ISSN 2377-8385

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PUBLISHERS

December 2021 | Volume 8 | Issue 1

OBESITY RESEARCH

Open Journal

www.openventio.org

ISSN 2377-8385

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Original Research

Association of *Fat Mass and Obesity Associated*, *Dopamine Receptor Type 2* and *Ankyrin Repeat and Kinase Domain Containing 1* Genes with Pediatric Obesity and Metabolic Risk: A Case-Control Study

Renata M. Pinto, PhD[1,2,3*]; Jakeline S. Fortes, MS[3]; Rúbia V. Monteiro, MS[3]; Nygell S. Alves, MS[3]; Maria P. Curado, PhD[1]; Lysa B. Minasi, PhD[3,4]; Daniela de M e Silva, PhD[5]; Aparecido D. da Cruz, PhD[3,4]

[1]*Federal University of Goiás, Health Science Pos Graduation Program, Goiânia, GO, Brazil*
[2]*Children´s Hospital of Goiás, Goiânia, GO, Brazil*
[3]*Pontifical Catholic University of Goiás, Replicon Research Center, Goiânia, GO, Brazil*
[4]*Department of the State of Goiás, Laboratory of Human Cytogenetics and Molecular Genetics, Secretary of State for Health of Goiás, Goiânia, GO, Brazil*
[5]*Federal University of Goiás, Goiânia, GO, Brazil*

[*]***Corresponding author***
Renata M. Pinto, PhD
Federal University of Goiás, Health Science Pos Graduation Program, Goiânia, GO, Brazil; E-mail: drarenatamachado@gmail.com

Article information
Received: April 11th, 2021; **Revised:** May 17th, 2021; **Accepted:** May 18th, 2021; **Published:** May 29th, 2021

Cite this article
Pinto RM, Fortes JS, Monteiro RV, Alves NS, Curado MP, Minasi LB, de M e Silva D, da Cruz AD. Association of *fat mass and obesity associated*, *dopamine receptor type 2* and *ankyrin repeat and kinase domain containing 1* genes with pediatric obesity and metabolic risk: A case-control study. *Obes Res Open J*. 2021; 8(1): 1-14.
doi: 10.17140/OROJ-8-145

ABSTRACT

Background
Genetic polymorphisms that affect the availability and secretion of dopamine can affect the risk of obesity.

Objectives
To investigate the relationship between pediatric obesity and cardiovascular risk factors (CRF) with the polymorphisms of "*Fat Mass and Obesity Associated*" (*FTO*) rs9939609, "*Dopamine Receptor type 2*" (*DRD2*) rs6277 and "*Ankyrin Repeat and Kinase Domain Containing 1*" (*ANKK1*) rs18000497 genes.

Methods
Case-Control study conducted with 226 pediatric patients from 5 to 16-years of age. The two main groups, Obese (O) and Eutrophic (E), were subdivided according to the value of HOMA-IR into obese with insulin resistance (ORI) or insulin sensitivity (OSI) and eutrophic resistant (ERI) or sensitive (ESI) to insulin. According to the presence of two or more *CRF*, they were subdivided into metabolically unhealthy or metabolically healthy groups: Obese Metabolically Unhealthy (OMU), Obese Metabolically Healthy (OMH), Eutrophic Metabolically Unhealthy (EMU) and Eutrophic Metabolically Healthy (EMH). Polymorphisms were determined by real-time Polymerase Chain Reaction (PCR) or Restriction Fragment Length Polymorphisms (PCR-RFLP).

Results
In the obese group, the higher the number of risk alleles of *FTO* and *ANKK1* genes isolated and the three genes combined, the higher the mean BMI (p<0.0001). Regarding the *FTO* gene: the frequency of the risk allele was: 57.7%-ERI, 37.4%-ESI (p=0.048), and the homozygous wild genotype was: 29.5%-OMU, 37.5%-OMH (p=0.02). Regarding the *DRD2* gene: the genotypes with the risk allele were present in 84.6%-OMU and 67.5%-OMH (p=0.031). Regarding the *ANKK1* gene: the frequency of the homozygous risk genotype was current in 15.4%-ERI and 13.5%-ESI (p<0.0001) and 62.5%-EMU and 41.5%-OMH (p=0.031).

Conclusion
Risk alleles of *FTO*, *DRD2* and *ANKK1* genes had an additive effect on the outcome of pediatric obesity in Brazilian children and conferred a higher risk of insulin resistance (*FTO* and *ANKK1*) and CRF.

Keywords
Childhood obesity; Genetic polymorphism; Insulin resistance; Metabolic syndrome; Dopamine.

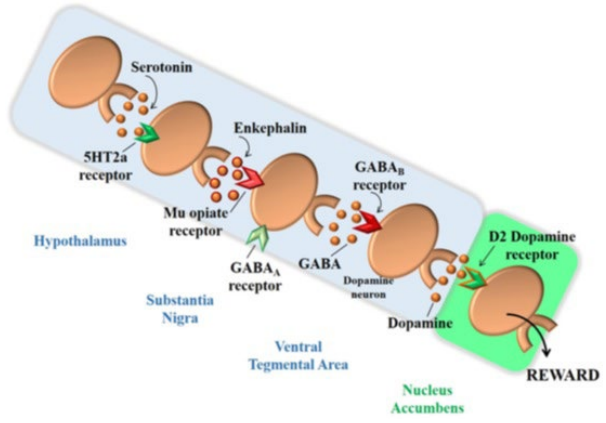
INTRODUCTION

Obesity is recognized by the World Health Organization (WHO) as one of the ten leading health problems in most diverse societies,[1] and in 2019 chronic non-communicable diseases: obesity, diabetes, cancer, and cardiovascular diseases reached the second position in the ranking world of health challenges.[2] The prevalence of obesity has increased worldwide, with epidemic proportions in many developed and developing countries.[3,4] Currently, obesity is considered a neurobehavioral disease in which hypothalamic changes occur in the control of hunger, satiety, and energy expenditure.[5] It is a condition of multifactorial etiology influenced by genetic, epigenetic, endocrine-metabolic, and behavioral factors.[6,7]

The regulation of food intake by the Central Nervous System (CNS) depends on the interaction of a homeostatic component that aims to balance energy and nutrients and a hedonic component, which seeks pleasure associated with food.[8,9] The ingestion of highly palatable foods (rich in sugar and fats) can deregulate the homeostatic control of appetite, perpetuating the stimulus to eat, which makes the intake primarily mediated by hedonic and non-homeostatic needs.[10]

Cortico-limbic-striated structures and circuits form the Brain Reward System (BRS). BRS is activated by various stimuli that trigger a cascade of neurotransmitter secretion that ultimately releases dopamine (DA), bringing a sense of pleasure and leading the individual to seek positive reinforcements.[10,11] 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 1).[12]

Figure 1. *Interaction of Various Neurotransmitters that forms the "Brain Reward Cascade*



Source: Pinto et al[12]

Considering that DA plays a crucial role in BRS, it is involved in eating behavior, and that genetic factors are responsible for up to 80% of the inter-individual variation of nutritional status,[13] the study of genetic polymorphisms that affect the availability and secretion of DA have gained space in the literature.[12]

Dopamine Receptor Type 2 gene (rs6277) Polymorphism

The *dopamine receptor type 2* (*DRD2*) is the main self-regulator of DA release in the brain reward dopaminergic system, with pre and postsynaptic effects.[14]

The *DRD2* gene, located at 11q22-q23, has several polymorphisms capable of influencing the quantity and functionality of receptors in the membrane of the postsynaptic cell, which directly implies in the predisposition of individuals to seek any sub stance or behavior that stimulates BRS[15] with a consequent higher propensity to gain weight in the future.[16]

The single nucleotide polymorphism (SNP) *rs6277* of the *DRD2* gene consists of the change of an Arginine (A) by Guanine (G) in the position 957pb in exon 7. There are only a few researches that studied this polymorphism from a metabolic point of view, but it is known that the presence of the risk allele (G) leads to a reduction in the affinity of the receptors and is associated with: psychiatric disorders related to the deficiency of dopaminergic function and processing of emotions,[17] a greater sense of reward even in individuals of normal weight,[18] higher consumption of sugar and increased body mass index (BMI) from normal to overweight/obesity in adults.[19]

Ankyrin Repeat and Kinase Domain Containing 1 gene (rs1800497) Polymorphism

The *Ankyrin repeat and Kinase domain containing 1* gene (*ANKK1*) is also located in the 11q22-q23 region. It's *rs1800497* SNP interferes with the availability of *DRD2* receptors and was considered part of the *DRD2* gene itself, under the name of Taq1A polymorphism of the *DRD2* gene.[20] This SNP influences the signaling of *DRD2* receptors: healthy individuals who have the risk allele (T) have a reduction of between 30-40% in the density of *DRD2* in the striatum when compared to those without T-allele, and reduced glucose metabolism in the regions of BRS that contain dopaminergic neurons.[20] This reduction in the BRS's dopaminergic action reflects clinically in a higher risk of obesity in adults,[16] greater weight gain,[16] more intense reinforcement (feeling of pleasure) after a meal, and increased energy intake, especially in obese.[21] There is a wide variation in genotype and allele frequencies in different countries, and even within the same country, there is inconsistency regarding the association or not with metabolic outcomes. Chart 1 shows the main results of these scientific researches.

