA appears a individual set at 200 bp. Current consequence groundss that no methylation was detected neither at corpulent or thin males. Both acknowledgment sites of BstUI were lost (TGTG) .

At least one of the two Cs appeared at the mark CpGIs was converted to thymine as they were unmethylated.

amazonaws. com/aaimagestore/essays/0928728. 011.

013. png”/> Chapter 4: DiscussionThe current experiment demonstrates that the examined booster part of 5HT $_{2C}$ Roentgen does non incorporate any methylation form between corpulent and thin control topics. No methylation was detected at the two bing CpGIs. Consequently, the undertaking hypothesis was rejected, and without the aid of a statistical analysis.

4.

1 Promoter MethylationStrong groundss from old surveies support that CpG methylation degree at booster part appears a negative association with the cistron look degrees. As the degree of methylation increased the look

degrees decreased *(new wave Eijk, et al. , 2012)* . Van Eijk et Al. (2012) from the scrutiny of the methylation and cistron look degrees in whole blood, shown that the methylation form at booster parts (Commonwealth of Independent States) normally (65. 4 %) appears negative correlativity with cistron activity degrees. Concurrently, the same survey detected that infrequently (34. 6 %) there is a positive association between methylation and transcript degrees, both degrees increased or decreased.

Regardless the type of correlativity the methylation form is an epigenetic marker important correlated with cistron look, advancing the activation or the inactivation of the cistron written text *(Woodfine, Huddleston and Murrell, 2011)* . As already mentioned, methylation occurs to CpGs dinucleotide that are mostly absent from cistron organic structure and chiefly present at booster part organizing CpG islands *(Weber, et al. , 2007)* . Have been found that methylation at booster rich of CpGs is incompatible with cistron activity *(Weber, et al.*

, 2007) . *4. 2 Methylation and Obesity* High DNA methylation variableness has been determined as causative factor of fleshiness. This epigenetic form seems to mirror the effects of the exposure to environmental factors *(Woodfine, Huddleston and Murrell, 2011)* .

The emphasis, smoke, physical activity, diet, infections, toxins and drugs are some environmental factors that can change methylation form increasing the hazard for fleshiness, malignant neoplastic disease and other complex diseases. Obesity is a multifactorial upset ensuing from the interaction between environmental and familial factors. The methylation form is

recommended as the molecular mechanism that intercede this interaction *(Woodfine, Huddleston and Murrell, 2011)* . Methylation marker can be examined at booster part or at cistron organic structure. Van Eijk et Al. (2012) detected higher per centum of negative correlativity between booster methylation and cistron activity, while methylation at cistron organic structure (trans) shown mostly per centum of positive correlativity (68. 2 %) between DNA methylation and cistron activity.

The DNA methylation appeared to be dominant at cistron organic structure on healthy persons *(Jjingo, et al. , 2012)* . There are groundss that maternal diet during gestation has effects on foetus methylation form.

Maternal folate inadequacy may take in loss of DNA methylation along cistrons organic structure of the fetus, which affects cistron look increasing the hazard to develop fleshiness. *4. 3 HT $_{2C}$ R and Obesity* Serotonin (5-hydroxytryptamine, 5-HT) is a monoamine neurotransmitter involved in transition of emotion, slumber, appetency and temper *(Schloss and Williams, 1998)* . The 5-hydroxytryptamine web consists of 14 G protein coupled receptors (GPCRs) and a 5-hydroxytryptamine transporter (5-HTT or SERT) . The 5-hydroxytryptamine receptors are classified at seven single categories, from 5-HT1 to 5-HT7, which are divided harmonizing their construction and operation *(Hoyer, Hannon and Martin, 2002)* .

Surveies on the 5-hydroxytryptamine synthesis and metamorphosis have shown the indispensable function of the neurotransmitter in ordinance of appetency and organic structure weight. Research data provide groundss exemplifying the opposite association between the degree of 5-

hydroxytryptamine in the encephalon and nutrient consumption *(Lam, et al. , 2010)* . When the degree of 5-hydroxytryptamine is increased the appetency is reduced and the antonym. The 5-HT $_{2C}$ R (receptor) , one of the 14 5-hydroxytryptamine receptor subtypes, takes portion in the intracellular transition of the neurotransmitter in the nervous system *(Quilter, et al. , 2012)* .

The look of the 5-HT $_{2C}$ Roentgen has been detected in legion encephalon countries. The 5-HT $_{2C}$ Roentgen has been established as the lone 5-hydroxytryptamine receptor that its familial lack leads at appetite mental unsoundness ensuing in hyperphagia and fleshiness. Thus, has been recognised the indispensable function of the 5-HT $_{2C}$ Roentgen in the transition of appetency and organic structure weight *(Quilter, et al. , 2012)* . Surveies on 5-HT $_{2C}$ Roentgen smasher mice have shown the development of hyperphagia (augment of frequence and continuance of repast) and fleshiness *(Holmes, Gallic and Seckl, 1997)* . Both pharmacological and familial surveies illustrate the 5-HT $_{2C}$ R as an indispensable medium of serotonin effects on nutrient consumption *(Lam, et al. , 2008)* .

