Openventio PUBLISHERS

ISSN 2377-8385

OBESITY RESEARCH

Open Journal

| March 2016 | Volume 2 | Issue 4 |

www.openventio.org

OBESITY RESEARCH

Openventio PUBLISHERS

ISSN 2377-8385

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Openventio PUBLISHERS

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-e006

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Volume 2 : Issue 4
Article Ref. #: 1000OROJ2e006

Article History
Received: December 30th, 2015
Accepted: December 31st, 2015
Published: January 4th, 2016

Citation
Brown CW. Diabetes prevention in African-American communities. *Obes Res Open J.* 2016; 2(4): e15-e16. doi: 10.17140/OROJ-2-e006

Diabetes Prevention in African-American Communities

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Type-2 Diabetes (T2D) has reached epidemic proportions with the number of people being diagnosed almost tripling in recent generations.[1] T2D is the most common form of diabetes and accounts for about 90% to 95% of all cases of diagnosed diabetes.[1] T2D currently affects 10% of Americans, predictions are that by 2050, 1 of every 5 Americans will be affected by T2D.[2] Sadly, the incidence of T2D is also among the rise in children and has been predicted to become the "new epidemic" in with an overall increase of 33% in incidence and prevalence during the past decade.[3] T2D is a major health threat that not only has devastating health and psychosocial effects, but will also significantly impact health care expenses in the future.[4-6]

The prevalence of T2D is higher in African-Americans and other ethnic minorities. African-Americans are 1.7 times more likely to have diabetes than non-Hispanic whites.[2] In addition, African-Americans are more likely to suffer complications from diabetes, such as end-stage renal disease and lower extremity amputations.[7] African-Americans are 3.4 times more likely to have end stage renal disease and 3.5 times likely to be hospitalized for lower limb amputations as compared to non-hispanic whites.[7]

Obesity and physical inactivity are two major risk factor for diabetes. African-Americans in general, and African-American women specifically, have higher obesity and physical inactivity rates. African-American women have the highest obesity rate and are the least active of any other ethnic or gender groups in the United States.[7] More than half of African-American women (58%) are overweight or obese as compared to one-third of the adult population.[1] African-American women's obesity is almost twice that of Caucasian women and significantly higher than that of Mexican-American women (44.9%).[1] Consequently, this domino effect of obesity and diabetes contributes to the growing gap in health disparities.

Despite the fact that T2D is preventable, T2D continues to be on the rise in African-American communities. African-Americans and other ethnic minorities continue to carry the heavy burden of the devastating effects of diabetes. Studies have shown that lifestyle interventions have been successful in preventing or delaying the onset of diabetes.[2,8] Diabetes prevention interventions have proven to work outside of research settings. In particular, the National Diabetes Prevention Program (NDPP) has been proven to work in community based settings. The program is a lifestyle change program designed for individuals who have prediabetes or are at risk for diabetes and has been proven to reduce their risk of developing diabetes by 58%. Prediabetes is a condition in which blood glucose levels are higher than normal, but not high enough for a diagnosis of diabetes, Ironically, despite the high prevalence of diabetes and prediabetes among African-Americans and other minorities, little is known about successful interventions for this population.[2,9,10] Thus, there is a real need to add to the body of knowledge information about successful diabetes prevention targeting African-Americans and other ethnic minorities.

IMPLICATIONS FOR FURTHER RESEARCH

Future diabetes prevention research studies that focus on African-Americans and other ethnic minorities is much needed. African-Americans, Native Americans and Latinos, and Native Americans are disproportionately at risk for diabetes, but underrepresented in studies of

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-e006

diabetes prevention programs.[9,10] African-American women carry the highest rates for physical inactivity and obesity, which places them at an extremely higher risk for T2D. Accordingly, evidence-based diabetes prevention and obesity interventions are critically needed for African-American women.[9] Community translations of NDPP have shown promise to be effective in African-Americans and other ethnic minority communities. However, there is limited evidence documenting the effectiveness of cultural adaptations. Sanders Thompson et al affirm that the lack of information about participants' responses to cultural elements is a "lost opportunity" to gain deep understandings of program acceptance and behavioral change.[11] Hence, there is still a need to develop and or modify health interventions and prevention programs that responsive to the cultural practices of the subcultural groups targeted.

CONCLUSION

In conclusion, T2D is one of the most serious health challenges facing the African-American community. The health, psychosocial and economic impact of T2D is well documented. What is needed is a greater understanding of the impact of awareness, early risk assessment and prevention measures, specifically in the African-American community. With almost 89 million Americans having prediabetes, prevention plays a critical role in combating the devastating impact of diabetes on African-Americans and other ethnic minority populations.

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ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-116

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Volume 2 : Issue 4
Article Ref. #: 1000OROJ2116

Article History
Received: November 16th, 2015
Accepted: November 24th, 2015
Published: November 24th, 2015

Citation
Bégin S, Guénard F, Tchernof A, et al. Impact of *NMT1* gene polymorphisms on features of the metabolic syndrome among severely obese patients. *Obes Res Open J*. 2015; 2(4): 101-110. doi: 10.17140/OROJ-2-116

Impact of *NMT1* Gene Polymorphisms on Features of the Metabolic Syndrome among Severely Obese Patients

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ABSTRACT

Introduction: N-myristoyltransferase (NMT) is implicated in myristoylation, required for biological activities of several proteins. Its gene N-myristoyltransferase 1 (*NMT1*) has been found to be overexpressed and hypermethylated in Visceral Adipose Tissue (VAT) of severely obese individuals with Metabolic Syndrome (MetS+) *versus* without (MetS-).
Objective: The aim of this study was to verify the associations between *NMT1* gene polymorphisms Single Nucleotide Polymorphisms (SNPs) and metabolic complications among obese subjects.
Methods: Associations between SNPs and determinants of MetS were tested with 1752 obese participants undergoing a bariatric surgery. The effect of selected SNPs on methylation, and correlation with expression levels of *NMT1* were verified in subgroups.
Results: Rs2239921 was significantly associated with systolic (p=0.03) and diastolic (p<0.0001) blood pressures. Rs2239923 was associated with plasma High Density Lipoprotein-Cholesterol or HDL-Cholesterol (HDL-C) levels (p=0.05), while rs2269746 was associated with Low Density Lipoprotein-Cholesterol or LDL-Cholesterol (LDL-C) (p=0.006) and Total-Cholesterol (Total-C) levels (p=0.004). Rs1005136 (p=0.03), rs8066395 (p=0.03) or rs2157840 (p=0.04) were associated with plasma concentrations of C-Reactive Protein (CRP). Phenotype-associated SNPs were associated with *NMT1* methylation levels of six CpG sites. *NMT1* methylation levels of one CpG site, cg10755730, correlated with gene expression levels (r=0.57; p=0.04).
Conclusion: These results suggest that the presence of *NMT1* SNPs is associated with altered plasma lipid levels as well as with increased inflammation markers and blood pressure among severely obese patients.

KEYWORDS: Obesity; Epigenetics; Genomics; Lipids; Insulin; Myristoylation.

ABBREVIATIONS: BMI: Body Mass Index; CRP: C-Reactive Protein; CVD: Cardiovascular disease; DBP: Diastolic Blood Pressure; HDL-C: High Density Lipoprotein-Cholesterol; HMZ: Homozygote; HTZ: Heterozygote; HWE: Hardy-Weinberg Equilibrium; LDL-C: Low Density Lipoprotein-Cholesterol; MetS: Metabolic Syndrome; *NMT1*: N-myristoyltransferase 1; SBP: Systolic Blood Pressure; SNP: Single Nucleotide Polymorphism; TG: Triglycerides; T2D: Type 2 Diabetes; VAT: Visceral Adipose Tissue; DNA: Deoxyribonucleic acid; NCEP-ATPIII: National Cholesterol Education Program Adult Treatment Panel III.

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ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-116

INTRODUCTION

Obesity is a major health problem worldwide due to the imbalance created by physical inactivity and the abundance of food, which are influenced by intrinsic and extrinsic factors.[1] This condition brings about significantly increased risk of Cardiovascular diseases (CVD) and Type 2 Diabetes (T2D)[2] through a constellation of risk factors characterizing the metabolic syndrome (MetS).[3] However, nearly 20% of severely obese individuals remain exempt of MetS.[3] This could be partly explained by genetic factors and epigenetic modifications. Epigenetics modulate gene expression and can also be involved in the process leading to increased comorbidities in certain obese individuals, as they are both transmissible and influenced by the environment.[4] It encompasses strong mechanisms that regulate gene expression without affecting the Deoxyribonucleic acid (DNA) sequence.[5] Also, epigenetic marks, such as CpG dinucleotides, are more frequent in promoter regions and first exons of specific genes,[6] consistent with a major role for methylation in the regulation of gene expression. We have previously shown that methylation levels are associated with MetS[7] and its features.[8]

The *NMT1* gene, located on chromosome 17, encodes the enzyme N-myristoyltransferase 1 (*NMT1*). It catalyzes the irreversible reaction of myristoylation,[9] which makes a specific covalent linkage between myristic acid, a 14-carbon saturated fatty acid, and the NH2-terminal glycine of a protein.[10] For many proteins, myristoylation is essential for stability and functions, such as protein-protein interactions, membrane attachment and cellular localization.[11] Myristoylation may also be involved in the regulation of gene transcription through modification of DNA-binding proteins.[11] *NMT1* has not been previously associated with MetS, but as it induces important protein modifications,[12] and is largely linked with disease state,[13] it may potentially be related to obesity comorbidities. Previous work by our team observed *NMT1* as being differentially expressed and methylated between subjects with MetS (Mets+) and without MetS (MetS-).[14,15] The aim of the current study was thus to test for specific associations between *NMT1* genetic variations and metabolic complications among severely obese patients. Afterwards, the association between the phenotype-associated SNPs and methylation sites, as well as between these sites and expression levels, were tested to further understand the potential underlying mechanisms relating *NMT1* to obesity-related complications. We therefore, hypothesize that genetic variations/SNPs in *NMT1* are associated with features of MetS and that *NMT1* gene methylation levels are associated with obesity-related metabolic complications, based on the role of methylation in the regulation of gene expression.[16]

MATERIAL AND METHODS

Subjects Selection

A total of 1752 severely obese men (N=545) and women (N=1207) were recruited among patients undergoing a bariatric surgery (biliopancreatic diversion with duodenal switch) since June 2000 at the Québec Heart and Lung Institute (Québec City, Québec, Canada). The surgical protocol has been described elsewhere.[17] Body weight, height, waist girth and resting Systolic Blood Pressure (SBP) and Diastolic Blood Pressures (DBP) were measured before the surgery using standardized procedures. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. C-reactive protein (CRP) concentrations in plasma were measured with an high-sensitivity C-reactive protein (hs-CRP) immunoassay using a monoclonal antibody coated with polystyrene particles.[18] The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATPIII) diagnosis criteria[19] were used to determine the presence of MetS. All subjects included in the study provided a written informed consent and Université Laval ethics committee approved the study. Tissue specimens were obtained from the Biobank of the Institut universitaire de cardiologie et de pneumologie de Québec according to institutionally-approved management modalities.