Fat Mass and Obesity Associated gene (rs9939609) Polymorphism

The *Fat Mass and Obesity Associated* (*FTO*) gene, located at 16q12.2, encodes a 2-oxoglutarate-dependent demethylase involved in post-translational modifications, deoxyribonucleic acid (DNA) repair, and fatty acid acidification.[22] The presence of at least one risk allele (A) of this SNP is associated with higher caloric intake, less satiety, and a higher frequency of episodes of compulsive eating when compared with homozygotes for the wild allele (T).[23-26]

Chart 1. *Summary of Studies Carried Out in the Pediatric Population that Evaluated the rs1800497 Polymorphism of the ANKK1 gene*

Reference	Population	Conclusion
Epstein et al[84]	26 Children from 8 to 12-years old with overweight and parents with obesity Country: USA	Presence of the T allele associated with higher BMI and also weight modification after a weight loss program lasting 0.5 to 1-year.
Ergum et al[85]	Children 46 obese 50 eutrophic Country: Turkey	There was no difference in the allelic distribution of the groups. The following proportion was observed: Obese: T- 51%, C- 49% Eutrophic: T- 52% C- 48%
Stice et al[16]	44 female teenagers Country: USA	Observation of MRI in a situation of food anticipation showed attenuated response of the CNS of patients with the presence of the T allele.
Strien et al[86]	279 Adolescents Country: Netherlands	Genotype frequency: TT-2.9%; CT - 30.8%; CC - 66.3% Allele frequency: T -18.3%; C- 81.7% The presence of the T allele increased the intake of emotional background
Duran-Gonzalez et al[87]	448 Adolescents Country: USA	Genotype and allele frequency (%): TT-15.2; CT- 46.5; CC- 38.3; T-38.5; C- 61.5 T allele associated with central obesity
Araz et al[42]	200 pediatric patients aged 2 to 17 years. 50% obese 50% eutrophic Country: Turkey	Genotypic and allele frequency (%) in Obese: TT-7; CT-26; CC- 67 T-20; C - 80 Genotype and allele frequency (%) in Controls: TT-4; CT-31; CC- 65 No association of T allele and BMI
Roth et al[88]	583 eutrophic adults 28 overweight children 423 children with obesity Country: USA	n children, the following genotypic and allelic frequencies were found: TT-2.4%; CT- 29.3%; CC- 68.3% T- 17%; C- 83% There was no association between elevated BMI with the presence of the T allele; however, the TT genotype showed a worse response after a lifestyle change program aimed at weight loss.
Pinto et al[76]	Pediatric patients from 5 to 16 years of age 55 obese 50 eutrophic Country Brazil	Genotype and allele frequency (%) in the obese: TT-14.5; CT-40; CC-45.5 T-34.5; C 65.5 Genotype and allele frequency (%) in controls: TT-10; CT-26; CC-64 T-23 allele; C-77 T allele associated with obesity and alteration of glycemic homeostasis
Yeh et al[77]	84 teenagers of Asian origin Country: USA	Genotype frequency (%) TT + CT 63%; CC 37% No difference in BMI, the presence of the T allele associated with increased consumption of carbohydrates and fast food
Yobregon et al[78]	258 Pediatric patients aged 8 to 14 years 115 obese, 42 overweight, 101 eutrophic Country: Chile	Genotype frequency (%) TT-9.7; CT- 38.4; CC- 51.9 No difference in BMI, T allele associated with greater pleasure in eating in obese boys.
Cartel et al[79]	286 Pediatric patients aged 7 to 12 years Country: USA	Genotype frequency (%) TT– 12.9; CT– 39.5; CC- 47.6 TT homozygotes reported 20% higher energy intake of carbohydrates, and greater deposition of visceral fat.

Currently, *FTO* is the gene that shows the most significant association with human polygenic obesity. After evaluating 3337 obese and 3159 controls, a recent meta-analysis study definitively confirms its correlation with obesity in European populations.[27] The association of *FTO* with obesity has been confirmed in other ethnicities[28] such as Asians[29,30] and Hispanics.[31-33] Few studies have evaluated this SNP in the Brazilian population, and only 7 have been carried out in the pediatric population of our country. Chart 2 shows the main findings of these studies.

Recently Sun et al[34] reviewed evidence that suggest a role of two common gene variants, *FTO* and *ANKK1*, in driving gene-environment interactions leading to obesity, metabolic dysfunc-

Chart 2. *Summary of Studies Conducted with Brazilian Children and Adolescents to Evaluate the rs9939609 Polymorphism of the FTO gene*

Reference	Population	Conclusions
Silva et al[59]	N = 348 Age: 0-8-years State: RS	Genotype and allele frequency (%) AA- 16.4;AT- 46.3;TT- 37.4 A - 40;T- 60 Association with higher BMI and adiposity
Lourenco et al[60]	N = 1088 Age: 0-10-years State: AM	Genotype frequency (%) AA- 15.6;AT- 44.8;TT- 39.6 Association with weight gain
Pereira et al[64]	195 obese 153 eutrophic State: MG	Allele frequency (%) Obese: A- 49.5;T-50.5 Eutrophic: A- 46.7;T-53.27 There was no difference between groups
Reuter et al[61]	N = 406 Age: 7-17-years State: RS	Genotype and allele frequency (%) AA-13.3;AT-44.3;TT- 42.4 A - 35.5;T- 64.5 AA genotype associated with obesity/overweight
Nascimento et al[62]	136 obese 172 eutrophic Age: 8-17-years State: PR	Genotype and allele frequency (%) Obese: AA- 14.7;AT- 46.3;TT- 38.9 Eutrophic: AA-9.9; AT-52.3;TT- 37.8 Allele A associated with lower AC reduction after intervention with diet and exercise
Rodrigues et al[65]	378 obesos 378 eutrophic Age:18-9-years State: MA	Genotype and allele frequency (%) Obese: AA -14.5;AT-44.9,TT-40.5 / A-36.8;T-63.2 Eutrophic: AA-13.5; AT- 47.9;TT-38.6 / A- 37.4; T-62.6 There was no difference between groups
Todendi et al[63]	N = 871 Age: 7-17-years State: RS	Allele A associated with increase in BMI

Legend: AC: Abdominal circumference; AM: Amazonas; BMI: Body mass index; MA: Maranhão; MG: Minas Gerais; PR: Paraná; RS: Rio Grande do Sul.

tion, and cognitive change *via* their influence on *DRD2* signaling. It was also demonstrated that the *FTO* protein regulates dopamine signaling *via DRD2*, and the polymorphisms *rs9939609* of the *FTO* gene and *rs18000497* of the *ANKK1* gene interact.[35] A study conducted in humans found that *FTO* risk variants affect the brain dopaminergic response and modify a pleasurable task's learning. The *FTO* gene modulates the BRS connectivity, suggesting that the increased risk for obesity is related to the processing of the sensation of pleasure.[35]

OBJECTIVES

The main objective of this study is to investigate the relationship between pediatric obesity and the polymorphisms of the *ANKK1* (*rs18000497*), *DRD2* (*rs6277*) and *FTO* (*rs9939609*) genes, in a population of Brazilians. We also intend to correlate the studied polymorphisms with the degree of insulin sensitivity and cardiovascular risk factors (CRF) components of the metabolic syndrome (MS): Blood pressure (BP), Abdominal circumference (AC), Triglycerides (TG), High-density cholesterol (HDL) and blood glucose.

METHODS

This research is a Case-Control study carried out jointly by LaGene - Laboratory of Human Cytogenetics and Molecular Genetics of the Health Department of the State of Goiás (LaGene/Lacen/SES-GO), NPR- Replicon Research Center (PUC-GO), and the Children's Hospital of Goiânia/Child Endocrinology Office and Federal University of Goiás (LabMut/FG). The project was approved by the Research Ethics Committee of PUC Goiás, with the number: 16303313.4.0000.0037. All parents or guardians were interviewed and signed the informed consent form consenting with their child's participation in the study and data for research. The researchers clarified the methodological research procedures before and during the study to all participants.

The study included pediatric patients from 5 to16-years of age who sought the pediatric endocrinology office of the Children's Hospital of Goiânia (Goiânia-Goiás-Brazil) years 2017 and 2018. The responsible researcher was personally assessed in complete pediatric consultation with thorough anamnesis and physical examination. Exclusion criteria were: overweight, malnutrition, severe chronic diseases, presence of genetic syndromes, use of medications known to alter weight.

The nutritional status diagnosis was based on the BMI with interpretation according to the reference values proposed by WHO.[36] According to the BMI, children were divided into two main groups: Obese (whose BMI was above +2 SD) and Eutrophic (whose BMI was between -2 SD and +1 SD) that constituted the control group.

The BP was evaluated according to the Hypertension Guidelines for children and Adolescents,[37] which follows the values of the Consensus of the American Academy of Pediatrics.[38] Arterial hypertension was considered if the systolic and/or dia-

stolic blood pressure values were equal to or higher than the 95th percentile for sex, age, and height percentile on three or more occasions.[37,38]

The AC was measured at the midpoint between the last fixed rib (10th) and the iliac crest's upper border. P90 was considered for age, sex, and ethnicity as the maximum value of normality, according to the values described by Freedman and collaborators.[39]

Biochemical Evaluation

After 8 to 12-hours of fasting, blood was collected to determine the lipid profile, blood glucose, and insulin. Through the equipment Architect c8000® Plasma total cholesterol, HDL-cholesterol, and TG were determined by enzymatic-colorimetric assay, and Glucose values were determined by the enzymatic method (Glucose Oxidase - Latest, SP, Brazil). Insulin was measured by the chemiluminescence technique using an immunometric immunoassay in a piece of automated equipment Architect i2000®.

The Brazilian Society of Pediatrics' recommendation for the Brazilian pediatric population was used as the reference for serum lipids.[40] The reference values for fasting blood glucose do not differ between children and adults, with typical values between 60 and 100 mg/dL, pre-diabetes between 101 and 125 mg/dL, and diabetes ≥ 126 mg/dL.[41] Fasting insulinemia is considered normal from 2.5 to 25 mIU/mL in adults and up to 15 mIU/mL in children.[42] The determination of fasting blood glucose and insulin in the same sample allows the calculation of the homeostatic model assessment insulin resistance (HOMA-IR) index, a method used to quantify insulin resistance (IR).[43] The interpretation of HOMA-IR values followed the parameters established by de Almeida et al[44] for the Brazilian pediatric population. HOMA was considered altered when the value was 2 standard deviations (SD) above the mean for age and sex.

According to the value of HOMA-IR, the main groups were subdivided into obese with insulin resistance (ORI) or insulin sensitivity (OSI) and eutrophic resistant (ERI) or sensitive (ESI) to insulin. According to the presence of two or more CRF, they were subdivided into metabolically unhealthy (MU) or metabolically healthy (MH) groups: OMU, OMH, EMU, EMH.