The 5-HT $_{2C}$ Roentgen seems to be involved at weight addition during antipsychotic intervention. Atypical major tranquilizers such as Clozaril and olanzapine addition 5-HT $_{2C}$ R hostility as binding at the receptor, and doing weight addition. On the other manus, the agonists of 2C receptor appeared to diminish nutrient consumption. Several 5-HT $_{2C}$ R agonists tested as selective anorexic drugs. The BVT.

X, a extremely selective 2C receptor agonist, illustrates significantly decrease of insatiate appetency in hyperphagic and corpulent mice *(Lam, et al. , 2008)* . Another recent survey implicates the 5-HT $_{2C}$ Roentgen with appetite transition demoing that a polymorphism at the booster of 5-HT $_{2C}$ R cistron is correlated with weight addition through antipsychotic intervention *(Reynolds, Zhang and Zhang, 2003)* . Finally, promoter part of 5-HT $_{2C}$ R cistron was studied from Yuan et Al. (2000) in order to place possible polymorphous venue associated with fleshiness. The experimental consequences (3 venue of individual nucleotide permutation and 1 dinucleotide repetition polymorphous venue) were consistent with the research suggestion that polymorphisms in 5-HT $_{2C}$ R booster are correlated with the change of written text degrees of the cistron, which play a cardinal function in the transition of appetency and organic structure weight. *4. 4 5-HT $_{2C}$ R booster methylation and Fleshiness* Promoter part of a cistron has a cardinal function in the cistron look.

The booster sequence offers the initial binding venue of the RNA polymerase and other written text factors in order to originate the written text. Possible mutants at booster sequence or variableness of methylation degrees have important impact on cistron look degrees. The hypothesis of the current research was based on this rule. Given that, the 5-HT $_{2C}$ R has an indispensable function in ordinance of appetency and organic structure weight, and that unnatural methylation degrees at 5-HT $_{2C}$ R booster can impact the cistron look, the current hypothesis was based on strong theoretical groundss to analyze a possible correlativity between these two

rules. As it mentioned above, the methylation degrees at the boosters in their bulk illustrate negative correlativity with cistron look degrees.

So, the expected result of this experiment would be hypermethylated boosters in the corpulent topics. On the other, the thin topics (command sample) were supposed to hold normal scope of the methylation degrees. Previous surveies associating to several mental upsets have examined the effects of methylation at serotonin receptors in the diseases onset.

Falkenberg et Al. (2010) detected an association between the methylation of 5-HT $_{2A}$ R booster and neuropsychiatric upsets. The same survey showed that possible polymorphisms in booster sequence can impact the methylation position at the booster. Carrard et Al. (2011) in bend showed that increased methylation degrees at booster part of 5-HT $_{1A}$ R cistron are important correlated with the development of schizophrenic disorder and dipolar upset. Sing the fleshiness Zhao et Al. (2013) have made a research proposing that methylation position at booster of the 5-hydroxytryptamine transporter (SLC6A4) cistron is correlated with fleshiness. Significant correlativity was observed between hypermethylation of the SLC6A4 booster and the fleshiness prevalence within the tried topics.

All the aforesaid examples lead at the suggestion that any defect of 5-hydroxytryptamine 2C receptor, like booster polymorphism, may do the decrease of cistron activity taking to hyperphagia and fleshiness. Consequently, the hypermethylation of booster can expose the same result in the appetite transition. *4.*

5 Methylation analysis The methyltransferase enzyme that presents in higher eucaryotic beings, transportations a methyl group at the C, which bind at the 5th C of the nitrogen-bearing base. Methylation patterns occur at a C that accompany by G (CpG dinucleotide) . The Deoxyribonucleic acid methylation is heritable molecular mechanism that involved at cell distinction and cistron look. CpG methylation is presented in low degree in normal tissues. Change of methylation degrees in cistrons is related with a scope of diseases such as malignant neoplastic disease, fleshiness, diabetes and several mental upsets.

In order to understand the impact of methylation mechanism on multifactorial upsets and to look into the potencies of this epigenetic form on intervention, several methylation analysis methods have been developed. For the demands of current experiment a sensitive, dependable and quantitative technique was used. COBRA is a quantitative method with high truth, which is able to specify CpG methylation degrees at specific cistron venue presented in little sum of genomic DNA.

The combined bisulfite limitation analysis method comprises by three cardinal molecular techniques, bisulfite alteration, polymerase concatenation reaction and limitation enzyme digestion. Through the bisulfite alteration each unmethylated C is converted to uracil, while methylated Cs maintained as C. The deaminization of C through bisulfite intervention converts the C to uracil. In this point the PCR elaboration comes to finish the transition of unmethylated Cs by changing each U to thymine. The combination of bisulfite alteration and PCR leads to the formation of new limitation enzyme sites or may retain the initial methylation dependant sites, such as CGE‡CG

that recognised by BstUI enzyme. Therefore, limitation enzyme digestion can uncover CpG methylation sites. The methylation degrees are described from the per centum of the comparative sums of digested and undigested sites of the tried sequence. The choice of limitation enzymes plays a cardinal function in the concluding result.