Genotyping

Genomic DNA was extracted from the blood buffy coat using the GenElute Blood Genomic DNA kit (Sigma, St Louis, MO, USA). Based on differential expression and methylation of the *NMT1* gene in a previous dataset,[14] 11 tag Single Nucleotide Polymorphisms (tSNPs) spanning promoter (2 kb), coding and intronic regions of the *NMT1* gene in addition to the 3' gene region (2 kb) were selected for analysis using the Tagger selection algorithm of the Haploview software (pairwise tagging, R2 ≥0.80).[20] This strategy allowed covering 100% of the genetic variability of the common polymorphisms (MAF≥1%) at the *NMT1* locus in the Caucasian population (CEU HapMap). In addition, rs2157840 SNP was included in this study due to its close localization to the cg00693004 CpG site previously found to be differentially methylated between MetS+ and MetS- subjects.[17] We thus ended up with a set of 12 SNPs analyzed in the current study. SNPs were genotyped, using the QuantStudio™ 12K Flex OpenArray® AccuFill™ system (Applied Biosystems) and analyzed with TaqMan Genotyper v1.3 (Life Technologies).

DNA Methylation Analysis

A second subgroup of 32 obese individuals chosen among the larger group was used for DNA methylation analysis, including the 14 subjects (MetS+, N=7; MetS-, N=7) selected for gene expression profiling.[14] The 18 obese individuals (MetS+, N=9; MetS-, N=9) added to the 14 initially studied[15] were selected to fulfil initial selection criteria[14] and to represent extremes of the MetS diagnosis criteria spectrum. Genomic DNA extraction was achieved from 200 mg of visceral adipose tissue (VAT) using the DNeasy Blood and Tissue kit (QIAGEN, Mississauga, Ontario, Canada). Bisulfite conversion was conducted on 1 µg of DNA, and quantitative DNA methylation analysis was carried out at the McGill University and Génome Québec Innovation Centre (Montreal, Canada). Infinium HumanMethylation450 BeadChip (Illumina, San Diego, CA, USA) were processed ac-

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-116

cording to the manufacturer's instructions. The BeadChips interrogate more than 485000 methylation sites at single-nucleotide resolution. Methylation data were visualized and analyzed with the GenomeStudio software version 2011.1 (Illumina Inc.) and the methylation module. Methylation levels (beta values; β) were estimated as the ratio of signal intensity of the methylated alleles to the sum of methylated and unmethylated intensity signals of the alleles (β value= C/(T+C)). We applied internal control probe pairs for data correction (background subtraction and normalization). CpG sites located within the *NMT1* locus and promoter region were extracted using the GenomeStudio Methylation Module, thus leading to a total of 26 CpG sites analyzed in this study. *NMT1* was identified as differentially methylated [(Diff score=341.86) based on the differentially methylated CpG site cg00693004 (chr17:43151433, according to genome build 37).

Gene Expression

Expression data for the *NMT1* gene presented here were retrieved from a previous study aimed at the identification of differentially methylated genes between MetS+ and MetS- severely obese men. The protocol leading to the identification of *NMT1* as being differentially expressed between severely obese (BMI >40 kg/m^2) MetS+ and MetS- men has been previously described.[14] Briefly, 14 severely obese men with and without MetS, who did not take any medication to treat MetS components, were matched as closely as possible for age, BMI and smoking status. Characteristics of individuals sorted by MetS groups are available elsewhere.[14] Even if it is not listed in the tables due to the large number of genes discovered, this investigation led to the determination of *NMT1* gene as being overexpressed within VAT of the MetS+ *versus* MetS- subsets (1.35-fold; p=0.04).

Statistical Analysis

At first, phenotypic differences between the MetS+ and MetS- groups were tested for the entire cohort. Gene expression microarray analysis was conducted using unpaired Student's t-test, and differentially expressed genes were compared between MetS+ and MetS- groups. Hardy-Weinberg Equilibrium (HWE) was verified. For SNPs showing a frequency of rare homozygotes below 5%, homozygotes for the rare allele were merged to heterozygotes for statistical analysis (6 SNPs: rs41484746, rs8066395, rs10491142, rs2239921, rs2239922 and rs2269746). The Generalized Linear Model (GLM) procedure was used to test associations between SNPs and MetS components, with adjustments for age, sex, BMI and medication to treat MetS features when appropriate. When a significant SNP effect was identified, all pairwise comparisons among genotype groups were performed using least square means and Student's t-tests. Pearson correlation co-efficients were computed to assess the relationship between methylation, expression and MetS components. P-values were calculated for all associations and were considered to be statistically significant if the P-value was less than 0.05. Statistical analysis were performed with Statistical Analysis Software (SAS) software version 9.3. Phenotypic data are presented as mean±SD.

RESULTS

Cohort Description

From the study sample of 1752 severely obese subjects, 1745 patients were classified as being MetS+ (n=1428) or MetS- (n=317 or 20.19%). All characteristics shown in Table 1 were significantly different between MetS+ and MetS- groups, except BMI and plasma CRP levels. The MetS+ group had a higher mean age and showed significantly higher values for waist girth, fasting glucose, Triglycerides (TG), total-C/HDL-C ratio, SBP and DBP. The MetS- group showed significantly higher plasma concentrations for total-C, LDL-C and HDL-C.

Identification of *NMT1* SNPs

Regarding the 12 SNPs, 8 were intronic and 4 were exonic, 2 of them being in the 3' untranslated region (exon 12) (Table 2). Exonic SNPs rs2239922 and rs2239923 were synonymous variations. These 12 tSNPs were further genotyped in the whole cohort of 1752 participants. Genotype distribution and HWE p-values are also shown in Table 2.

Association of *NMT1* SNPs with features of the MetS

Associations between tSNPs, and plasma fasting glucose, lipid, CRP levels as well as blood pressure, taking into account the confounding effects of age, sex, BMI and medication, were tested. Significant associations were observed for rs2239921, rs2239923, rs2269746, rs8066395, rs2157840 and rs1005136 (Table 3). Carriers of the rare allele for rs2239921 showed lower systolic (p=0.03) and diastolic (p<0.0001) blood pressures than wild-type homozygotes. Rare homozygotes for rs2239923 displayed elevated HDL-C levels compared to the other genotype groups (p=0.05), while carriers of the wild-type genotype for rs2269746 demonstrated higher levels of LDL (p=0.006) and total-C (p=0.004) than carriers of the rare allele. Homozygotes of the wild-type allele for rs1005136, rs8066395 and rs2157840 displayed elevated plasma CRP levels (p=0.03, p=0.03 and p=0.04 respectively). Two trends were also found, the first for association between rs12449933 with elevated plasma TG concentrations (p=0.08; 2.15±1.58 *vs.* 1.82±1.08 *vs.* 1.78±0.94 mmol/L) in rare homozygotes, and the second in carriers of the wild-type genotype for rs41484746 with higher BMI (p=0.06; 51.8±8.7 *vs.* 50.8±8.2 kg/m^2) and waist girth (p=0.06; 140.8±17.9 *vs.* 139.1±16.8) than carriers of the rare allele.

Gene Methylation and Expression Analysis

With the perspective of trying to better understand the mechanism underlying the association between *NMT1*'s SNPs and CVD risk factors, we investigated gene methylation and expression levels. Between genotype group differences in methyl-

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ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-116

	All	MetS+	MetS-	P value
Number of Subjects (% male)	1752(31.1)	1428(33.61)	317(20.19)	—
Age (years)	43.0±10.6	44.0±10.6	38.7±9.8	<0.0001
BMI (kg/m^2)	51.8±8.9	52.0±9.0	51.2±8.7	0.32
Waist girth (cm)	140.6±17.7	141.7±17.4	135.5±18.5	<0.0001
CRP (mg/l)	11.04±9.12	11.22±9.16	10.42±9.00	0.05
Fasting glucose (mmol/L)	6.53±2.32	6.85±2.41	5.11±0.98	<0.0001
Lipid profile (mmol/L)				
TG	1.83±1.08	1.98±1.12	1.17±0.37	<0.0001
Total-C	4.70±0.95	4.68±0.98	4.80±0.82	0.05
LDL-C	2.67±0.83	2.65±0.84	2.80±0.75	0.02
HDL-C	1.24±0.35	1.18±0.32	1.49±0.37	<0.0001
Total-C/HDL-C	4.03±1.29	4.17±1.34	3.33±0.74	<0.0001
Blood Pressure (mmHg)				
SBP	139.0±17.1	140.0±17.3	134.6±15.6	0.0006
DBP	83.8±11.5	84.0±11.7	83.1±10.3	0.04

Values are presented as mean±SD.
Abbreviations: MetS: Metabolic Syndrome; BMI: Body Mass Index; CRP: C-Reactive Protein; TG: triglycerides; Total-C: Total Cholesterol; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SD: Standard Deviation.

Table 1: Subjects characteristics.

SNPs	Number of genotypes	Common HMZ (WT)	HTZ	Rare HMZ	Localization[a]	Other designation[b]	Region	MAF	HWE P values
rs12449933	1737	1060	587	90	chr17:43137201	c.-1497C>T	Intron 1	0.22	0.46
rs41484746	1740	1458	268	14	chr17:43142664	c.131+3836A>G	Intron 1	0.09	0.66
rs8066395	1716	1108	547	61	chr17:43147582	c.131+8754A>G	Intron 1	0.19	0.52
rs2157839	1734	772	765	197	chr17:43151400	c.132-7612T>C	Intron 1	0.33	0.72
rs2157840	1731	530	843	358	chr17:43151473	c.132-7539G>T	Intron 1	0.45	0.50
rs1005136	1725	550	834	341	chr17:43164246	c.385+226G>T	Intron 3	0.44	0.44
rs10491142	1738	1308	400	30	chr17:43167863	c.386-3190G>C	Intron 3	0.13	0.93
rs2239921	1735	1585	149	1	chr17:43170907	c.386-146C>T	Intron 3	0.04	0.19
rs2239922	1740	1492	234	14	chr17:43175906	c.870C>T	Exon 7	0.08	0.15
rs2239923	1733	850	728	155	chr17:43176804	c.916C>T	Exon 8	0.30	0.96
rs1053733	1730	585	829	316	chr17:43183028	c.*21G>A	Exon 12 (3'-UTR)	0.42	0.46
rs2269746	1740	1535	198	7	chr17:43185023	c.*2016C>A	Exon 12 (3'-UTR)	0.06	0.82

[a]Position according to genome build 37. [b]Reference sequence : NM_021079.
Abbreviations: SNP: Single Nucleotide Polymorphism; HMZ: Homozygote; WT: Wild-type; HTZ: Heterozygote; MAF: Minor Allele Frequency; HWE: Hardy-Weinberg Equilibrium.

Table 2: Genotype distribution and localization of selected *NMT1* SNPs.