We choose to use the individualized evaluation of CRF and not the presence or absence of MS as there are no reference criteria for MS before ten-years of age, and even after that age, the criteria for MS remain controversial. More than 40 different cut-off points have already been used,[45] and even after the consensus attempt by the International Diabetes Federation, there is still no consensus on which criteria should be used for the diagnosis of MS in adolescence.[46] Thus, this research followed the American Academy of Pediatrics's recommendation to focus on the CRF individually instead of focusing on the diagnosis of MS.[47]

Determination of the Genetic Polymorphisms

The polymorphisms of *DRD2* and *FTO* genes were determined by the real-time polymerase chain reaction (PCR) method. The *rs9939609* and *rs6277* SNPs were genotyped using the TaqMan Real-Time PCR® kit (SNP Genotyping kit, from AppliedBiosystems, USA), following the manufacturer's concentration guidelines. This protocol included the primer oligonucleotide sequences and two fluorophore-labeled TaqMan® minor groove-binding (MGB) probes, named VIC® and FAM™.

To evaluate the polymorphism of the *ANKK1* gene, the polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) strategy was used, using the restriction enzyme TaqIA whose cutting site contains an SNP C/T (*C32806T*), following the methodology proposed by Behravan et al.[48]

The Primers used were: GGTTCCTTGCGACTGCTGTGAATTT [T/A] GTGATGCACTTGGATAGTCTCTGTT (Sequence 5´→3´) for FTO; TCTTCTCTGGTTTGGCGGGGCTGTC [G/A] GGAGTGCTGTGGAGACCATGGTGGG (Sequence 5´→3´) for DRD2, and ACCCTTCCTGAGTGTCATCA (Forward) and ACGGCTGGCCAAGTTGTCTA (reverse) for *ANKK1*.

RESULTS

Two hundred twenty-six children and adolescents were evaluated, 124 (54.9%) had obesity, and 102 (45.1%) were eutrophic. The two groups were similar in age and sex distribution (49.2% were female in the obese group and 50.9% in the eutrophic group). Table 1 shows the clinical and biochemical parameters and the proportion of obese and eutrophic patients with altered parameters used for the main groups' subdivision.

All patients were genotyped for the *ANKK1* gene; however, due to an insufficient amount of DNA, two eutrophic and six children with obesity were not genotyped for the *FTO* and *DRD2* genes. For the combined analyzes of the three genes, these eight patients were excluded.

There was no statistical difference between the obese and eutrophic groups in the allelic and genotypic distribution of the three genes studied (Table 2). The risk allele of the three genes was more frequent in the obese group concerning the eutrophic group, and the homozygous genotypes for the wild allele of the *FTO* and *ANKK1* genes were more frequent in the eutrophic group, but none of these differences reached statistical significance.

Table 3 and Figure 2 show the evolution of the mean Z Score of the BMI (Z-BMI) within the obese group, according to the number of risk alleles. In the combined evaluation of the three genes, the presence of 0, 1, and 6 risk alleles was disregarded due to the small number of patients. The highest number of risk alleles correlated with a higher Z-BMI for the *FTO* and *ANKK1* genes individually and in the sum of the three genes studied, showing an upward curve of the lowest number of alleles and a lower Z-BMI for the highest number of alleles and higher Z-BMI (Figure 2), with a statistically significant difference for all ranges of the number of risk alleles considered.

Table 1. *Clinical and Biochemical Parameters Observed in the Obese and Eutrophic Groups*

Variables	Obese		Eutrophic		*p* value
	Mean	SD	Mean	SD	
Age (years)	9.7	2.4	10.1	2.6	0.114
Weight (kg)	55.6	19.8	30.4	10.1	< 0.0001*
Height (cm)	142.7	13.6	135.3	15.7	0.121
Z-BMI	3.05	0.97	- 0.45	0.81	< 0.0001*
Mother´s BMI (kg/m²)	29.7	6.3	24,2	3.3	< 0.0001*
Father´s BMI (kg/m²)	32.4	6.9	26.9	4.7	0.003*
Glucose (mg/dL)	87.08	6.4	86.4	6.7	0.337
Insulin (µui/mL)	12.68	6.7	6.28	2.7	< 0.0001*
HOMA	2.77	1.53	1.32	0.6	< 0.0001*
TC (mg/dL)	170.34	29.9	162.05	26.5	0.13
HDL (mg/dL)	43.25	8.67	48.23	10.01	0.1
LDL (mg/dL)	107.19	29.02	97.37	24.08	0.186
TG (mg/dL)	94.18	44.57	73.65	27.12	< 0.0001*
Variables	**Obese N (%)**		**Eutrophic N (%)**		***p* value**
	Altered	**Normal**	**Altered**	**Normal**	
TG	64 (51.6)	60 (48.4)	31 (30.4)	71 (69.6)	0.0012*
HDL	83 (66.9)	41 (33.1)	40 (39.2)	62 (60.8)	0,0003*
LDL	50 (40.3)	74 (59.7)	25 (24.5)	77 (75.5)	0.0119*
Glucose	2 (1.6)	122 (98.4)	1 (0.9)	101 (99.1)	0.679
BP	4 (3.2)	120 (96.8)	0 (0)	102 (100)	NA
AC	107 (86.3)	17 (13.7)	0 (0)	102 (100)	NA
MS#	83 (66.9)	41 (33.1)	24 (23.5)	78 (76.5)	<0.0001*
Subgroup	OMU	OMH	EMU	EMH	
HOMA	78 (62.9)	46 (37.1)	13 (12.7)	89 (87.3)	<0.0001*
Subgroup	ORI	OSI	ERI	ESI	
Total: N (%)	124 (100%)		102 (100%)		

*Legend: AC: Abdominal circumference; BMI: Body mass index; EIS: Eutrophic with insulin sensitivity; ERI: Eutrophic resistant to insulin resistance, EMH: Eutrophic metabolically healthy; EMU: Eutrophic metabolically unhealthy; HDL: High density cholesterol; LDL: Low density cholesterol; HOMA: Homeostatic Model Assessment Insulin Resistance; ORI: Obese resistant to insulin; OSI: Obese sensitive to insulin; OMH: Obese metabolically healthy; OMU: Obese metabolically unhealthy; BP: Blood pressure; MS: Metabolic syndrome; TC: Total cholesterol; TG: Triglycerides; ; SD: Standard deviation; Z-BMI: Z score of the BMI; # considering 2 or more parameters; *p level with statistical significance.*

Subgroups According to Insulin Sensitivity Criteria

Table 4 shows the genotypic and allelic distribution of the *FTO*, *DRD2* and *ANKK1* genes in the subgroups that were divided according to insulin sensitivity criteria: ORI, OSI, ERI, and ESI.

Regarding the *FTO* gene, in the eutrophic subgroups, the wild homozygous genotype (TT) was more prevalent in the insulin-sensitive group-ESI than in the insulin-resistant group-ERI (36.8×23%, p=0.039). The risk allele (A) was more prevalent in the ERI group (57.7%) than in the ESI group (37.4%), with an odds ratio (OR) of 2.29 for IR when the A allele was present (p=0.048).

Regarding the *DRD2* gene, we did not observe any statistically significant difference in the evaluation of the groups together or separately.

Regarding the *ANKK1* gene: the ERI group showed a higher proportion of the homozygous genotype for the risk allele (TT) when compared to ESI (15.4%×13.5%, p<0.00001).

Subgroups According to the Metabolic Health Criteria

The evaluation of the genotypic and allelic distribution of the *FTO*, *DRD2* and *ANKK1* genes in the groups subdivided according to the CRF number is shown in Table 5.

Regarding the *FTO* gene, in the evaluation of the obese subgroups, we observed that the wild homozygous (TT) genotype is present in 37.5% of the metabolically healthy group (OMH) and 29.5% of the metabolically sick group (OMU) (p=0.02).

Regarding the *DRD2* gene, in the evaluation of the obese subgroups, the homozygous genotype for the non-risk allele (AA) is more prevalent in the healthy subgroup – OMH than in the subgroup with two or more CRF-OMU (32.5%×15.4%, p=0.053). In

Table 2. *Genotypic and Allelic Distribution of FTO, DRD2 and ANKK1 genes in the Obese and Eutrophic Groups. (Risk alleles: FTO=A, DRD2=G, ANKK1=T)*

Gene	Obese N (%)	Eutrophic N (%)	X ²	*p* value
***FTO* rs9939609**				
Genotypes				
AA	25 (21.2)	15 (15)	1.38	0.500
AT	55 (46.6)	50 (50)		
TT	38 (32.2)	35 (35)		
Total	118 (100)	100 (100)		
Alleles				
A	105 (44.5)	80 (40)	0.89	0.344
T	131 (55.5)	120 (60)		
Total	236 (100)	200 (100)		
***DRD2* rs6277**				
Genotypes				
GG	43 (36.4)	29 (29)	1.594	0.450
AG	50 (42.4)	50 (50)		
AA	25 (21.2)	21 (21)		
Total	118 (100)	100 (100)		
Alleles				
G	136 (57.6)	108 (54)	0.577	0.4471
A	100 (42.4)	92 (46)		
Total	236 (100)	200 (100)		
***ANKK1* rs1800497**				
Genotypes				
TT	13 (10.5)	14 (13.7)	1.763	0.414
CT	45 (36.3)	29 (28.5)		
CC	66 (53.2)	59 (57.8)		
Total	124 (100)	102 (100)		
Alleles				
T	71 (28.6)	57 (28)	0.026	0.871
C	177 (71.4)	147 (72)		
Total	248 (100)	204 (100)		

Legend: A: Adenine; C: Cytosine; G: Guanine; T: Thymine.