The BstUI (CGE‡CG) enzyme is normally used on methylation analysis. The limitation site of the BstUI contains two CpG dinucleotides. Consequently, merely if both CpGs are methylated will be retained the acknowledgment site of the enzyme. In order to uncover different methylation degrees in the same limitation site, a 2nd enzyme can be used incorporating a individual CpG at its acknowledgment site. In the current experiment the usage merely of BstUI reduces the methylation degree that can be detected. The 2nd enzyme, HaeIII (GGE‡CC) , acts as control enzyme, was used to find the bisulfite transition along the tried sequence. As every experimental method and bisulfite intervention appears several restrictions.

The uncomplete transition of unmethylated Cs can take at false positive methylation degrees. Bisulfite alteration marks and converts the 5-methylcytosine, while 5-hydroxymethylcytosine, a different DNA alteration form, blocks the transition retaining unmethylated C as C. Consequently, the being of 5-hydroxymethylcytosine may besides look false positive methylation degree. The bisulphite transition requires high temperature and long incubation clip, factors that can do DNA debasement. Increased bisulfite concentration may besides take to DNA debasement. The EZ DNA Methylation-Gold Kit ^{Thulium} , which was used for the bisulfite alteration,

reduces significantly the incubation clip at 3 hours from 16-20 hours. This kit provides transition efficaciousness & gt ; 99 % guaranting the transition of unmethylated Cs while retains methylated Cs. Concurrently, this kit besides appears & gt ; 75 % DNA recovery.

4. 6 Research restrictions The reported survey appears few restrictions. The methylation form and cistron look are wholly dependent on the type of tissue that is examined *(Falkenberg, Gurbaxani, Unger and Rajeevan, 2010)* . The methylation form was analysed in cheek epithelial cells and non in the primary involved tissues.

Consequently, the methylation degrees at 5-HT $_{2C}$ R booster in cheek cells may exemplify an indirect contemplation of fleshiness pathogenesis. Nevertheless, the detected methylation degrees at 5-HT $_{2C}$ R booster, as present in cheek cells, can be used as a paradigm in order to understand the methylation mechanism at 5-HT $_{2C}$ R booster as existed in other tissues such as encephalon. The 2nd restriction is the absence of cistron look informations in order to compare with the methylation degrees at corpulent and non-obese control topics.

The tight agenda of the undertaking limited the optimization of the experimental procedure. Finally, the chief restriction was the little sample size. Practical troubles and the limited funding restricted the enlisting and analysis of larger sample. *4. 7 Conclusion and Future research* In drumhead, this experimental undertaking tried to look into the methylation degrees at the booster of 5-HT $_{2C}$ receptor cistron and how they related with fleshiness, comparing methylation degrees and BMI between corpulent and thin control

topics. The experimental consequences showed 0 % of methylation at the two analysed CpGIs in both corpulent and control samples.

This result contrasts with the initial hypothesis, which relied on strong theoretical background. Consequently, including the mentioned limitations the initial hypothesis should be investigated once more. For a hereafter survey the size of voluntaries should be significantly increased and besides generalised to females topics. Sing the chief disadvantage of COBRA, this is the sensing of CpG methylation merely at specific venue and non at the whole mark sequence, is inferred that a different methylation analysis method should be used in order reveal the entire methylation degrees at booster part of 5-HT $_{2C}$ R cistron. The mark sequence of the 201 bp at 5-HT $_{2C}$ R booster contains 16 CpG dinucleotides (12 individual CpG sites and 2 braces of CpGs) where merely four of them were tested in this survey.

The pyrosequencing check is a quantitative method based on bisulfite alteration, PCR and sequencing check. The bisulfite intervention together with PCR, as already mentioned at COBRA method, converts unmethylated C to thymine and retain the methylated C as it is. Then the modified sequence is analysed from pyrosequencing package. The methylation form of each CpG is analysed as an unreal T/C individual nucleotide polymorphism *(Zhao, Goldberg and Vaccarino, 2013)* . Therefore, the methylation position of the 16 CpGs can be detected and quantified. The bisulfite pyrosequencing can supply high quality of methylation informations, for both corpulent and control topics, capable to uncover possible correlativity between methylation at 5-HT $_{2C}$ R booster and fleshiness. MentionsAbbott, A.

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solution 1. 0 protocol as provided from the maker. *Appendix III* : EZ DNA Methylation-Gold kit protocol as provided from the maker. *Appendix IV* : Short IPAQ format every bit provided to the voluntaries. *Appendix V* : Demographic, diet and clinical questionnaire as provided to the voluntaries. *Appendix VI* : Measures and Answers that characterise each voluntary.