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-116

	rs12449933				rs41484746		
	Means[a]				Means		
Phenotypes	Common HMZ (WT)	HTZ	Rare HMZ	*P* values[b]	Common HMZ (WT)	Rare allele carrier	*P* values
Number of subjects (N)	1055	584	90	—	1450	281	—
BMI (kg/m^2)	51.8± 8.8	51.5±8.0	50.8±10.1	0.54	51.8±8.7	50.8±8.2	0.06
Waist girth (cm)	140.7±17.9	140.7±17.0	138.0±20.3	0.25	140.8±17.9	139.1±16.8	0.06
CRP Protein (mg/l)	10.88± 8.32	10.53±8.31	11.67±9.11	0.87	10.96±8.39	10.01±8.10	0.49
Fasting glucose (mmol/l)	6.53±2.33	6.52±2.29	6.85±2.47	0.42	6.53±2.33	6.61±2.33	0.45
Lipid profile (mmol/l)							
TG	1.82±1.08	1.78±0.94	2.15±1.58	0.08	1.83±1.06	1.82±1.09	0.22
Total-C	4.68±0.93	4.69±0.95	4.84±0.87	0.49	4.69±0.92	4.70±1.01	0.97
LDL-C	2.67±0.83	2.69±0.82	2.72±0.75	0.36	2.67±0.81	2.71±0.88	0.51
HDL-C	1.23±0.32	1.24±0.38	1.25±0.48	0.54	1.24±0.36	1.22±0.28	0.81
Total-C/HDL-C	4.01±1.22	4.03±1.42	4.19±1.30	0.27	4.02±1.32	4.01±1.10	0.90
Blood pressure (mmHg)							
SBP	138.9±16.6	138.8±16.7	137.5±17.8	0.33	138.7±16.6	139.4±17.3	0.56
DBP	83.8±11.5	83.9±10.7	82.6±11.3	0.41	83.9±11.3	82.96±10.8	0.30

	rs2239921			rs2239923			
	Means			Means			
Phenotypes	Common HMZ (WT)	Rare allele carrier	*P* values	Common HMZ (WT)	HTZ	Rare HMZ	*P* Values
Number of subjects (N)	1578	148	—	846	725	155	—
BMI (kg/m^2)	51.7±8.5	52.0±9.7	0.66	51.5±8.6	51.7±8.4	52.5±9.5	0.30
Waist girth (cm)	140.5±17.5	141.9±20.1	0.18	140.8±17.5	140.3±18.0	141.0±17.8	0.81
CRP Protein (mg/l)	10.72±8.25	11.83±9.47	0.73	10.64±8.42	10.96±8.38	11.57±7.93	0.27
Fasting glucose	6.56 ±2.35	6.31±2.04	0.69	6.52±2.27	6.53±2.30	6.62±2.56	0.87
Lipid profile (mmol/l)							
TG	1.81±1.00	1.93±1.64	0.45	1.82±1.06	1.82±1.09	1.80±1.02	0.85
Total-C	4.68±0.94	4.76±0.89	0.81	4.70±0.94	4.69±0.93	4.64±0.92	0.74
LDL-C	2.67±0.82	2.74±0.81	0.93	2.69±0.82	2.67±0.83	2.59±0.79	0.96
HDL-C	1.24±0.36	1.21±0.29	0.71	1.24±0.37	1.23±0.35	1.26±0.27	0.05
Total-C/HDL-C	4.01±1.30	4.15±1.23	0.47	4.06±1.43	4.02±1.16	3.82±1.02	0.40
Blood pressure (mmHg)							
SBP	138.9±16.7	138.1±17.6	0.03	139.3±17.3	138.7±16.6	136.9±13.9	0.82
DBP	83.9±11.1	81.2±11.5	<0.0001	83.9±11.4	83.4±11.1	84.2±10.5	0.34

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Phenotypes	rs2269746 Means			rs8066395 Means		
	Common HMZ (WT)	Rare allele carrier	*P* values	Common HMZ (WT)	Rare allele carrier	*P* values
Number of subjects (N)	1527	204	—	1102	605	—
BMI (kg/m^2)	51.6±8.5	52.3±9.2	0.39	51.8±8.9	51.5±8.0	0.50
Waist girth (cm)	140.5±17.7	140.9±18.0	0.88	140.4±18.0	140.8±17.2	0.46
CRP Protein (mg/l)	10.79±8.34	10.89±8.48	0.29	11.21±8.52	10.03±7.99	0.03
Fasting glucose	6.56±2.34	6.40±2.22	0.93	6.61±2.39	6.40±2.20	0.26
Lipid profile (mmol/l)						
TG	1.83±1.10	1.77±0.83	0.97	1.85±1.15	1.78±0.91	0.58
Total-C	4.71±0.94	4.56±0.89	0.004	4.69±0.93	4.71±0.94	0.70
LDL-C	2.69±0.82	2.56±0.81	0.006	2.67±0.82	2.70±0.83	0.93
HDL-C	1.24±0.36	1.21±0.32	0.61	1.23±0.34	1.25±0.37	0.84
Total-C/HDL-C	4.03±1.29	3.99±1.32	0.18	4.05±1.33	3.99±1.23	0.64
Blood pressure (mmHg)						
SBP	138.7±16.7	140.1±17.4	0.12	138.6±16.7	139.2±16.8	0.61
DBP	83.6±11.1	84.8±11.7	0.42	83.7±11.1	83.9±11.3	0.78

Phenotypes	rs2157840 Means				rs1005136 Means			
	Common HMZ (WT)	HTZ	Rare HMZ	*P* values	Common HMZ (WT)	HTZ	Rare HMZ	*P* values
Number of subjects (N)	527	838	357	—	547	829	340	—
BMI (kg/m^2)	52.2±9.2	51.5±8.2	51.5±8.6	0.26	52.1±9.2	51.5±8.2	51.5±8.7	0.23
Waist girth (cm)	140.9±17.8	140.1±17.6	141.2±17.8	0.63	140.6±17.8	140.2±17.8	141.3±17.7	0.59
CRP Protein (mg/l)	11.78±8.59	10.36±8.29	10.57±8.13	0.04	11.81±8.60	10.21±8.23	10.63±8.19	0.03
Fasting glucose	6.55±2.30	6.54±2.38	6.50±2.22	0.72	6.53±2.31	6.55±2.38	6.47±2.12	0.43
Lipid profile (mmol/l)								
TG	1.86±1.25	1.79±0.95	1.86±1.06	0.78	1.86±1.25	1.79±0.96	1.86±1.02	0.85
Total-C	4.67±0.93	4.69±0.95	4.73±0.93	0.98	4.67±0.94	4.69±0.93	4.75±0.94	0.86
LDL-C	2.64±0.81	2.68±0.84	2.72±0.80	0.74	2.64±0.82	2.68±0.83	2.73±0.80	0.43
HDL-C	1.23±0.29	1.23±0.35	1.25±0.44	0.38	1.23±0.29	1.23±0.35	1.26±0.44	0.68
Total-C/HDL-C	3.98±1.11	4.03±1.32	4.08±1.46	0.77	3.99±1.17	4.02±1.30	4.09±1.47	0.71
Blood pressure (mmHg)								
SBP	139.0±16.4	138.5±17.0	139.5±16.5	0.92	138.8±16.3	138.7±17.2	139.0±16.3	0.99
DBP	84.1±11.0	83.4±11.3	84.0±11.3	0.67	83.9±11.0	83.5±11.2	83.9±11.4	0.68

[a]Values presented (means±SD) are untransformed and unadjusted. [b]*P* values obtained are adjusted for the effect of age, sex, BMI and medication use except for BMI and waist girth which were adjusted for age and sex.
Abbreviations: HMZ: Homozygote; WT: Wild-type; HTZ: Heterozygote; N: Number; BMI: Body Mass Index; CRP: C-Reactive Protein; TG: Triglycerides; Total-C: Total cholesterol; LDL-C: Low Density Lipoprotein Cholesterol; HDL-C: High Density Lipoprotein Cholesterol; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; SD: Standard Deviation.

Table 3: Significant genotype differences identified between *NMT1* SNPs, subjects' characteristics, CRP, fasting glucose, lipid profile and blood pressure.

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-116

ation and expression levels were tested only for CVD risk factor-associated SNPs (rs2239921, rs2239923, rs2269746, rs8066395, rs2157840 and rs1005136). Methylation levels were available for 26 CpG sites distributed within or near the gene; the precise locations are presented in Table 4. Six CpG sites were significantly associated with phenotype-associated SNPs. There was an association between methylation and gene expression levels only for cg10755730 (r=0.574; p=0.04; Table 5).

DISCUSSION

Only a few studies on *NMT1* are related to obesity or T2D[21] with most studies related to cancer.[22] The tyrosine-kinases c-Src family, which includes important oncogenesis-related molecules,[23] was identified among its main substrates.[24] For this reason, it represents a therapeutic target for cancer treatment,[22] and has drawn a lot of attention recently. Moreover, known *NMT1* substrates are kinases, phosphatases and G-proteins,[21] involved in numerous signal-transduction cascades.[10] *NMT1* is thus implicated in several metabolic processes, from cell development to apoptosis.[25] In a transcriptomic experiment, we observed that the *NMT1* gene was overexpressed in VAT of MetS+ obese patients, so it was compelling to examine the associations between variations in this gene and metabolic complications of obesity. For instance, the reaction of myristoylation involves myristic acid, a saturated fatty acid,[10] which has been associated with the Western high-fat diet and related disease conditions.[26] Additionally, lipid-modified proteins may be integrated in the initiation of atherosclerosis.[27] A link between variants of this gene and obesity-related comorbidities is of interest. Moreover, an analysis of sequences coding for novel proteins potentially associated with MetS showed that they had numerous myristoylation sites,[28] suggesting that this type of modification can affect their activity. In this study, we reveal associations between genetic variations of the *NMT1* gene and components of MetS.

Considering that *NMT1* was found to be differentially expressed and methylated in VAT of MetS+ *vs.* MetS- obese men, associations of *NMT1* SNPs with obesity-related metabolic complications (BP, CRP, glucose and lipid levels) were tested. Six gene polymorphisms of *NMT1* (rs2239921, rs2239923, rs2269746, rs8066395, rs2157840 and rs1005136) were associ-

SNP	cg16594296 Promoter	cg10755730 Promoter	cg21452443 Promoter	cg24354954 Promoter	cg07481784 Promoter	cg21554013 Promoter	cg02077631 Promoter	cg21132931 Promoter	cg22356484 Exon 1	cg06208294 Intron 1	cg00405568 Intron 1	cg09377882 Intron 1	cg09214551 Intron 1
rs2239921	0.80	0.39	0.47	0.57	0.71	0.06	0.21	0.38	0.55	0.62	0.63	0.53	0.59
rs2239923	0.12	0.03	0.31	0.45	0.81	0.44	0.14	0.51	0.78	0.60	0.77	0.98	0.34
rs2269746	0.76	0.28	0.51	0.99	0.34	0.83	0.20	0.74	0.72	0.44	0.56	0.93	0.17
rs8066395	0.93	0.41	0.90	0.48	0.50	0.63	0.67	0.82	0.04	0.68	0.79	0.76	0.59
rs2157840	0.15	0.57	0.37	0.19	0.12	0.68	0.18	0.78	0.89	0.97	0.45	0.46	0.43
rs1005136	0.15	0.57	0.37	0.19	0.12	0.68	0.18	0.78	0.89	0.97	0.45	0.46	0.43

SNP	cg03287877 Intron 1	cg08860622 Intron 1	cg00693004 Intron 1	cg04013970 Intron 1	cg02888886 Intron 3	ch_17_1184801R Intron 3	cg17942929 Intron 3	cg05349016 Intron 3	cg16080654 Exon 8	cg24136288 Exon 12 (3'-UTR)	cg22542420 Exon 12 (3'-UTR)	cg11583751 Exon 12 (3'-UTR)	cg05322982 Exon 12 (3'-UTR)
rs2239921	0.21	0.57	0.71	0.34	0.34	0.83	0.24	0.11	0.04	0.77	0.92	0.40	0.32
rs2239923	0.36	0.54	0.04	0.38	0.28	0.93	0.37	0.38	0.17	0.98	0.14	0.08	0.91
rs2269746	0.39	0.41	0.07	0.73	0.98	0.35	0.74	0.40	0.52	0.004	0.67	0.34	0.83
rs8066395	0.71	0.78	0.07	0.90	0.82	0.81	0.51	0.32	0.92	0.39	0.26	0.92	0.72
rs2157840	0.36	0.86	<0.0001	0.13	0.17	0.62	0.33	0.07	0.20	0.02	0.0004	0.41	0.71
rs1005136	0.36	0.86	<0.0001	0.13	0.17	0.62	0.33	0.07	0.20	0.02	0.0004	0.41	0.72

P values for associations were obtained from a subset of 14 obese subjects (7 MetS+ and 7 MetS-). CpG sites positions according to genome build 37.