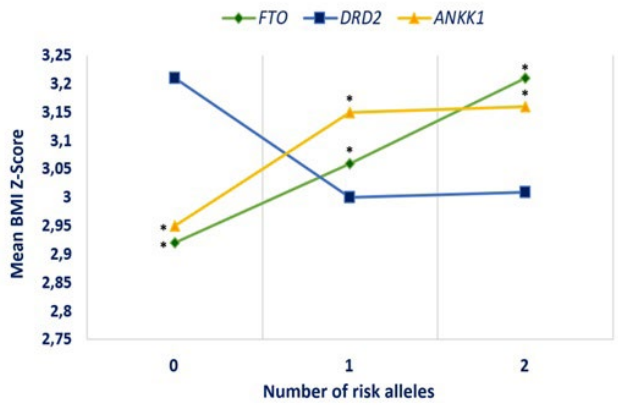
Table 3. *Mean BMI Z score in the Obese Group, According to the Number of Risk Alleles of the FTO, DRD2 and ANKK1 Genes Individually and of the 3 Genes Combined*

Number of Risk Alleles	Mean Z-BMI	SD	*p* value
***FTO* rs9939609**			
0 (TT)	2.92	0.68	0.000*
1 (AT)	3.06	1.09	0.000*
2 (AA)	3.21	1.05	0.000*
***DRD2* rs6277**			
0 (AA)	3.21	1.12	0.453
1 (AG)	3.00	1.05	0.574
2 (GG)	3.01	0.76	0.404
***ANKK1* rs1800497**			
0 (CC)	2.95	0.84	0.000*
1 (CT)	3.15	1.17	0.000*
2 (TT)	3.16	0.76	0.000*
FTO+DRD2+ANKK1			
2	2.89	0.93	0.000*
3	3.09	1.08	0.000*
4	3.13	1.09	0.000*
5	3.45	0.64	0.000*

*Legend: A: Adenine; C: Cytosine; G: Guanine; SD: Standard deviation; T: Thymine; Z-BMI: Z score of the BMI; *p level with statistical significance.*

Figure 2. *Mean BMI Z-Score in the Obese group According to the Number of Risk Alleles of the FTO, DRD2 and ANKK1 Genes*

A) FTO, DRD2 and ANKK1 genes Separately



B) FTO, DRD2 and ANKK1 genes Combined

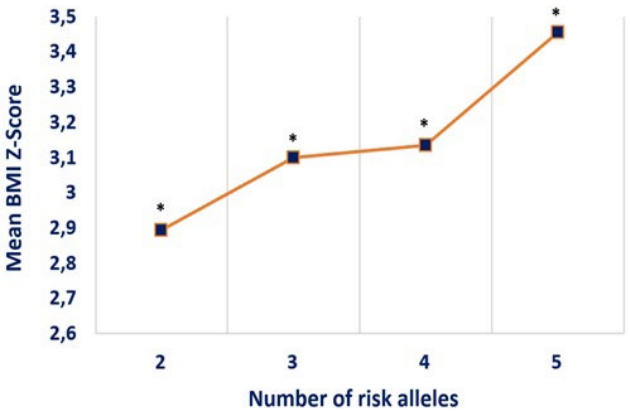


Table 4. *Genotypic and Allelic Distribution of FTO, DRD2 and ANKK1 genes in the Groups: ORI (Obese Resistant to Insulin), OSI (Obese Sensitive to Insulin), ERI (Eutrophic Resistant to Insulin Resistance) and ESI (Eutrophic Sensitive to Insulin)*

Gene	ORI N (%)	OSI N(%)	ERI N(%)	ESI N(%)	X ²	*p* value
***FTO* rs9939609**						
Genotypes						
AA	15 (20)	10 (23.3)	5 (38.5)	10 (11.5)		
AT	36 (48)	19 (44.2)	5 (38.5)	45 (51.7)	7.15	a0.039
TT	24 (32)	14 (32.5)	3 (23)	32 (36.8)		
AA+AT	51 (68)	29 (67.5)	10 (77)	55 (63.2)	1.14	0.765
TT	24 (32)	14 (32.5)	3 (23)	32 (36.8)		
Total	75 (100)	43 (100)	13 (100)	87 (100)		
Alleles						
A	66 (44)	39 (45.3)	15 (57.7)	65 (37.4)		
T	84 (56)	47 (54.7)	11 (42.3)	109 (62.6)	4.76	a0.048
Total	150 (100)	86 (100)	26 (100)	174 (100)		
***DRD2* rs6277**						
Genotypes						
GG	27 (36)	16 (37.2)	5 (38.5)	24 (27.6)		
AG	28 (37.3)	22 (51.2)	5 (38.5)	45 (51.7)	6.54	0.3649
AA	20 (26.7)	5 (11.6)	3 (23)	18 (20.7)		
GG+AG	55 (73.3)	38 (88.4)	10 (77)	69 (79.3)	3.75	0.289
AA	20 (26.7)	5 (11.6)	3 (23)	18 (20.7)		
Total	75 (100)	43 (100)	13 (100)	87 (100)		
Alleles						
G	82 (54.7)	54 (62.8)	15 (57.7)	93 (53.4)		
A	68 (45.3)	32 (37.2)	11 (42.3)	81 (46.6)	2.20	0.530
Total	150 (100)	86 (100)	26 (100)	174 (100)		
***ANKK1* rs1800497**						
Genotypes						
TT	9 (11.5)	4 (8.7)	2 (15.4)	12 (13.5)		
CT	28 (35.9)	17 (37)	3 (23.1)	26 (29.2)	2.18	0.414
CC	41 (52.5)	25 (54.3)	8 (61.5)	51 (57.3)		
TT+CT	37 (47.5)	21 (45.7)	5 (38.5)	38 (42.7)	0.60	0.895
CC	41 (52.5)	25 (54.3)	8 (61.5)	51 (57.3)		
Total	78 (100)	46 (100)	13 (100)	89 (100)		
Alleles						
T	46 (29,5)	25 (27,2)	7 (26,9)	50 (28,1)		
C	110 (70,5)	67 (72,8)	19 (73,1)	128 (71,9)	0.19	0.978
Total	156 (100)	92 (100)	26 (100)	178 (100)		

Legend: A: Adenine; C: Cytosine; EIS: Eutrophic with insulin sensitivity; ERI: Eutrophic resistant to insulin; G: Guanine; ORI: Obese resistant to insulin; OSI: Obese sensitive to insulin; T: Thymine; a – p value in the comparison between ERI and ESI.

Table 5. *Genotypic and Allelic Distribution of the FTO, DRD2 and ANKK1 Genes in the Groups: OMU (Obese MetaBolically Unhealthy), OMH (Obese Metabolically Healthy), EMU (Eutrophic Metabolically Unhealthy) and EMH (Eutrophic Metabolically Healthy)*

Gene	OMU N (%)	OMH N(%)	EMU N(%)	EMH N(%)	X ²	*p* value
***FTO* rs9939609**						
Genotypes						
AA	12 (15.4)	13 (32.5)	4 (17.4)	11 (14.3)		
AT	43 (55.1)	12 (30)	12 (52.2)	38 (49.3)	9.88	a0.020
TT	23 (29.5)	15 (37.5)	7 (30.4)	28 (36.4)		
AA + AT	55 (70.5)	25 (62.5)	16 (69.6)	49 (63.6)	1.23	0.745
TT	23 (29.5)	15 (37.5)	7 (30.4)	28 (36.4)		
Total	78 (100)	40 (100)	23 (100)	77 (100)		
Alleles						
A	67 (42.9)	38 (47.5)	20 (43.5)	67 (42.9)		
T	89 (57.1)	42 (52.5)	26 (56.5)	89 (57.1)	1.63	0.650
Total	156 (100)	80 (100)	46 (100)	154 (100)		
***DRD2* rs6277**						
Genotypes						
GG	28 (35.9)	15 (37.5)	7 (30.4)	22 (28.6)		
AG	38 (48.7)	12 (30)	12 (52.2)	38 (49.4)	7.53	a0.053
AA	12 (15.4)	13 (32.5)	4 (17.4)	17 (22)		
GG + AG	66 (84.6)	27 (67.5)	19 (82.6)	60 (88)	4.88	a0.031
AA	12 (15.4)	13 (32.5)	4 (17.4)	17 (22)		
Total	78 (100)	40 (100)	23 (100)	77 (100)		
Alleles						
G	94 (60.3)	42 (52.5)	26 (56.5)	82 (53.2)		
A	62 (39.7)	38 (47.5)	20 (43.5)	72 (46.8)	2.02	0.567
Total	156 (100)	80 (100)	46 (100)	154 (100)		
***ANKK1* rs1800497**						
Genotypes						
TT	49 (59)	4 (9.8)	5 (20.8)	44 (56.4)		
CT	25 (30.1)	20 (48.8)	4 (16.7)	25 (32.1)	9.01	b0.031
CC	9 (10.9)	17 (41.4)	15 (62.5)	9 (11.5)		
TT + CT	34 (41)	24 (58.5)	9 (37.5)	34 (53.6)	4.18	0.242
CC	49 (59)	17 (41.5)	15 (62.5)	44 (46.4)		
Total	83 (100)	41 (100)	24 (100)	78 (100)		
Alleles						
T	43 (25.9)	28 (34.1)	14 (29.2)	43 (27.6)		
C	123 (74.1)	54 (65.9)	34 (70.8)	113 (72.4)	0.9	0.591
Total	166 (100)	82 (100)	48 (100)	156 (100)		

Legend: A: Adenine; C: Cytosine; EMH: Eutrophic metabolically healthy; EMU: Eutrophic metabolically unhealthy; G: Guanine; OMH: Obese metabolically healthy; OMU: Obese metabolically unhealthy; T: Thymine; a – p value in the comparison between OMU and OMH, b – p value in the comparison between OMH e EMU.

patients with obesity, the genotypes with the presence of the risk allele of the *DRD2* gene (GG+AG) increased the risk for metabolic disease by 2.65 times (p=0.031).

Regarding the *ANKK1* gene, when we compared the "healthy obese" group with the "unhealthy eutrophic" group, we observed that the homozygous genotype for the risk allele (TT) was twice as frequent in the EMU group as in the OMH group (20.8%×9.8%, p=0.031).

DISCUSSION

The polygenic nature of common obesity discovers risky genes and their variants a challenging task. Genome-Wide Association Study (GWAS) studies have brought new insights into the understanding of polygenic obesity, but even today, specific genes' contribution

to BMI variability is still poorly understood.[6] No GWAS study has included the Brazilian population, and studies in the pediatric age group are scarce. Although carried out with a small number of patients and with groups composed following the simple random sampling strategy without replacement, the present research contributed to the knowledge of genetic factors that interfere in the phenotype of pediatric obesity in Brazilians.