Table 4: Association of phenotype-associated SNPs with gene methylation levels.

CpG site ID	Localization[a]	Region	Correlation coefficient[b]	*P* value	Number of individuals tested
cg10755730	chr17:43138257	Promoter	0.574	0.04	14
cg22356484	chr17:43138772	Exon 1	-0.079	0.81	13
cg00693004	chr17:43151433	Intron 1	0.277	0.36	14
cg16080654	chr17:43176852	Exon 8	-0.428	0.14	14
cg24136288	chr17:43183150	Exon 12 (3'UTR)	-0.008	0.98	14
cg22542420	chr17:43183176	Exon 12 (3'UTR)	-0.112	0.71	14

[a]CpG sites positions according to genome build 37. [b]Pearson's r correlation coefficient.

Table 5: Correlation of gene methylation with gene expression levels for SNPs-associated CpG sites in a subset of 14 severely obese subjects.

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-116

ated with obesity-related phenotypes. Our results suggest that *NMT1* is affecting inflammatory markers and blood pressure, as well as the plasma lipid profile. Thus, it contributes to the inter-individual variability observed in metabolic complications among obese patients. Although, no other study has considered *NMT1* as a candidate gene for the MetS. The involvement of *NMT1* in a genetic syndrome has, however, been demonstrated through the example of the N-myristoylated mutant form of the protein SHOC2 leading to the Noonan-like syndrome.[29] Whereas the wild-type protein is not myristoylated, a missense mutation in the SHOC2 gene results into its myristoylation, leading to the disorder. As proposed by Martin and coll,[25] this suggests that it is possible for other proteins that are not normally myristoylated to undergo this modification under certain circumstances and lead to damaging health conditions. Conversely, myristoylation can induce protection, such as in the case of cardiac ischemia-reperfusion injury where this protein modification protects against oxidation.[30]

To our knowledge, the present study is the first to present data suggesting a potential role for *NMT1* in lipoprotein and cholesterol metabolism, and it is particularly relevant as plasma lipid alterations are major elements of obesity and associated health risk factors.[31] Gene methylation and expression levels were thus examined to better understand the link between *NMT1* gene SNPs and MetS risk factors. Also, SNPs may influence gene methylation and expression levels.[32] Further, analysis were conducted with the six phenotype-associated SNPs and all of them were found to be significantly associated with at least one of the 26 CpG sites selected. This result suggests that *NMT1* SNPs affect gene methylation levels. Among the six significant CpG sites, only one (cg10755730) was significantly correlated with *NMT1* gene expression. The SNP rs2239923 was significantly associated with this methylation site (p=0.03), so it may be associated with *NMT1* gene expression. The link of *NMT1* phenotype-associated SNPs with gene methylation levels of CpG sites indicates a potential mechanism affecting gene expression and protein activity. Additional studies are needed to confirm the present findings and to better understand how *NMT1* SNPs, gene methylation and expression levels are linked to obesity and MetS.

Besides the absence of studies reporting associations between *NMT1* gene SNPs and plasma lipid levels, a possible link between *NMT1* and insulin has been put forward.[21,33,34] It is thus tempting to speculate that a possible mechanism relating *NMT1* to obesity and MetS might involve insulin. First, the known substrates of *NMT1* and insulin are indicated to be similar.[21] King, et al. has also suggested that *NMT1* is regulated by insulin, because the protein activity was observed to be inversely proportional to insulin levels in plasma.[21,34] As well, it has been reported that the insulin receptor can be myristoylated.[33] Afterwards, the observation that *NMT1* is overexpressed in MetS+ *vs.* MetS- obese subjects is in agreement with a possible insulin resistance associated with MetS. The demand of myristoylation by myristoylated proteins can modulate gene expression, like it is demonstrated in tumorigenesis.[35] Taken together, these observations would suggest that the differential expression of the *NMT1* gene between MetS+ and MetS- groups might be coming from an altered insulin pathway/function, and affect any of its constituents that need to be myristoylated. Indeed, insulin is considered to play a key role in the pathogenesis of the MetS,[36] even if it is not fully understood. Insulin resistant adipose tissue with limited expandability and lipid storage capacity eventually leads to systemic insulin resistance, involving of course other important sites of glucose uptake such as skeletal muscle, due to excessive postprandial non-esterified fatty acid spillover to non-adipose tissues and inflammatory mechanisms.[37] Hyperglycemia and dyslipidemia eventually emerge as major consequences of these alterations.[37] Similar mechanisms may also partly apply to hypertension which is related to obesity and insulin resistance.[38]

The present results support the potential role of *NMT1* in MetS. Nevertheless, this study is based on associations that need to take into account some potential limitations. First, gene methylation and expression in VAT were measured on a relatively small number of subjects. However, these analyses were only preliminary to find a potential mechanism relating gene variations to their associations with phenotypes. Additionally, for gene methylation and expression, VAT samples in their entirety were used for analyses, meaning that all cell types such as endothelial cells, fibroblasts and macrophages were included in addition to adipose cells. Between subjects differences in tissue composition could affect the results. Measurements on isolated cell fractions in future studies are needed. The results presented here were obtained by testing samples from severely obese individuals. This condition is known to modulate systemic inflammation[39] which, may also have altered the effects of *NMT1* SNPs.

CONCLUSION

Knowing that *NMT1* is overexpressed and hypermethylated in VAT of MetS+ compared to MetS- obese patients, the current study reveals the associations between SNPs within this gene and obesity-related metabolic complications. Specifically, SNPs of *NMT1* are associated with an altered lipid profile as well as with increased inflammatory marker levels and blood pressures. Additionally, the data related to gene methylation and expression levels suggest potential mechanisms linking *NMT1* gene variations to MetS risk factors.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests. AT receives research funding from Johnson & Johnson Medical Companies for studies unrelated to the present publication.

ACKNOWLEDGEMENTS

The authors acknowledge the inestimable collaboration of surgeons Frédéric-Simon Hould, Stéfane Lebel and Picard Marceau

from the Québec Heart and Lung Institute Bariatric Surgery Team, who have also sampled adipose tissue for this project and contributed to the organization of the clinical setup needed to perform their studies. Authors express their gratitude to Christine Racine and Caroline Gagnon from the Québec Heart and Lung Institute biobank. The authors also thank Catherine Raymond, Alain Houde for sample management and technical assistance. M.C.V. is Canada Research Chair in Genomics Applied to Nutrition and Health recipient. This work was supported by a grant-in-aid from the Heart and Stroke Foundation of Canada.

AUTHORS' CONTRIBUTIONS

SBégin wrote the article; SBégin and FG completed the statistical analyses; MCV, AT, YD and LP established study design; SBiron, OL, LB and SM recruited patients, collected clinical data and samples; SBégin and MCV have principal liability for final content.
All authors read and approved the final manuscript.

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Research

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Volume 2 : Issue 4
Article Ref. #: 1000OROJ2117

Article History
Received: November 16th, 2015
Accepted: December 14th, 2015
Published: December 14th, 2015

Citation
Hajek P, McRobbie H, Snuggs S, Peerbux S, Myers Smith K, Miler JA. Effects of protein load prior to the main meal of the day: a pilot trial. *Obes Res Open J*. 2015; 2(4): 111-116. doi: 10.17140/OROJ-2-117

Effects of Protein Load Prior to the Main Meal of the Day: A Pilot Trial

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ABSTRACT

Background: High protein diets increase satiety and may decrease energy intake. Many overweight people overeat in the evening. We hypothesized that ingesting protein prior to the evening meal may limit successive calorie intake and generate weight loss.
Aims: To explore whether protein pre-load before the evening meal will lead to weight loss compared to eating as usual.
Methods: 129 adults with a Body Mass Index (BMI) ≥25 reporting eating large evening meals were randomized to either consume a 20 g protein bar 30 minutes before their evening meal daily for two weeks (Protein pre-loading (PP) arm) or not (No protein pre-loading (NP) arm). Hunger ratings were recorded, immediately prior to each evening meal. Participants returned at the end of weeks one and two to provide their weight and rating of hunger and any changes in evening food consumption since baseline.
Results: There was no significant difference in weight loss between the study arms (Week1 PP: -0.13 kg, [SD=0.74] *vs.* NP: -0.06 kg, [SD=0.75], not significant (NS); Week2 PP: +0.06 kg, [SD=0.82] *vs.* NP: -0.005 kg, [SD=0.82], NS). Participants in the PP arm reported less hunger before evening meals than those in the NP arm (Week1: 4.97 [SD=0.94] *vs.* 3.72[SD=0.65], $p<.001$; Week2: 4.95 [SD=0.94] *vs.* 3.69[SD=0.71], $p<.001$). They also reported eating less at their evening meals (Week1: 2.59[SD=0.53] *vs.* 2.11[SD=0.54], $p<.001$; Week2: 2.63[SD=0.49] *vs.* 2.10[SD=0.50], $p<.001$).
Conclusion: Consuming 20 g of protein before the evening meal reduced hunger and self-reported food intake in the evening, but had no effect on weight.

KEYWORDS: Weight loss; Protein; Hunger; Randomized-controlled trial.

ABBREVIATIONS: BMI: Body Mass Index; WAP: Weight Action Programme; RDA: Recommended Dietary Allowance; ANOVA: Analysis of variance; GCP: Good Clinical Practice; NS: Not Significant.

INTRODUCTION

Weight management programs often advise dieters to avoid skipping meals.[1,2] The advice seems to be linked to an observation that obese women consume fewer calories in the morning compared to lean women, but consume more calories in the evening.[3] It is not clear whether this implies a causal link between skipping meals and obesity, but it has been proposed that breakfast-skipping and prolonged fasting may lead to an increase in blood insulin levels, which may promote lipogenesis.[4] Another possible causal route would be if the accumulated calorific deficit leads to overcompensation at the next meal.

If it is true that the caloric deficit accumulated by skipping meals generates weight gain due to overeating later, interventions which reduce hunger prior to main meals may provide a weight management benefit.

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ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-117

Protein increases satiety and decreases subsequent energy intake more than the other macronutrients, which is the usual explanation for high protein/low carbohydrate diets leading to greater weight loss than high carbohydrate/low protein diets.[5,6] Apart from effects on satiety, increased thermogenesis[7] and enhanced glycaemic control[8] could also be contributing to this effect.

We tested the hypothesis that consuming protein prior to the evening meal reduces appetite and subsequent food intake. This was a 'proof-of-principle' exploratory study to inform a possible future trial with a larger sample and longer follow-up.

MATERIALS AND METHODS

Participants

Participants were recruited through local advertising and from a pool of service users who enquired about joining free local weight management courses (Weight Action Programme (WAP))[9] Participants were included if they had a Body Mass Index (BMI) ≥25 and if they reported that their largest meal of the day was the evening meal. Exclusion criteria were: age<18 years, diagnosis of diabetes, and allergies to nuts.

Study Design

This was a randomized controlled trial with two arms. Participants in the experimental arm were asked to eat a protein bar 30 minutes before their evening meal (Protein pre-loading (PP)) and rate their hunger immediately prior to their evening meal for two weeks. Participants in the control arm were asked to rate their hunger only (No protein pre-loading (NP)). The study used commercially available protein bars (Body Build, Boots Pharmacy, UK). The bars are stated by the manufacturer to provide 20 g of protein per bar. The bars provide 154 kcals and 3.5 g of saturated fat, 11 g of carbohydrates and 3 g of dietary fiber. They were purchased from Boots pharmacy. The bars were selected to provide a sufficient dose of protein to generate the hypothesized effect while trying to keep the daily protein intake reasonably close to the Recommended Dietary Allowance (RDA), which is around 60 grams per day.[10]

Procedures

Prospective participants were mailed study information prior to the baseline session. At the baseline session, eligibility criteria were checked and informed consent and baseline weight were collected. The study was approved by London City & East Research Ethics Committee (REC number 09/H0703/114).

Participants in both conditions were asked to monitor their hunger immediately before their evening meal (the main meal of the day for all participants). In addition to this, those in the PP condition were provided with a one-week's supply of protein bars to eat 30 minutes prior to their evening meal each day.

Both groups received an identical explanation of the study. It stated that the effects of hunger-monitoring and protein-pre-load prior to evening meals on caloric consumption during the meal are not known. They were also told that the study was investigating how practicable these two interventions are, how well clients adhere to them and whether they have any effect on weight in people who make no other changes to their lifestyle or daily routines.

Participants were asked not to change any of their usual routines, and not to go on a diet, or take any other steps to lose weight over the next two weeks. They were invited to attend the WAP programme immediately after completion of the study.

Two further sessions took place one and two weeks after the baseline session. At Week one, participants were weighed and asked to complete ratings of the procedures and report their adherence to them. PP participants were provided with a second batch of bars and both groups were asked to continue with the hunger monitoring exercise. At Week two, final weights and ratings were collected.

Participants were paid £10 for attending each session, contingent on adhering to the study procedures for at least 6 of the 7 days.

Measures

The same set of Omron Body Fat Scale BF400 was used with each volunteer on all three occasions.

The pre-meal hunger was rated on a 10-point scale ranging from 1='Starving' to 10='full to the point of feeling sick'. Participants were given a card, on which to record their hunger rating (Figure 1).

Front

Hunger rating card 1

Name: **Date:**

Please rate every day how hungry you feel (using the rating scale on the back on the back of the card) immediately before you eat your evening meal.

	Rating 1-10
Day 1	
Day 2	
Day 3	
Day 4	
Day 5	
Day 6	
Day 7	

Back

10 = Stuffed to the point of feeling sick
9 = Very uncomfortably full, need to loosen your belt
8 = Uncomfortably full, feel stuffed
7 = Very full, feel as if you have overeaten
6 = Comfortably full, satisfied
5 = Comfortable, neither hungry nor full
4 = Beginning signals of hungry
3 = Hungry, ready to eat
2 = Very hungry, unable to concentrate
1 = Starving, dizzy, irritable

Figure 1: The hunger rating card.

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-117

The ratings of the previous week's experience included the following: Participants rated on a 3-point scale (More than before, Same as before, Less than before) the amount of food they had eaten during the day over the previous week and separately if the amount of food they had eaten at their evening meals had changed since starting the study. Participants were also asked to score how helpful they found the study procedures and how easy it was to follow them on a scale of 1-5 (1=not very helpful; 5=very helpful; and 1=very difficult; 5=very easy). Adherence to study procedures was recorded.

Randomization

The randomization list was generated by an independent researcher not involved in the study. Participants were sequentially allocated an opaque envelope which assigned them to one of the two conditions.

Sample Size

The awareness of taking part in a weight loss study together with the hunger monitoring exercise was expected to generate a small weight loss of some 0.5 kg. Increasing this to 1.5 kg would indicate a potentially useful effect. From data on previous WAP attendees, we estimated the average weight of participants as 94 kg (SD=18.7). To have 85% probability of detecting a difference of 1 kg ($p<.05$, one-tailed test) 52 participants would be needed in each arm. The study aimed to randomize 130 participants.

Data Analysis Plan

The weight change in the two groups and subjective ratings of hunger and amount of food consumed were compared by analysis of variance (ANOVA). Any significant differences between the study groups at baseline were entered as covariates. If the number of people who did not attend the session at Week two differed between the two study arms, an Intention-to-treat analysis would be performed with the assumption that study drop-outs lost no weight (i.e. baseline weight would be carried forward). If there were no difference in proportion of study drop-outs between the two groups, the per-protocol analysis would be used including only participants who provided 2-week weight data. This would prevent a risk of the imputed data from drop-outs masking a real study effect.

Ethics and Risk Assessment

The study was approved by London City & East Research Ethics Committee (REC number 09/H0703/114) and conducted in compliance with the principles of the World Medical Association (WMA) Declaration of Helsinki and ICH Good Clinical Practice (GCP). Participants signed informed consent and study records were kept confidential.

The trial was not expected to pose any risks to participants. The protein bars were standard bars available for over-the counter purchase in pharmacies and health food shops; and the study participation was expected to possibly generate a small weight loss.

RESULTS

129 participants were randomized, of whom 118 completed the study. 11 participants dropped out (did not attend appointments), 8 in the PP arm and 3 in the NP arm.

Table 1 shows baseline characteristics of the sample. The sample had the usual characteristics of people seeking help with weight loss in East London, i.e. they were mostly women in their 40 s (age range 19-68 years), with about half belonging to ethnic minorities.

	PP(N=65)	NP(N=64)	Difference
Age(SD)	42.98(11.50)	45.22(12.36)	NS
Weight(SD)	93.13(17.03)	91.14(16.14)	NS
% Women	80%	89.1%	NS
BMI(SD)	34.04(5.77)	33.69(5.73)	NS
In paid employment	52(80%)	49(77%)	NS
Educated to degree level or equivalent	26(40%)	30(47%)	NS
White British	28(43%)	33(52%)	NS
Entitled to free prescriptions	23(37.50%)	21(32.80%)	NS
Smokers	8(12.30%)	7(10.90%)	NS
Heart disease	2(3.2%)	3(4.7%)	NS
Concurrent medication	39(60.9%)	34(54%)	NS

Table 1: Baseline characteristics of the sample.

There were no significant differences between the study arms in any of the baseline characteristics reported above.

106 (82%) participants followed the study procedures on at least 12 of the 14 study days (49 in PP and 57 in NP arms). Both groups showed good adherence to hunger card completion in Week one, but in Week two, completion decreased somewhat in the PP group and, increased in the control group. In the PP condition, the adherence to the protein bar task decreased somewhat from Week one to Week two but this difference did not reach statistical significance (see Table 2).

Table 3 shows the weight change in the two study arms. The PP arm lost about 0.04 kg more than the control group, but the difference was not statistically significant.

The Table includes participants who provided complete data. The 'intention to treat' analysis with baseline weight carried forward (for participants who dropped out) yields very

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-117

	PP (N=60, Wk 1; N=57, Wk2)		NP (N=63, Wk 1; N=61, Wk 2)		
Hunger cards	**Mean**	**SD**	**Mean**	**SD**	***p* value**
Days hunger cards completed (Week 1)	6.77	0.65	6.75	0.93	.89
Days hunger cards completed (Week 2)	6.56	1.34	6.84	0.93	.20
Protein bars	Week 1 (N=57)		Week 2 (N=57)		*p* value
Days protein bar consumed (PP arm)	6.54	0.91	6.33	1.07	.12

Table 2: Adherence to study procedures.

	PP (N=61 at Wk1 and N=56 at Wk 2)	NP (N=63 at Wk 1 and 61 at Wk 2)	Difference
Week 1 kg (SD)	-0.14(0.77)	-0.06(0.76)	NS
Week 2 kg (SD)	+0.06(0.82)	-0.005(0.82)	NS
	PP(N=56)	NP(N=61)	
Overall kg (SD)	-0.10(1.08)	-0.06(1.03)	NS

Table 3: Weight change from baseline.

similar results (weight loss of 0.13 *vs.* 0.06 kg in week one and a gain of 0.05 kg *vs.* loss of 0.005 kg in week two (PP *vs.* NP), NS, and overall weight loss of 0.08 kg *vs.* 0.06 kg in PP and NP, respectively, NS).

Table 4 shows the hunger ratings in the two study arms. The PP group reported significantly less hunger prior to the evening meal in both weeks.

	PP		NP		Difference
	Mean	SD	Mean	SD	
Week 1	4.97	0.94	3.72	0.65	*p*<.001
Week 2	4.95	0.94	3.69	0.71	*p*<.001

*Note that higher values indicate lower hunger.

Table 4: Effect of protein bars on hunger ratings prior to evening meal.

Protein bars also significantly reduced retrospective ratings of food consumption in the evening over the previous week (see Table 5). Those in the PP arm also tended toward reporting reduced eating during the day compared with NP arm, but these differences did not reach statistical significance.

Table 6 shows ratings of the ease of use and helpfulness of the two interventions. Participants in the NP arm found the study procedure easier to follow in the first week than those in the PP arm, but by the end of the second week this difference was no longer significant. Although, the PP group tended towards higher ratings of helpfulness of the intervention compared with the NP group, the difference was not significant.

DISCUSSION

The main finding of this study is that the protein pre-load significantly reduced ratings of pre-meal hunger (see Table 4) and also the self-reported amount of food eaten at the evening meals (see Table 5). This however had no effect on weight (there was virtually no weight change in either study arm, see Table 3). This was despite good adherence to study procedures (see Table 2) which were rated as reasonably easy to follow (see Table 6).

The study results provide further indirect evidence contradicting the hypothesis that hunger generates overeating leading to weight gain. We studied the effect of skipping meals on weight change previously using a more direct approach, looking at weight changes over the Ramadan fast.[11] During four weeks of Ramadan, religious Muslims do not eat during the day and only begin eating in the evening by which point they feel very hungry.[12] Literature search suggested that it is in fact not known whether Ramadan fasting generates weight loss or weight gain.[10] Only a small number of studies were identified, which used small samples, mostly of students and pregnant women, and reported inconsistent results: one found weight gain,[13] three reported weight loss,[14-16] and five found no significant weight change.[12,17-20] In order to investigate this further we took weight measurements in 202 Ramadan observers at baseline, at the end of Ramadan, and a month later. Ramadan generated a weight loss of about 1 kg, which was re-gained within the next four weeks.[10]

Correlational studies that link weight gain and being overweight with skipping meals,[21,22] have led to weight management programmes commonly suggesting that skipping meals undermines weight loss or generates weight gain, while regular spacing of food intake can help.[1-2,23-25] This is probably incorrect. Our two studies suggest that reducing hunger prior to the evening meal does not generate any reduction in overall calorie intake, and that skipping of meals may in fact generate a modest weight loss. The results of the present study however are only tentative and need to be interpreted with caution.

The study had several limitations. Participants

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-117

	PP (N=61 Wk1, N=57 Wk2)		NP (N=63 Wk1, N=61 Wk2)		
	Mean	SD	Mean	SD	p value
Change in eating over week 1 (evening)	2.59	0.53	2.11	0.54	<.001
Change in eating over week 1 (daytime)	2.21	0.45	2.10	0.47	.16
Change in eating over week 2 (evening)	2.63	0.49	2.10	0.50	<.001
Change in eating over week 2 (daytime)	2.22	0.50	2.05	0.50	.07

*Note: higher values indicate eating less than before.