The presence of higher-levels of parent's BMI of the obese children corroborates studies that indicate a vital genetic component in human obesity.[49-51] In addition to the genetic factor, these children live in the same environment as their family, therefore, they are exposed to their lifestyle. It is known that children whose both parents are obese have an 80% possibility of being obese, 50% when one parent is obese, and 9% when the parents are not obese.[52] It is worth mentioning that the father's average BMI is above normal in both groups. The group of eutrophic children has overweight fathers, a fact consistent with the advancement of overweight and obesity in Brazilians, which affects 56.9% of the adult population.[53]

Both obese and eutrophic groups had similar blood glucose levels. The obese group, however, showed higher-levels of insulin and HOMA-IR. These insulin sensitivity changes are attributed to obesity per se, which is a significant risk factor for diabetes mellitus (DM) type 2. The worsening of the situation of the pancreatic malfunction is related to obesity evolves to DM.[54-56]

We observed that TG levels were higher in the obese group, an expected result since these children also had elevated insulin and HOMA. Hypertriglyceridemia is a pervasive alteration in patients with obesity and is related to insulin resistance: obese patients with IR, even without DM2 installed, have selective hepatic IR: insulin cannot suppress hepatic glucose production while continuing to perpetuate lipogenesis, resulting in hypertriglyceridemia.[57,58]

The other lipid profile parameters, the mean TC, high-density cholesterol (HDL), and low-density cholesterol (LDL), did not show a statistical difference. The mean levels of TC and LDL were higher in the obese group but still within the normal range, while the mean value of HDL was lower in the obese group and was in the sub-normal range. When assessing the number of individuals in each group with LDL and HDL outside the normal range (Table 1), a significantly higher proportion of obese people was in this condition. Indeed, a similar means between the obese and eutrophic groups might be due to the presence of 33.1% of "Healthy Obese" (OMH group) and 23.5% of "Sick Eutrophic," also called "false thin" or "obese eutrophic," that composed the EMU group.

It was observed that the risk allele of the three genes was more frequent in the obese group and that the homozygous genotype for the non-risk allele of the *FTO* and *ANKK1* genes was more frequent in the eutrophic, but none of these differences reached statistical significance. The small number of participants evaluated may justify the lack of statistical significance.

When evaluating the effect of the presence of the risk alleles within the obese group, we observed that the higher the number of risk alleles, the greater the Z-BMI for the *FTO* and *ANKK1* genes alone, and in the sum of the 3 genes studied. The upward curve of the lowest number of alleles and lowest Z-BMI for the highest number of alleles and the highest Z-BMI, with a statistically significant difference for all ranges of the number of risk alleles considered, indicate that the studied polymorphisms have an additive effect on the outcome of childhood-onset obesity. This result is unprecedented in the literature.

Considerations Regarding the FTO gene rs9939609

The *rs9930506* polymorphism of the FTO gene is the SNP that shows the most significant association with human polygenic obesity: a recent meta-analysis study after evaluating 3337 obese and 3159 controls confirms its correlation with obesity in European populations.[27] Few studies have evaluated this SNP in the Brazilian population, and only seven were carried out in the pediatric population of our country, five have found an association with adiposity[59-63] and 2 showed no difference between patients with or without obesity.[64,65]

Our study showed that in the Eutrophic group, the presence of wild homozygous genotype (TT) associates with insulin sensitivity, and the presence of the risk allele (A) associates with an increase of 2, 29 times in the risk for IR. This SNP has already been associated with insulin sensitivity changes in insulin sensitivity.[66] but this is the first time this result is presented in Brazilian children. Within the obese group, the TT genotype was more frequent in the OMH group (37.5%) than in the OMU group (29.5%); this difference is statistically significant, so we can say that the T allele is associated with the absence of CRF, which can be considered a protective factor within the obese group, a result also unprecedented in the literature for the Brazilian population.

Considerations Regarding the *DRD2* gene rs6277

The *rs6277* is a poorly studied SNP: only six scientific studies have evaluated the rs6277 variant of the *DRD2* gene in children or adolescents to assess cognitive control and behavioral nuances[67-71] and choke risk.[72]

From the metabolic point of view, the *rs6277* polymorphism of the *DRD2* gene was evaluated in only four articles, all performed in adults. This SNP was associated with compulsive eating disorder,[17,18] weight gain,[19] and higher sugar consumption.73,74 The prevalence of the GG genotype varied between 12 and 21% in these studies. Our series found a higher frequency of genotypes with the risk allele (G), GG-36.4 and 29%, AG-42.4%, and 50% in obese and eutrophic individuals respectively, and AA - 21% for both groups. The highest frequency of the G allele found in our study is probably due to the great miscegenation of the Brazilian population, data corroborated by the 1000 Genome Project,75 which shows a frequency of the G allele of 12.5% in Europeans, 8% in Asians, and 93% in Africans.

Ramos-Lopez et al[73] associated the GG genotype with

significantly higher-levels of TG in adults. The present study associated the G allele of the SNP rs 6277 of the *DRD2* gene with CRF in obese children and adolescents, which was an unprecedented result in the literature.

Considerations Regarding the *ANKK1* gene rs1800497

We observed that the frequency of the homozygous genotype for the wild allele (CC) was slightly higher in the eutrophic group (57.8%) than in the obese group (53.2%). However, there was no statistically significant difference in the allelic or genotypic distribution. This result disagrees with a previous study carried out by our team that found an association of the T-allele with the presence of obesity in children.[74,75] However, when assessing the evolution of the mean Z-BMI in the obese group, we observed that the higher the number of risk alleles, the higher the Z-BMI, corroborating the previous finding that the T allele is associated with increased BMI.

Few studies have been carried out to verify the association of the *rs1800497* polymorphism of the *ANKK1* gene in children and adolescents. A considerable variation in allele frequencies was observed in the literature, even within populations of the same country, and inconsistency regarding the association with metabolic outcomes.[12,76-79]

In evaluating the subgroups using the HOMA values criterion, the risk allele's homozygous genotype (TT) was associated with insulin resistance in eutrophic individuals. This result corroborates the finding of previous research carried out by our group, which for the first time in the literature, associated the T-allele of the *ANKK1* gene with alteration of glycemic homeostasis.[76]

Clinical and animal studies evidence the relationship between *DRD2* receptors and glucose metabolism. Clinical research conducted in diabetic patients[80] and animal study[81] demonstrated improved glycemic control using Bromocriptine, a dopaminergic agonist that acts *via DRD2* receptors. In 2005, RUBI et al[82] demonstrated for the first time that *DRD2* receptors are expressed in the pancreatic beta-cell and modulate insulin secretion. A study carried out in knockout rats for the *DRD2* gene revealed that *DRD2* receptors play a crucial role in insulin secretion and glycemic homeostasis; rats with no *DRD2* receptor showed a failure in insulin response in the face of glucose overload, higher fasting glucose, glucose intolerance, and reduced beta-cell mass.[83] Such results show that *DRD2* is essential for the proliferation of beta cells and insulin secretion and can be considered a growth factor essential for glycemic homeostasis.[83]

Regarding the presence of CRF, the risk allele of the *ANKK1* gene was associated with the presence of two or more CRF, as shown by the presence of the homozygous risk genotype (TT) in 20.8% of EMU and only 9.8% of OMH, a result also found to be unprecedented in the literature.

CONCLUSION

In conclusion, our results found that the risk alleles of the *FTO*, *DRD2* and *ANKK1* genes interfered with the outcome of pediatric obesity in Brazilian children:

- The higher number of the risk alleles of *FTO* and *ANKK1* genes and the 3 genes combined significantly increased the mean Z-BMI of the obese group.
- The risk alleles of the *FTO* and *ANKK1* genes were positively associated with IR in eutrophic children.
- The T allele of the *FTO* gene has a protective effect against CRF in children with obesity
- The risk alleles of the *DRD2* and *ANKK1* genes conferred a higher risk of CRF in children with obesity and normal weight, respectively.

For being a case-control, our findings cannot ascribe causality, only association. Further studies are needed, with a more significant number of patients and longitudinal follow-up, to confirm the possible causality between the risk alleles and our findings.

It is hoped that in the future, more excellent knowledge of the specific contribution of each genetic variant will be a useful tool in clinical practice, being able to guide preventive measures and specific treatment according to genetic risk scores.

ACKNOWLEDGEMENTS

This research was supported by grants of CAPES and FAPEG/DECID/MS

- RMP and ADC designed the research (project conception, development of overall research plan, and study oversight).
- RMP conducted the clinical evaluation JSF, RVM, and LBM conducted the genetic protocols (hands-on conduct of the experiments).
- RMP, LBM, and ADC analyzed data or performed statistical analysis.
- RMP, MPC, and ADC interpreted data findings.
- RMP, JSF, MPC, and ADC wrote the paper.
- RMP and ADC had primary responsibility for final content. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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ISSN 2377-8385

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Case Report

Chylous Ascites Associated with Internal Hernia Post-Roux-en-Y Gastric Bypass: A Case Report

Ahmad E. Al-Mulla, MD[1*]; Mohammad Y. Saleh, MBChB, MSc, DIC[2]; Mohammad Zanki, MD[2]

[1]Department of Surgery, Farwaniya Hospital, Ministry of Health Kuwait, Sabah Al-Nasser, Block 6, P. O. Box 13373, Farwaniya 81004, Farwaniya, Kuwait
[2]Department of Surgery, Al-Amiri Hospital, Ministry of Health Kuwait, Al-Amiri Hospital, Arabian Gulf Street, P. O. Box 13041, Kuwait City, Kuwait

[*]***Corresponding author***
Ahmad E. Al-Mulla, MD
Senior Specialist General Surgery and Bariatric Surgery, Kuwait Board of General Surgery, Fellowship Minimal Invasive and Bariatric and Endoscopy, Department of Surgery Farwaniya Hospital, Ministry of Health Kuwait, Sabah Al-Nasser, Block 6, P. O. Box 13373, Farwaniya 81004, Farwaniya, Kuwait; Tel. 0096524888000, 00965-99833454; E-mail: draalmulla2007@gmail.com

Article information
Received: July 5th, 2021; **Revised:** July 23rd, 2021; **Accepted:** July 23rd, 2021; **Published:** July 23rd, 2021

Cite this article
Al-Mulla AE, Saleh MY, Zanki M. Chylous ascites associated with internal hernia post-roux-en-Y gastric bypass: A case report. *Obes Res Open J.* 2021; 8(1): 15-17. doi: 10.17140/OROJ-8-146

ABSTRACT

Chyloperitoneum is a rare intra-abdominal finding in internal hernia, only a few cases reports mentioned in the literature. It presents around 0.001-0.005% of hospital admissions. The presence of chylous ascites and swirl sign in a patient is a good indication of internal hernia and the bowel's validity.

Keywords
Chylous ascites; Chyloperitoneum; Internal hernia; Roux-en-Y gastric bypass.

INTRODUCTION

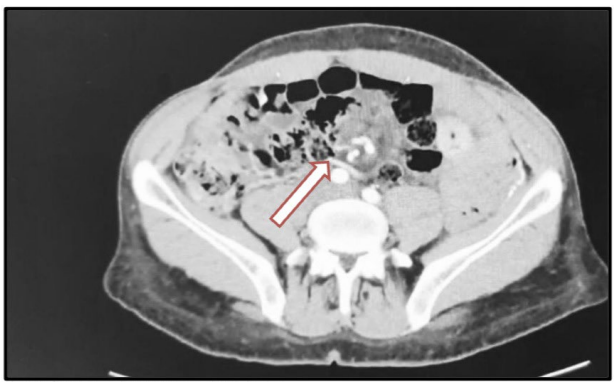
Chylous ascites or chyloperitoneum are defined as a milky fluid collection in triglycerides in the abdominal cavity.[1] It is responsible for 0.001-0.005% of hospital admissions.[2] Chylous ascites can be associated with many conditions such as malignancy, trauma, lymphatic injury, radiation, cirrhosis, and fibrosis but are rarely associated with bowel obstruction.[3] We discuss the course and management of a 31-year-old male patient who presents with chylous ascites due to small bowel obstruction post-Roux-en-Y gastric bypass.

CASE REPORT

A 31-year-old gentleman admitted *via* accident and emergency complaining of 8-months history of intermittent abdominal pain, which became severe in the past three-days, with 10-year background history of laparoscopic Roux-en-Y gastric bypass. The pain was severe colicky, associated with nausea and vomiting. He was vitally stable upon admission (Pulse 88 bpm, BP 130/88 mmHg and Temperature 37 °C). Abdominal examination, mild tenderness at the epigastric region, and distention. Laboratory investigation sent, the result was unremarkable, except for an increase in C-reactive protein (CRP) which was 9.9 mg/L (normal 0-8).

A computer tomography scan (CT-scan) urgently done, showing dilated and thickened small bowel loops, and swirling of the it to vessels and fat at the centre of the abdomen, mild pelvic fluids and enlarged mesenteric lymph node consisted of internal hernia (Figure 1). The surgical team discussed the result with the patient, and he agreed to go for surgery.

Figure 1. *CT-Scan Showing Central Abdominal Swirl Sign*



Intra-operatively we found a moderate amount of milky fluid at the pelvis and between bowel loops (Figure 2), no signs of perforation nor ischemia. As we reached Peterson's space, the mesentery and the small bowel loops engorged at the minor defect created from the previous laparoscopic Roux-en-Y gastric bypass (LRYGB) covered with thick white fluid (Figure 3). The bowel loops released were viable, with no ischemia or perforation.

Figure 2. *Intra-Abdominal Chylous Ascites*

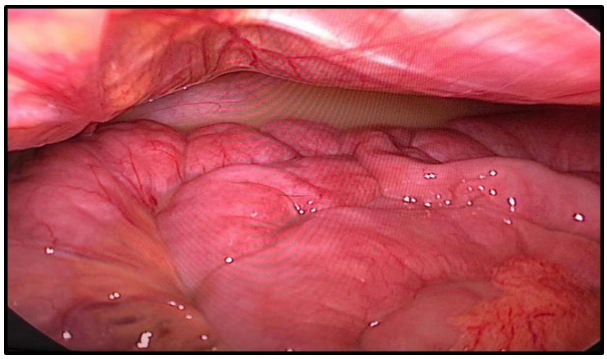
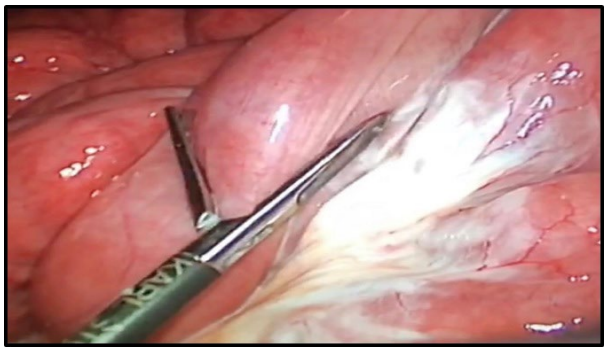


Figure 3. *Engorged Mesentery with Chyle Leaking at the Peterson's Space*



The defect was sutured continuously with a non-absorbable thread, and the fluids were aspirated and sent for culture and analysis.

POST-OPERATIVELY

Patient recovery was uneventful, and he received four days of broad-spectrum antibiotics (Tazocin 4.5 gm IV and metronidazole 500 mg IV). We discharged him after resuming a regular diet and opened his bowel.

First 2-weeks visit, uneventful recovery at home, with regular bowel habit and diet. Abdominal examination was unremarkable, and the wound was clean and healing well. Laboratory of abdominal fluid consist of diverse organisms, and fluid analysis was high for triglycerides 300 d/L consistent with chyle.

DISCUSSION

The disruption of abdominal cavity lymphatics causes chylous ascites. Lymphatic drainage starts from the intestinal trunk and further to the chyle cistern and then upwards to the thoracic trunk.[4] Interference to this pathway leads to chylous ascites. There is three mechanism of disruption which are: (1) Exudation of the lymphatics directly to the peritoneal cavity (2) obstruction of the lymphatic system due to neoplastic lesion (3) direct damage to the lymphatics due to trauma of surgery.[5] Clinical presentation of chylous ascites as described by Browse et al[6]: (1) painless abdominal distension (75%), (2) malnutrition/hypoproteinemia (60%), (3) dyspnea (46%), and (4) steatorrhea (46%). Diagnosed chylous ascites can be with CT-scans imaging by identifying the density of the intra-peritoneal fluid or directly *via* paracentesis, which appears as a turbid milky fluid, with a triglyceride level of >110 d/L.[1]

Chyloperitoneum due to small bowel obstruction because of Roux-en-Y gastric bypass is rare; just a few case reports mentioned in the literature.[6] Reconstruction of bowel limbs creates novel spaces, namely Peterson's space between the Roux limb and the transverse colon and jejuno-jejunostomy space. The incidence of internal hernia post laparoscopic Roux-en-Y gastric bypass is 0.2-11%.[7]

Chyloperitoneum due to small bowel obstruction is due to mesenteric engorgement with chyle, resulting in the white staining seen in the case report.[8] Akrama et al[3] described the presence of chyle as due to obstruction of the lymphatic, only with no disruption of the arteriovenous system. Hence, in internal hernia due to volvulus obstruction of the small bowel, the low-pressure systems are initially obstructed example, the lymphatics and venous and the high-pressure system are patent, such as the arterial. Thus, the presence of chylous ascites bowel indicated bowel viability, as we can confirm from the case report. Nevertheless, delay intervention in such cases may lead to sequential compromise of the arterial system leading to ischemia and perforation. Thus, prompt intervention is required.

CONCLUSION

Chylous ascites or chyloperitoneum is a rare presentation in patients with internal hernia. Only a few cases report mentioned in the literature, and it is a good sign of bowel obstruction and indicator of bowel viability.

INSTITUTIONAL BOARD PERMISSION

Yes.

CONSENT

The authors have received written informed consent from the patient.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Effects of *Garcinia Cambogia* Compounded Supplements on the Formation of Body Fat Induced by a High Energy Diet in Obese Rats

Wan-Li Chu, BSc[1*]; Wen-Chuan Lin, PhD[2]; Li-Chan Yang, PhD[2]

[1]*Health Take Corporation, 14 F, No. 192, Zhonggong 2nd Road, Xitun Dist., Taichung City 407, Taiwan, ROC*
[2]*Department of Pharmacy, China Medical University, Taichung, Taiwan, ROC*

[*]***Corresponding author***
Wan-Li Chu, BSc
Health Take Corporation, 14 F, No. 192, Zhonggong 2nd Road, Xitun Dist., Taichung City 407, Taiwan, ROC; Tel. 04-23585228 ext.109;
E-mail: sales8@healthtake.com.tw

Article information
Received: July 7th, 2021; **Revised:** August 13th, 2021; **Accepted:** August 20th, 2021; **Published:** September 18th, 2021

Cite this article
Chu W-L, Lin W-C, Yang L-C. Effects of *Garcinia cambogia* compounded supplements on the formation of body fat induced by a high energy diet in obese rats. *Obes Res Open J.* 2021; 8(1): 18-25. doi: 10.17140/OROJ-8-147

ABSTRACT

Background
Obesity is a public health concern in many countries. Obesity is often accompanied by other diseases and, in addition to its effects on personal health, also increases national health expenditure and medical costs. Currently, weight loss can be achieved through several medical means, such as gastric bypass surgery, liposuction, or the use of weight loss drugs. However, these options may lead to side effects or increased mortality. As such, the development of anti-obesity supplements that are natural and safe merits greater research attention. *Garcinia cambogia* extract, green coffee bean extract, mulberry leaf extract, chromium yeast, and wakame extract are known to have the potential to combat obesity and adjust physical constitutions; however, the effect on fat loss of these agents in a compound supplement has not been researched or discussed.

Objective
This study investigated the effects of a compound supplement (hereafter referred to as *Garcinia cambogia* compounded supplements (GC)) containing *Garcinia cambogia* extract, green coffee bean extract, mulberry leaf extract, chromium yeast, and wakame extract on fat accumulation induced by a high energy (HE) diet in rats.

Design
Six-week-old, male Sprague–Dawley rats were assigned to a control group or an experimental (HE) group. The control group comprised 12 rats who were given regular feed. The HE group comprised 36 rats who were given HE diet and were further divided according to whether they received carboxymethyl cellulose (CMC) or GC (305 and 1220 mg/kg, denoted as GC-L and GC-H, respectively) for 5-weeks. Starting from the sixth-week, the rats were tube-fed various dosages of GC. After the ninth-week, the rats' body weight, food intake, body fat mass, serum biochemical properties, and liver fat were analyzed.