Table 5: Effect of protein bars on ratings of eating over the previous week.

	PP (N=61 Wk1, N=57 Wk2)		NP (N=62 Wk1, N=60 Wk2)		
	Mean	SD	Mean	SD	Difference
Ease following procedure (Week 1)	3.92	1.17	4.35	0.82	*p*=.02
Did you find it helpful? (Week 1)	3.73	0.94	3.51	0.93	*p*=.18
Ease following procedure (Week 2)	4.02	0.95	4.32	0.70	*p*=.06
Did you find it helpful? (Week 2)	3.89	0.88	3.61	0.97	*p*=.10

Table 6: Ease of use and helpfulness ratings.

were only followed up over a period of two weeks, the sample size was relatively modest, and the protein preload was limited to 20 g of protein. It could be argued that an effect could possibly emerge over a longer period of time (to allow the experimental manipulation to exert its influence), with a larger sample (to detect smaller effects), and/or with a higher protein pre-load (to enhance the pre-loading effect). The trial lasted for only two weeks, but the weight in both groups remained stable, with no sign of the two groups diverging during the second week. It is also unlikely that an effect would emerge with a larger sample size, because there was virtually no difference between the two study arms. Regarding the size of the protein pre-load, the dose was sufficient to generate a significant reduction in hunger, which was the key hypothetical mediator of any effect.

Another potential limitation is that the trial was not blinded, but expectations seem to have played little role as the weight in both study arms remained stable throughout the two week period. The lack of blinding could have in theory, influenced the subjective ratings, although this too seems unlikely. The PP arm reported a reduction in subsequent food intake and the protein pre-load seems to have indeed generated this as otherwise the additional 154 kcal consumed with the protein bar would induce a small weight gain. The reduction however seems to have been limited to maintaining the habitual overall calorie intake.

In conclusion, our two studies together suggest that increased hunger prior to evening meal does not generate weight gain, and reduction of hunger prior to evening meal does not generate weight loss. The advice to dieters to space eating episodes regularly throughout the day may have a good health rationale, but it may not contribute to weight loss.

DISCLOSURE

There were no competing interests and the study required no external funding.

AUTHORS CONTRIBUTIONS

PH, HJM, SJS and KEMS conceived the study and contributed to data analysis and study write-up, SJS and KEMS contributed to data collection and data analysis, SP and JAM contributed to data analysis and study write up.

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ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-118

Letter to the Editor

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Volume 2 : Issue 4
Article Ref. #: 1000OROJ2118

Article History
Received: March 10th, 2016
Accepted: March 14th, 2016
Published: March 15th, 2016

Citation
Demerdash HM. Possible mechanisms of insulin resistance in obese subjects. *Obes Res Open J*. 2016; 2(4): 117-118. doi: 10.17140/OROJ-2-118

Possible Mechanisms of Insulin Resistance in Obese Subjects

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Obesity is characterized by excessive triglyceride accumulation in adipose tissue cells (adipocytes); the adipocytes are not just a reservoir for storage of energy in the form of triglyceride, but more importantly act as endocrine cells; they secrete several hormones as leptin and adiponectin, also secrete adipokines as TNF-α, Interleukin 6 (IL-6) and Plasminogen activator inhibitor-1 (PAI-1). In obese subjects, adipocytes release high levels of free fatty acids (FFA) and its metabolites; Diacylglycerol (DAG) and ceramide.[1]

Acquired insulin resistance is associated with obesity. Insulin resistance is classically defined as impaired insulin-mediated glucose disposal in skeletal muscle. There are several mechanisms responsible for insulin resistance in obese subjects, which can be classified into either to activation of inflammatory pathways or changes in lipoproteins and apoprotein concentrations as result of associated dyslipidemias.[1,2]

Those mechanisms include; inflammatory pathways through activation of (IKKβ) (inhibitor of nuclear factor κB) and c-Jun N-terminal kinase 1 (JNK1): They play a role in feedback inhibition of the insulin signaling cascade. Their activation occur by increased secretion of free fatty acids (FFA) and its metabolites, which together adipokines (TNF-α), reactive oxygen species in both liver and adipose tissue. The resultant activation of JNK1 stimulates serine phosphorylation of insulin receptor substrate 1 (IRS-1) with a resultant decline in insulin signaling.[3] The second is inhibition of Peroxisome proliferator-activated receptor gamma (PPARγ) by TNF-α; PPARγ is a nuclear receptor that stimulates enzymes and/ proteins involved in fatty acid esterification and triglyceride synthesis and degradation. Its inhibition decreases triglyceride storage in adipocytes and increases lipid distribution to skeletal muscle and liver which consequently contributes to insulin resistance.[4] Also Leptin and adiponectin under normal conditions promote FA oxidation, lower lipid stores. Those effects are mediated through AMP-activated protein kinase. However; some degree of resistance to each of these adipokines in skeletal muscle develops in obese subjects leading to accumulation of DAG and ceramide, with resultant increase in FFA uptake and decreased oxidation, leading to impaired insulin signaling.[5]

Moreover, hepatocytes and adipocytes are the major sources of apolipoproteins. Apoproteins are the proteins of lipoproteins. They have several functions, for example some are known to regulate lipolytic enzyme activities and lipoprotein uptake into cells. Also some ratios of those apoproteins may be used as indicators of dyslipidemias. In addition, they exert an influence on insulin sensitivity either directly or through lipoproteins.[2]

There are several types of apoproteins:

Apolipoprotein (Apo A) is a major component of high density lipoproteins (HDL-C); apolipoprotein A-I (ApoA1) indirectly modulates insulin sensitivity through their antioxidant and anti-inflammatory action. apoA-I and apoA-II are reported to have incretin-like properties; incretins such as glucagon-like peptide-1 (GLP-1) and the glucose-dependent insulinotropic (GIP), are small peptides secreted from the gut in response to glucose, they stimulate insulin secretion from β-cells of pancreas. In addition, apoA-I and apoA-II both increase insulin secretion under basal as well as high glucose concentrations. In contrast to incretins which

ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-118

stimulate insulin secretion from β-cells only under high glucose concentrations. Also apoA-IV is similar to endogenous incretins, it stimulates insulin secretion from β-cells only under high glucose concentrations.[2,6]

The mechanism of apoA-I and apoA-II in insulin secretion is dependent on the ATP-binding cholesterol transporter, ABCA1, which is expressed on the β-cell surface, which through a series of reactions promote insulin synthesis and secretion. In addition, the effect on insulin resistance may be through decreased FA oxidation in skeletal muscle.[2]

Apolipoprotein (ApoB 100) is the major constituent of very low density lipoprotein (VLDL) and low density lipoprotein (LDL). Insulin decreases ApoB 100 secretion by promoting its degradation in the hepatocyte. Also insulin promotes clearance of circulating ApoB 100 particles by the hepatocytes through low-density lipoprotein receptor (LDLR), LDLR-related protein 1 (LRP1). Consequently, the insulin-resistance is associated with increased concentration and decreased clearance of ApoB100 and LDL-Cholesterol.[7]

Recently it was taken into consideration that ApoB and ApoB/ApoA-I ratio rather than on low density lipoprotein-cholesterol (LDL-C) is considered as early predictors of insulin resistance and reflects the balance of cholesterol transport ; ApoB/ApoA-I ratio (≥1.12 in men and ≥1.0 in women).[6] Apolipoprotein (ApoCIII) is produced by hepatocytes,it modulates the lipoprotein metabolism by inhibiting lipoprotein lipase. Increased expression of ApoCIII results in impaired regulation of pancreatic β-cell function; with increased cytoplasmic free Ca^{2+} concentration, inflammation and hyperglycemia.[8]

Apolipoprotein (Apo E) is one of the most widely studied apoproteins; There are three common isoforms of (Apo) E; (E2, E3, and E4) ApoE3 is the most common isoform with frequency of about 80%, while ApoE4 (12%) and ApoE2 (8%).[9] (ApoE) is plays a major role in lipid and lipoprotein transport. It is mainly involved in the metabolism of dietary lipids and the removal of chylomicron remnants and very low density lipoproteins (VLDL), from the circulation, through binding to LDL-receptor (LDLr). In obesity, associated dyslipidemias is characterized by slow rate of lipolysis, leading to lower and undetectable apoE exchange between lipoproteins fractions. Also increased free fatty acid plasma level, enhances hepatic VLDL and thus VLDL apoE production, with further contribution to insulin resistance and hyperglycemia.[9]

In conclusion there are several hypotheses for the pathogenesis of obesity-associated insulin resistance, including chronic inflammation and its contribution to decreased insulin secretion and dyslipidemia. Moreover, the possible role added by lipoproteins and their associated apoproteins; that help in modulating insulin sensitivity. Better understanding the above mentioned mechanisms would facilitate the development of new pharmacological strategies targeting those pathways that prevent obesity-associated diabetes and its complications.

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OBESITY RESEARCH

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ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-119

Mini Review

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Volume 2 : Issue 4
Article Ref. #: 1000OROJ2119

Article History
Received: March 16th, 2016
Accepted: March 21st, 2016
Published: March 24th, 2016

Citation
Pinto RM, Cominetti C, da Cruz AD. Basic and Genetic Aspects of Food Intake Control and Obesity: Role of Dopamin Receptor D2 TAQIA Polymorphism. *Obes Res Open J*. 2016; 2(4): 119-127. doi: 10.17140/OROJ-2-119

Basic and Genetic Aspects of Food Intake Control and Obesity: Role of Dopamin Receptor D2 TaqIA Polymorphism

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ABSTRACT

Regulation of food intake, energy expenditure and store are strikingly linked to obesity. Homeostatic control of food intake, hunger and satiety involves adipose and gastrointestinal hormones, such as leptin, insulin and ghrelin, which eventually affect neuronal signaling in the hypothalamus arcuate nucleus. On the other hand, hedonic control of food intake relates to substances such as opioids, endocannabinoids, gamma-aminobutyric acid, serotonin and dopamine, which act on the motivation and reward mechanisms. Dopamine is a precursor of noradrenaline and adrenaline and modulates a number of physiological functions, such as appetite, depending on the brain area and the type of receptor stimulated. It has been established as the main neurotransmitter of the hypothalamic reward system. Beyond the homeostatic and hedonic energy balance control, genetic aspects are also tightly involved in obesity pathophysiology. In this context, some Single Nucleotide Polymorphism (SNP) has been linked to common obesity. Here, we highlight the role of the dopamine receptor D2 gene TaqAI polymorphism, which affects the D2 receptor availability and has been associated to obesity. Therefore, the aim of this mini review is to cover basic aspects of food intake, energy balance, dopamine-related aspects, including genetic ones, and the relation with obesity.

KEYWORDS: Dopamine; *DRD2* gene; Genetic polymorphism; Obesity; Nutrigenetics.