Results
The results demonstrated that the HE+GC-L rats had significantly lower weight and body fat mass (569.5±51.3 g; 36.6±9.6 g) than the HE+CMC rats (618.5±57.1 g; 46.3±12.2 g). Food efficiency and calorie utilization were also significantly lower in the HE+GC-L rats than in the HE+CMC group ($p<0.01$). Compared with the HE+CMC group, food efficiency, calorie utilization, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and concentration of free fatty acids were also significantly lower in the HE+GC-H rats ($p<0.05$).

Conclusion
The GC supplementation significantly reduced body weight, body fat mass, body fat percentage, food efficiency, and calorie utilization in rats, and it thus has potential as a natural and safe plant extract dietary supplement. Its long-term effects on the human body should be investigated in the future.

Keywords
Obesity; *Garcinia cambogia* extract; Green coffee bean extract; Mulberry leaf extract; Chromium yeast; Wakame extract.

Obes Res Open J. 2021; 8(1): 18-25. doi: 10.17140/OROJ-8-147

INTRODUCTION

According to a World Health Organization (WHO) survey, the number of obese people in the world has tripled since 1975. In 2016, over 1.9 billion adults aged 18-years or older were overweight; 650 million of those overweight people were obese. In countries with the largest part of the world's population, more people die from being overweight or obese than from being underweight.[1] Among overweight or obese men and women, the prevalence of hypertension, type II diabetes, gallbladder disease, and osteoarthritis all rise sharply with increased weight.[2] Many obese people seek surgery or drug treatments, but gastric bypass surgery may lead to long-term vitamin and mineral deficiencies, post-operative complications, or even premature death.[3,4] Weight-loss drugs have also been reported to lead to uncomfortable side effects in the human body: for instance, Qsymia® is a combination of phentermine and topiramate, the adverse effects of the two medications individually including headache, insomnia, depression,[5] concentration; the side effects of taking orlistat include oily stools, diarrhea, stomach aches, and adverse reactions in the liver[6]; and Contrave® is a combination of bupropion and naltrexone, which may cause headache, vomiting, constipation, insomnia.[7] To prevent obesity and ensure the dietary safety of obese people, the development of natural and safe weight-loss supplements with no side effects merits further study, in the hopes that natural ingredients can help improve fat metabolism, control body weight, and reduce fat accumulation in obese people.

Garcinia cambogia is a plant that is commonly found in Southeast Asian countries and in India. There are approximately 400 Garcinia species are distributed around the world[8] and *Garcinia cambogia* is the species that can be legally used in Taiwan.[9] *Garcinia cambogia* is known for lowering blood lipids, combatting diabetes, and anti-inflammation.[8] *Garcinia cambogia* is rich in hydroxycitric acid (HCA), particularly the (−)-hydroxycitric acid isomer, which has antiobesity properties and can regulate serotonin, which is related to satiety, to reduce food intake. Furthermore, adenosine triphosphate citrate lyase (ATP citrate lyase) is a catalyst for the conversion of citric acid into acetyl-coenzyme A (Acetyl-CoA); it plays a key role in the synthesis of fatty acids, cholesterol, and triglycerides (TG). The HCA in *Garcinia cambogia* is an inhibitor of ATP citrate lyase and can reduce fat synthesis.[10]

Green coffee beans contain chlorogenic acid (CGA), which is the main polyphenol in coffee. This polyphenol can be found in various fruits and vegetables, such as grapes and strawberries.[11] Raw coffee beans have a higher CGA content, with *Coffee arabica* containing 3.5 to 7.5% in dry mass.[12] A related paper revealed that CGA can inhibit cholesterol synthesis in the liver, improve the balance of obesity hormones, and inhibit the absorption of cholesterol in the intestines.[13]

Wakame extract (*Undaria pinnatifida*) contains fucoxanthin, which can upregulate energy consumption and reduce excess lipids within white adipose tissue (WAT). These effects are partly induced by uncoupling protein 1 (UCP 1) in abdominal WAT. The body weight of mice with high energy (HE) diet–induced obesity was effectively reduced through the use of wakame extract containing fucoxanthin.[14]

The amount of glucose in the blood is also related to obesity. Excess glucose enters fat cells and is stored as fat, and obesity tends to lead to a decrease in insulin sensitivity, resulting in an increase in insulin resistance and blood sugar, which can cause more damage to the human body. As such, adding mulberry leaf extract (*Morus alba L.*) and chromium yeast, which can regulate blood sugar, in supplements can help obese people regulate their blood sugar. Mulberry leaf extract is known to contain l-deoxynojirimycin (DNJ), which is an effective inhibitor of α-glycosidases (sucrase, maltase, and glucoamylase) in the intestines.[15] Chromium supplements can increase insulin metabolism and reduce the risk factors of cardiovascular diseases among obese people.[16]

The benefits of these ingredients to the human body are already known, but no research has investigated the effect of these ingredients in a compound supplement (hereafter GC) on fat and blood sugar. The purpose of this study was to evaluate the effects of GC in reducing body fat accumulation, using Sprague–Dawley rats who were given HE diet to induce body fat accumulation.

MATERIALS AND METHODS

Supplement Composition

Garcinia cambogia compounded supplements (GC) was provided by HealthTake Corporation (738.8 mg per tablet). The formula contains *Garcinia cambogia* extract, green coffee bean extract, wakame extract, mulberry leaf extract, and chromium yeast. Each tablet also contains 220 to 330 mg of HCA and 20 to 30 mg of CGA.

Study Design

This animal experiment project was approved by the Institutional Animal Care and Use Committee (approval number: CMUIACUC-2018-357) of China Medical University (CMU) and was conducted in accordance with CMU laboratory animal ethics and guidelines. Six-week-old male Sprague–Dawley rats were purchased from BioLASCO Taiwan and kept in an animal room at CMU. The animal room had a set temperature of 22 °C±2 °C and 12-hours of light starting from 8 AM and 12-hours of darkness starting from 8 PM. After allowing the animals 1 week of adaptation, the HE group was started on HE diet (formula: D12492 ; Research Diets Inc., New Brunswick, NJ, USA), which contained 5.24 Kcal/g of energy, comprising 20% protein, 60% fat, and 20% carbohydrates. The control group was given a regular maintenance diet (formula: Altromin 1320; Altromin Spezialfutter GmbH & Co. KG; Lage, Germany). Altromin 1320 contains 2.85 Kcal/g of energy, comprising 24% protein, 11% fat, and 65% carbohydrates. The drinking water was autoclaved.

The experiment animals were divided into 2 groups, with 12 rats in the control group and 36 rats in the HE group.[17] The control group rats were given regular feed, and the HE group was given HE diet. During the first 4-weeks, the rats were 3 to a cage; from the fifth to ninth week, each rat was in its own cage

to facilitate the measuring of its food intake and food efficiency. After 5-weeks on the HE diet, the control group continued receiving regular feed, whereas the HE group was further divided into 3 groups of 12 rats on the basis of whether they received supplements and the relevant dosage. One group was given 0.5% carboxymethyl cellulose (CMC). The oral dosage of GC for the rats was calculated according to the human-to-rat metabolism conversion rate of 6.2 and the recommended daily GC dosages for humans (4 tablets, 738.8 mg per tablet). The daily dosage for rats was 2955.2 mg/60 kg (human body weight)×6.2=305 mg/kg. Thus, 305 mg/kg was the rat equivalent of one human dose. The daily dosages in this study were 305, and 1220 mg/kg, which were equivalent to 1, and 4 times the human dosages and were denoted as GC-L, and GC-H, respectively. The samples were prepared using a 0.5% CMC solution into suspensions of 30.5, or 122.0 mg/mL concentrations. The rats were fed using gastric tubes once a day, and the cast volume was 1 mL/100 g body weight. The control group were given the same volume of CMC solution. After 4-weeks of casting, the experiment animals were euthanized; prior to being euthanized, the animals fasted for 1 night. While the rats were under anesthesia, blood samples were collected from the celiac artery for blood biochemical analysis. The epididymal, perirenal, and mesenteric adipose tissue within the peritoneal cavity was also extracted and weighed on a precision scale to determine the body fat percentage. The experiment design was showed in Figure 1.

Body Weight and Food Intake

During the experiment period, the rats were weighed once a week to determine the test substance amount to be administered that week and for comparing the rats' weights at the start and end of the experiment. During the fifth to ninth week, the rats' daily food intake was also recorded to calculate the daily average food intake of each rat each week. At the end of the experiment, the rats' food efficiency during the 4-weeks of consuming the test substance was calculated using the following formula: food efficiency(%)=(weight gain/food intake)×100.

Caloric Intake

When converting food intake into caloric intake, the conversion was 2.85 Kcal/g for the regular feed and 5.24 Kcal/g for the HE diet. For the groups that were administered the test substance, the caloric conversion for GC was 3.07 Kcal/g, and the amount that was administered was based on the rat's body weight that week. At the end of the experiment, the caloric efficiency of the rats during the 4-weeks of consuming the test substance was calculated using the following formula : Kcal efficiency(%)=(weight gain/Kcal intake)×100.