ABBREVIATIONS: CNS: Central Nervous System; ARC: Arcuate Nucleus; GI: Gastrointestinal; GRP: Gastrin-releasing peptide; CCK: Cholecystokinin; PYY: Peptide YY; GLP1: Glucagon-like peptide-1; ApoAIV: apolipoprotein AIV; CART: Cocaine and amphetamine-regulated transcript; POMC: proopiomelanocortin; α-MSH: alpha-melanocyte-stimulating hormone; NPY: neuropeptide Y; AgRP: agouti-related protein; PC1: prohormone Convertase 1; GABA: gamma-aminobutyric acid; DA: dopamine; NAc: accubens nucleus; LHA: Lateral Hypothalamic Area; VMH: Ventromedial hypothalamic nucleus; RDS: Reward Deficiency Syndrome; ADHD: Attention Deficit Hyperactivity Disorder; SNP: Single Nucleotide Polymorphism; *NPY*: neuropeptide Y gene; *FTO*: fat mass and obesity-associated gene; *PPAR*: peroxisome proliferator-activated receptor gene; *APOE*: apolipoprotein E gene; *APOA1*: apolipoprotein A1 gene; *PLIN*: perilipin gene; *UCP1*: uncoupling protein 1 gene; *UCP2:* uncoupling protein 2 gene; *INSR*: insulin receptor gene; *ADIPOQ*: adiponectin gene; *IL6*: interleukin-6 gene; *RETN:* resistin gene; GWLS: Genome-Wide Linkage Studies; GWAS: Genome-Wide Association Studies; GIANT: The Genetic Investigation of ANthropometric Traits Consortium; *ANKK1:* ankyrin gene; BMI: Body Mass Index.

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ISSN 2377-8385 Open Journal http://dx.doi.org/10.17140/OROJ-2-119

FOOD INTAKE REGULATION AND OBESITY: HOMEOSTATIC AND HEDONIC CONTROL

Obesity is a multifactorial condition influenced by genetic, endocrine-metabolic, environmental and psychological factors. A delicate balance between three main biochemical and behavioral processes maintains body weight: food intake, energy expenditure control and energy storage control.[1]

Regulation of food intake by the Central Nervous System (CNS) depends on the interaction of a homeostatic component that aims the balance between energy and nutrients, and a hedonic component, which seeks food-associate pleasure (Figure 1).

Homeostatic control of intake depends on the hormonal peripheral signaling produced in response to changes in nutrient concentrations. Leptin and insulin are the main hormonal adiposity signals, and by reaching the CNS trigger mechanisms that promote inhibition of food intake and increased energy expenditure.[2] On the other hand, hunger and satiety sensations are communicated to the CNS by gastrointestinal hormones. During prolonged fasting, the stomach produces ghrelin that acts on the hypothalamus as an orexigenic signal. After food intake, ghrelin concentration falls, giving rise to the secretion of anorectic hormones, such as cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP1).[3,4]

The main targets of peripheral adiposity, hunger and satiety signaling are neurons in the hypothalamus arcuate nucleus (ARC). In this nucleus there are orexigenic neurons that produce neuropeptide Y (NPY) and agouti-related protein (AgRP), in addition to anorectic neurons producing cocaine and amphetamine-regulated transcript (CART) and proopiomelanocortin (POMC), precursor of alpha-melanocyte-stimulating hormone (α-MSH) by the action of prohormone convertase 1 (PC1).[1,5] The melanocortin 4 receptor (MC4R) plays an important role in the intricate hypothalamic appetite control. When leptin binds to its receptor on POMC neurons, α-MSH binds to MC4R, which produces a satiety signal. On the other hand, binding of AgRP to MC4R promotes increased intake.[2,6] Leptin activates the POMC neurons and inhibits AgRP neurons.[2,6,7] A scheme on the interaction of homeostatic intake control is depicted in Figure 2.

In humans, nutrition has not only physiological, but also social and behavioral roles. Food hedonic value is influenced by taste and previous experiences.[4] Intake of highly palatable foods (high in sugar and fat) is able to "deregulate" appetite homeostatic control, perpetuating the stimulus to eat, which causes the intake to be primarily mediated by hedonic and not homeostatic needs.[7] For decisions on food search, initiation and termination of meal to be properly taken, it is necessary the right integration between hypothalamic signals and cortical centers where substances such as opioids, endocannabinoids, gamma-aminobutyric acid (GABA), serotonin and dopamine (DA) act on the mechanisms of motivation and reward.[2]

Endogenous opioids as β-endorphin, enkephalin and dinorphin activate receptors in the accubens nucleus (NAc) disinhibiting orexigenic neurons in the Lateral Hypothalamic Area (LHA). Endocannabinoids impair leptin signaling, and interact with dopaminergic and opioid systems through the activation of CB1 receptors that inhibit melanocortin pathway.[2,7] Only the role of eating facilitator through its action on NPY neurons and consequent blockage of POMC transmission was attributed to GABA.[8] It is now known that GABA released by AgRP neurons is necessary to maintain a minimum level of appetite and the normal regulation of energy balance.[9]

Serotonin promotes satiety by acting directly in the ARC neurons, activating POMC and inhibiting AgRP neurons.

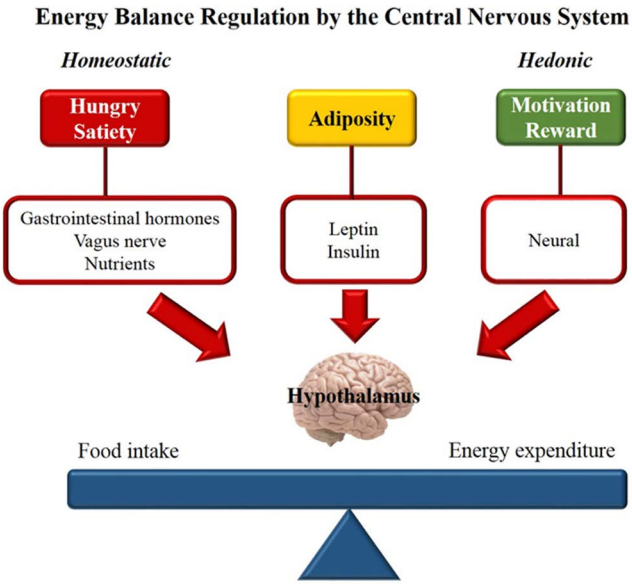

Figure 1: Homeostatic and hedonic control of energy balance.

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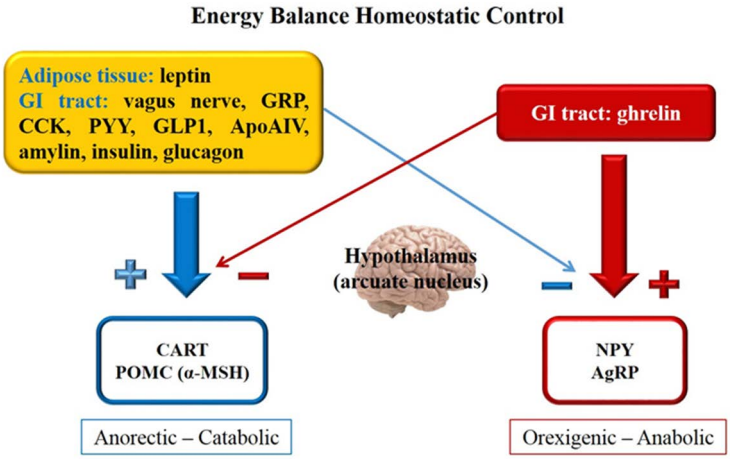

GI, gastrointestinal; GRP, gastrin-releasing peptide; CCK, cholecystokinin; PYY, peptide YY; GLP1: glucagon-like peptide-1; ApoAIV, apolipoprotein AIV; CART, cocaine and amphetamine-regulated transcript; POMC, proopiomelanocortin; α-MSH, alpha-melanocyte-stimulating hormone; NPY: neuropeptide Y; AgRP, agouti-related protein.

Figure 2: Homeostatic regulation of energy balance.

It also inhibits orexins-producing neurons in LHA.[2]

DA is a catecholamine precursor of noradrenaline and adrenaline and is an endogenous neurotransmitter that modulates a number of physiological functions, including behavior, ion transport, vascular tone and blood pressure. Several experimental studies established DA as the main neurotransmitter of the reward system.[10] It is currently considered the "pleasure molecule" or the "anti-stress molecule." Previc[11] stablished the concept of "dopaminergic society" and affirms that high DA concentrations was part of a general physiological adaptation to the increased meat consumption occurred two million years ago. The theory says that the "dopaminergic society" is characterized by high intelligence, personal destiny sense, religious/cosmic concerns and obsession with achieving goals.[11]

Regarding appetite, DA has varying effects depending on the brain area and the type of receptor stimulated. It has anorectic effect when it operates in the ARC, LHA and NAc, but acts as orexigenic in the ventromedial hypothalamic nucleus (VMH).[2] Several studies have related the dopaminergic brain circuits in eating behavior.[7] Animal studies reveal that the consumption of high-sugar or high-fat meals promotes the DA release in NAc.[7,12] The intake of a tasty meal for humans induces the DA release in a magnitude proportional to the meal degree of pleasure.[13]

OBESITY AS PART OF THE "REWARD DEFICIENCY SYNDROME – RDS"

Structures and cortico-limbic-striatal circuits form the brain reward system. Pleasurable stimuli activate this system and lead the individual to seek positive reinforcement of every type, not only food.[14] The brain reward cascade starts in the hypothalamus, where serotonin acts as a neurotransmitter stimulating the enkephalin release, which in turn inhibits GABAergic neurons in the substantia nigra. These GABAergic neurons act in the fine adjustment of DA amount that will be released in the NAc, the brain reward site (Figure 3).[10]

Studies show that low brain DA concentrations relate to greater vulnerability to substance abuse and abnormal behavior. It is known that all addictive drugs, as well as gambling, sex, food and even music promote DA release in the brain reward site.[10] In 1996, the term "Reward Deficiency Syndrome – RDS" was stablished, in order to define hypodopaminergic states-associated behaviors, which predispose to obsessive-compulsive behaviors and to impulsiveness.[15] The following changes are included in the RDS: a) Addictive behaviors: alcoholism, multiple substances abuse, obesity, smoking; b) Impulsive behaviors: attention deficit hyperactivity disorder (ADHD), Tourette's syndrome, autism; c) Compulsive behaviors: abnormal sexual behavior, addiction to gambling and betting; d) Personality disorders: conduct disorder, antisocial personality, aggressive behavior and generalized anxiety.[15]

GENETIC FACTORS RELATED TO OBESITY

Common obesity, also named exogenous obesity, is a complex disease with multifactorial etiology. Pleiotropic genetic syndromes and monogenic diseases account for only 1% of obesity cases.[16,17] The most common forms of monogenic obesity occur due to mutations in genes related to hypothalamic control system of energy balance, as leptin-melanocortin system, which result in changes in the concentration and/or activity of hormones, receptors and enzymes, including leptin and its receptor, POMC, MCR4 and PC1.[18] In addition, there may be mutations in genes affecting the hypothalamus development and therefore, promoting obesity.[6] It is also important to note that obesity may be a central component of several pleiotropic syndromes, such as Alstrom, Albright, Pader-Willi, Bardet-Biedel, Fragile X, among other syndromes.[17]

In complex diseases such as common obesity, it is

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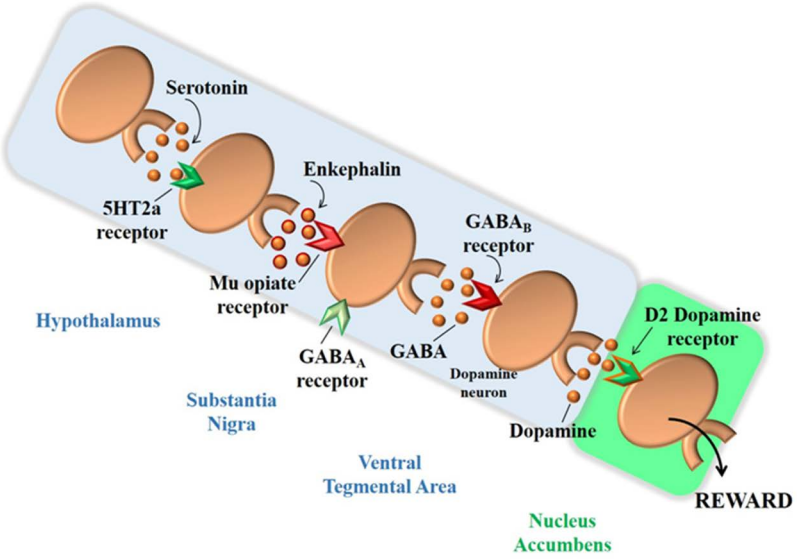

Figure 3: Interaction of various neurotransmitters that forms the "Brain Reward Cascade". Adapted from Blum et al[10]

necessary that genetic factors are associated with a favorable environment for the phenotype emergence. The "thrifty genotype" hypothesis, described by Neel[19] proposes that genetic variations that result in higher capacity to store energy as fat were positively selected in food deprivation times. It is believed that over thousands of years this "thrifty genotype" has perpetuated and was essential in mankind evolution. This theory suggests that genes included in the "thrifty genotype" are responsible for the great ability to accumulate energy as fat, the ability to save energy at critical periods, the capacity to "turn off" non-essential metabolic pathways and to facilitate the intake of large amounts of food whenever they are available.[20] Currently, this same "thrifty genotype" has become disadvantageous, due to the easy access to energy-dense foods and to the low energy expenditure, which could explain the current obesity epidemic.

In 2007 Speakman published the "predation release" hypothesis, as an alternative to the "thrifty genotype" theory.[21] Based on anthropological and epidemiological evidence, genetic screening and experimental research, the theory suggests that the greater skill of lean individuals selected those best adapted to the search for food and to escape from predators; until fire was discovered in the Paleolithic period, and there was a significant increase in body weight over time. The theory attributes this increase in weight not only to the cooking capacity and better palatability of foods, but mainly to the fact that the fire was able to keep out the main predators, reducing energy expenditure. It also suggests that the initial genetic network responsible for low weight and high body performance has been suppressed and lost over the millennia.[20,21]

Common forms of obesity result from the interaction between variations in different genes and a favorable environment. Generally, many studies suggest a strong genetic component in human obesity.[22-27] Studies report that in response to low calorie diets, some individuals lose weight more easily than others, and those carrying the same genotype respond in a similar manner when exposed to the same diet. Researches with monozygotic twins show that heredity accounts for 40 to 70% of inter-individual variation in cases of common obesity.[28] Differences between individuals and their predisposition to weight gain indicate that common variations in genomic DNA sequence, represented mainly by the Single Nucleotide Polymorphisms (SNP), may be responsible for the weight gain.[27,29] However, despite its great importance, the search for genes that raise the risk for obesity has not been easy.[28,30] It is still a challenge for the scientific community to separate the genetic component from the environmental one in the etiology of this disease. Individuals who are more susceptible to accumulating fat can carry risk variants in genes that influence appetite control (*NPY*, *POMC*, *MC4R*, etc.), cellular machinery regulation (*FTO, DRD2*, etc.), lipid metabolism and adipogenesis (*PPAR*, *APOE*, *APOA1*, *PLIN*, etc.), energy expenditure (*UCP1, UCP2*), insulin signaling (*INSR*, etc.) and inflammation (*ADIPOQ*, *IL6*, *RETN*, etc).[18,27]

The polygenic nature of common obesity makes the discovery of risk genes and their variants a challenging task. Different approaches have been developed to elucidate the genetic component of obesity, such as GWLS (Genome-Wide Linkage Studies), that include co-segregation studies of certain chromosomal regions with a trait or disease[30]; analysis of candidate genes involved in plausible physiological pathways; and GWAS (Genome-Wide Association Studies), that track markers throughout the genome to identify associated polymorphisms.[28] Through a meta-analysis of 37 GWLS, Saunders and co-workers concluded that this is not an effective approach to identify genetic variants for common obesity, as

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they did not locate any locus with conclusive evidence.[31]

Studies on candidate genes intended to identify the relation between one or more polymorphisms and a phenotype. In obesity, genes involved in the regulation of food intake, energy expenditure, lipid and glucose metabolism and adipose tissue development have been studied. In addition, genes described in monogenic forms of obesity have been investigated for a possible role in the common obesity genesis.[6] However, replication of most results has been somehow inconsistent, and so the findings of candidate gene studies remain obscure.[32]

In GWAS, unlike in the candidate gene approach, no assumption of the investigated gene function is made. These studies are based on the association of several markers, usually SNP and the approach is particularly useful in common complex diseases, such as obesity and diabetes.[6]

The latest update of "the human obesity gene map", performed by Rankinen and co-workers associated obesity with 253 loci after analysis of 61 GWAS.[33] The number of associations between SNPs and obesity has 127 candidate genes described, and of these, 22 genes are supported by more than five studies. The map shows loci in all chromosomes, except for the Y.[33]

A more recent meta-analysis from GIANT (The Genetic Investigation of ANthropometric Traits) Consortium conducted in adults[34] established 32 loci of susceptibility to Body Mass Index (BMI), several of which were confirmed in French and German children with severe obesity.[35] In 2015, 97 BMI-associated loci were identified in a GWAS with 339,224 subjects (~95.0% of European and ~5.0% of non-European descent) from 125 studies (82 with GWAS and 43 with Metabochip results). From those 97 loci, 56 were novel.[36]

Specifically for the childhood early-onset obesity, studies show that heredity is an important factor.[37,38] A large study of the childhood obesity genetics evaluated 5,530 cases and 8,318 controls through the analysis of 14 scientific papers, and the strong genetic influence in the childhood obesity development was definitely verified.[39] Three novel loci were identified in a meta-analysis with 47,541 children from 33 studies (discovery and replication phases).[40]

GENETIC VARIATIONS IN THE DOPAMINERGIC REWARD SYSTEM: DOPAMINE RECEPTOR D2 GENE (*DRD2*)

Taking into account that DA plays a crucial role in the brain reward circuit and is involved in food behavior, the study of genetic polymorphisms that affect the availability and secretion of DA has been standing out.

The *DRD2* gene is located on chromosomal region 11q22-q23, with about 66,097 base pairs. This gene encodes the D2 subtype receptor, a transmembrane protein that couples to G proteins and inhibits the activity of adenylate cyclase. In an alternative splicing mechanism, the *DRD2* encodes two molecularly distinct protein isoforms – D2S and D2L – which are co-expressed, although the D2L production is favored. These two isoforms differ by the presence of 29 additional amino acids at D2L.[41] Both forms of D2 receptor have distinct physiological functions. The D2L acts mainly in postsynaptic regions while the D2S has a presynaptic self-receiving function.[42] This gene was included in "the human obesity gene map" supported by five candidate genes studies performed only with adults.[33]

DRD2 TAQIA POLYMORPHISM

The *DRD2* is highly polymorphic and there are already many SNPs cataloged and described (Figure 4). However, increasing attention has been given to C32806T SNP (rs1800497), characterized by the exchange of a cytosine for a thymine in a repetitive region in *ANKK1* (gene codifying ankyrin), located downstream of the *DRD2*.[43] This SNP is also known as TaqIA and appears to affect the D2 receptor availability. Variant allele A1 (T) is associated to a reduced metabolic rate of glucose in dopaminergic areas of the human brain, which indicates a low activity of dopaminergic neurons.[44]

Variations in the expression and activity of dopaminergic receptors and in DA release are related to overeating and obesity. Mice with reduced density of *DRD2* receptors in the striatum

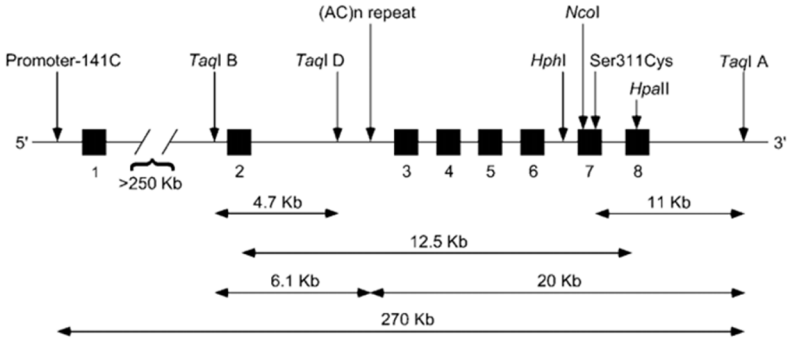

Figure 4: *DRD2* human gene with location of the most studied polymorphisms. Boxes represent exons and lines represent introns. Adapted from Noble, 2003.[44]

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dorsal side, when fed with fat-rich diet gained more weight than those with normal density *DRD2*. The increase in *DRD2* mRNA expression in the nucleus accumbens and in the putamen ventral part of obese mice, when compared with obesity-resistant mice, seems to be a compensatory response to the *DRD2* pathway lower activation induced by overeating.[45,46]

Studies have suggested that obese individuals may have decreased DA availability through a mechanism of dopaminergic D2 receptors downregulation in the dorsal and lateral striatum.[47-49] Drugs blocking D2 receptors increase the appetite, and those that raise central AD concentration have anorectic effects.[48]

In addition, researches with adult humans suggest that increases in body mass are associated with the *DRD2* A1 allele.[15,50,51] In studies with positron emission tomography, the A1 allele was associated with lower density of the *DRD2*[52] and with reduction of glucose metabolism in human brain dopaminergic regions.[53] All RDS components, including obesity, were related to a low dopaminergic function due to the association with the *DRD2* A1 allele.[10,15,54]

The SNP C32806T in *DRD2* is also associated with a reduced dopaminergic brain activity[55] and the A1 allele was initially associated with BMI increase.[15,56] However, there are few studies[28] verifying the association of this polymorphism in children and adolescents.

It has been observed large variation in the allelic frequencies regarding the *DRD2* TaqIA SNP, even in populations of the same country. For example, in two studies with Turkish obese children the variant allele frequency was 51.0% in one[57] and only 20.0% in the other research.[58] In the Netherlands, the A1 allele frequency was 18.3% in obese children[59] and in North-American studies with obese children, it ranged from 17.0%[60] to 38.5%.[61] In a Brazilian study with obese and normal weight (controls) children, the A1 allele frequencies were 34.5% and 23.0%, respectively, and a statistically significant association between the A1 allele and obesity was verified.[62]

FINAL REMARKS

Food intake is controlled by an interrelation between homeostatic and hedonic factors. The search for food-associated pleasure involves the same neuronal pathway that is stimulated by addictive/compulsive behaviors (alcohol, gambling, sex and drugs), the so called "hypothalamic reward system", that ends in DA. In this context, the minor A1 allele of the *DRD2* TaqIA polymorphisms has been associated with dopaminergic activity and increased obesity risk. Recognition of individuals predisposed to developing obesity through the determination of risk polymorphisms can guide prevention and treatment actions.

ACKNOWLEDGMENTS

We would like to thank Mr. Andre Hedlund for the English review.

AUTHOR'S CONTRIBUTIONS

RMP, CC and ADC wrote and approved the final manuscript.

CONFLICTS OF INTEREST (COI) STATEMENT

The authors declare no conflicts of interest.

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