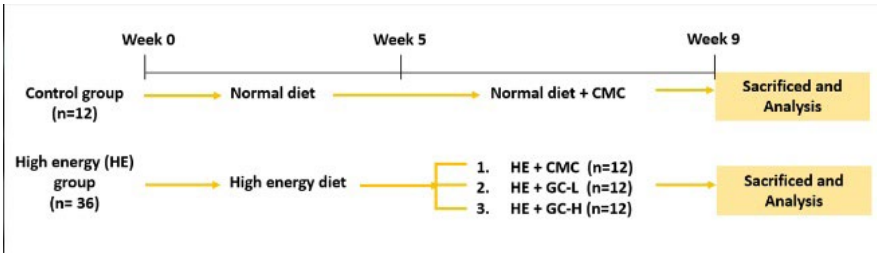
Measurement of Liver Fat Concentrations

After blood samples were collected from the celiac artery, the livers were rinsed with saline and stored at -80 °C for later use. Following the method employed by Folch et al,[18] after the lipids were extracted, the concentrations of TG and cholesterol in the liver were measured[18] by taking 0.1 g of liver tissue and adding 2 mL of extraction solvent (chloroform : methanol=2 : 1) from each rat. After the samples were homogenized with a homogenizer, they rested at room temperature for 60-minutes; the samples were subsequently placed in a centrifuge at 5000 rpm for 5-minutes. The top solution was placed in clean 1.5 mL centrifuge tube, and 0.2 mL of 0.9% sodium chloride (NaCl) was mixed into the solution. At this point, the liquid became cloudy. After being centrifuged at 2000 rpm for 5-minutes, the solution was divided into 2 layers. The bottom solution was retained and placed in a dry heater, where it was blown dry at 55 °C using nitrogen. After the solution was dried, the dried substance was combined with 100 μL of a solution of tert-butyl alcohol : triton X-100 : methanol (2 : 1 : 1) and heated at 65 °C for 15-minutes until completely dissolved. The new solution was tested using commercially available cholesterol and TG reagents (Roche).

Measurement of Serum Biochemical Values

Blood samples from the rats were processed in the centrifuge at 4700 rpm for 15-minutes to extract the plasma for the biochemical tests. Alanine aminotransferase (ALT), aspartate aminotransferase, TG, uric acid (UA), and creatinine in the serum were analyzed using commercially available diagnostic reagents (Roche, Germany) and a chemistry analyzer (Cobas Mira Plus, Roche, Switzerland). Free fatty acids (FFA) were measured using a non-esterified fatty acid kit (RANDOX, County Antrim, UK). Total cholesterol (T-Cho), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured using commercially available reagent kits (Fortress Diagnostics Limited, Antrim, UK). Ketone bodies were tested using a commercially available β-Hydroxybutyrate colorimetric assay kit (Cayman Chemical, MI, USA). Concentrations of sodium (Na) and potassium (K) in the

Figure 1. *Schematic Representation of the Experimental Design Timeline*



Control, normal diet with CMC; HE + CMC, high energy diet with CMC; HE + GC-L, high energy diet with 305 mg/kg/day of GC; HE + GC-H, high energy diet with 1220 mg/kg/day of GC.

blood were measured using enzyme methods with commercially sold reagent kits (Fortress Diagnostics Limited, Antrim, UK). Blood sugar was analyzed using a glucose analyzer (model 1500: Sidekick Glucose Analyzer; YSI. Yellow Spring, OH, USA).

Tissue Section Dyeing

The fat tissue around epididymis was extracted, set with formalin, and subsequently embedded in paraffin and cut into sections. The fat tissue sections were applied with a hematoxylin and eosin (H&E) stain and placed under a light microscope for observation.

Statistical Analysis

Results are expressed as the mean±SD. All experimental data were analyzed by one-way analysis of variance using the Dunnett's test, provided the data passed a normality test. A value of $p<0.05$ was used to indicate statistical significance between groups.

RESULTS

Body Weight Changes

The control group and the 3 HE groups exhibited no differences in body weight at the beginning of the experiment (Table 1). From the first to ninth-week, the rats in the HE+CMC group exhibited significantly higher body weights than the control group. After being fed an HE diet for 5-weeks, the 3 HE groups exhibited no differences in average body weight. The 2 groups who were administered GC had significantly lower average body weights than the HE+CMC group after continuing the HE diet from the seventh to ninth-week. In the ninth week, the rats in the HE+GC-L group had significantly lower average body weights than those in the HE+CMC group ($p<0.05$). After continuing on the HE diet from the seventh to ninth-week, the rats in the HE+GC-L group had significantly lower average body weights than rats in the HE+CMC group ($p<0.05$).

Table 1. *Effects of Supplements on Body Weight in Rats Fed with HE Diet*

Weeks	Body Weight (g)			
	Control	HE+CMC	HE+GC-L	HE+GC-H
Week 0	187.7±4.1	188.4±4.1	188.3±6.9	190.5±4.8
Week 1	256.6±9.9	272.6±10.3$^{\#\#}$	271.9±9.5	271.1±10.6
Week 2	280.5±13.7	310.1±16.7$^{\#\#}$	313.8±35.9	312.9±15.6
Week 3	346.0±19.8	392.3±22.5$^{\#\#\#}$	391.1±36.5	389.3±22.4
Week 4	376.3±20.7	437.7±26.8$^{\#\#\#}$	433.1±38.4	431.8±23.8
Week 5	399.8±21.7	479.7±28.7$^{\#\#\#}$	472.8±41.1	472.2±26.7
Week 6	427.0±30.6	532.9±42.1$^{\#\#\#}$	499.7±46.9	500.1±29.9
Week 7	451.5±33.9	568.4±45.4$^{\#\#\#}$	529.7±49.0	522.9±30.9*
Week 8	467.5±38.8	594.0±48.7$^{\#\#\#}$	552.7±52.1	543.8±33.8*
Week 9	477.9±42.0	618.5±57.1$^{\#\#\#}$	569.5±51.3*	561.9±37.1*

Control, normal diet with CMC; HE+CMC, high energy diet with CMC; HE+GC-L, high energy diet with 305 mg/kg/day of GC; HE+GC-H, high energy diet with 1220 mg/kg/day of GC. Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared with the control group. $^{*}p<0.05$ as compared with the HE+CMC group.

Food Efficiency and Caloric Efficiency

The HE rats were given HE diet for 9-weeks. After the first 5-weeks on the HE diet, the rats were started on 0.5% solution of CMC or GC for the remaining 4-weeks. The rats' weight gains were calculated by subtracting their body weight at the end of the first 5-weeks from their body weight at the end of the total 9-weeks; total food intake was the sum of the food intake from the sixth to ninth-week. As presented in Table 2, the total weight gain in the HE+CMC group was significantly higher than in the control group ($p<0.001$). The total weight gains in the GC groups were significantly lower than in the HE+CMC group ($p<0.01$). From the sixth to ninth-week, the total food intake of the HE+CMC group was significantly lower than that of the control group, but the total caloric intake was significantly higher in comparison. The GC groups and the HE+CMC group demonstrated no difference in total food intake and total caloric intake. Food efficiency and caloric efficiency were calculated according to total weight gain, total food intake, and total caloric intake during the fifth to ninth-week. The HE+CMC group demonstrated significantly higher food efficiency and caloric efficiency than the control group ($p<0.001$), and the GC groups' food efficiency and caloric efficiency were significantly lower than those of the HE+CMC group ($p<0.01$).

Changes in Body Fat and Liver Indicators

As indicated in Table 3, the weights of perirenal and mesenteric adipose tissue and the total weight of body fat were significantly lower among the rats in the HE+GC-L group than in the HE+CMC group ($p<0.05$). In the HE+GC-H group, the weights of epididymal, perirenal, and mesenteric adipose tissue and the overall weight and percentage of body fat were significantly lower than those in the HE+CMC group ($p<0.05$). The HE+CMC group exhibited significantly higher absolute liver weight, cholesterol levels in the

Table 2. *Effects of Supplements on Food Efficiency and Caloric Efficiency in Rats Fed with HE Diet*

Treatments	Weight Gain (g)	Food Intake (g)	Feed Efficiency (%)	Calorie Intake (Kcal)	Calorie Efficiency (%)
Control	78.2±28.4	866.4±75.5	8.9±2.5	2469.0±214.8	3.1±0.9
HE+CMC	138.8±34.4$^{\#\#\#}$	605.2±30.5$^{\#\#\#}$	22.8±4.8$^{\#\#\#}$	3170.7±160.9$^{\#\#\#}$	4.4±0.9$^{\#\#\#}$
HE+GC-L	96.8±20.3**	594.1±57.0	16.3±3.1***	3125.0±296.6	3.1±0.6**
HE+GC-H	89.8±21.6***	584.9±47.4	15.3±3.6***	3118.3±248.8	2.9±0.7***

Control, normal diet with CMC; HE+CMC, high energy diet with CMC; HE+GC-L, high energy diet with 305 mg/kg/day of GC; HE+GC-H, high energy diet with 1220 mg/kg/day of GC. Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#\#\#}p<0.001$ as compared with the control group. $^{**}p<0.01$, $^{***}p<0.001$ as compared with the HE+CMC group.

Table 3. *Effects of Supplements on Body Fat Mass and Body Fat Percentages in Rats Fed with HE Diet*

Treatments	Weight of Adipose Tissue			Body Fat (g)	Body Fat Ratio (%)
	Epididymal Adipose Tissue (g)	Perirenal Adipose Tissue (g)	Mesenteric Adipose Tissue (g)		
Control	4.9±1.2	5.4±1.4	2.7±0.8	13.0±3.0	2.7±0.5
HE+CMC	15.1±4.6$^{\#\#\#}$	22.5±5.6$^{\#\#\#}$	8.6±2.6$^{\#\#\#}$	46.3±12.2$^{\#\#\#}$	7.4±1.4$^{\#\#\#}$
HE+GC-L	12.9±3.3	17.1±4.9*	6.7±1.9*	36.6±9.6*	6.4±1.1
HE+GC-H	11.4±2.7*	17.3±5.0*	6.1±1.7**	34.8±8.6*	6.2±1.2*

Control, normal diet with CMC; HE+CMC, high energy diet with CMC; HE+GC-L, high energy diet with 305 mg/kg/day of GC; HE+GC-H, high energy diet with 1220 mg/kg/day of GC. Body fat defined as the sum of epididymal adipose tissue, perirenal adipose tissue, mesenteric adipose tissue. Body fat ratio (%) defined as (Body fat (g)/Body weight (g))×100. Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#\#\#}p<0.001$ as compared with the control group. $^{*}p<0.05$, $^{**}p<0.01$, as compared with the HE+CMC group.

liver, and TG concentrations in the liver than the control group (Table 4). Absolute liver weight, cholesterol levels in the liver, and TG concentrations in the liver were lower in the GC groups than in the HE+CMC group.

Table 4. *Effects of Supplements on Liver Concentrations of Triglycerides and Cholesterol in Rats Fed with HE Diet*

Parameters	Control	HE+CMC	HE+GC-L	HE+GC-H
Liver (g)	12.2±1.9	15.3±2.8$^{\#\#}$	14.1±2.7	14.1±1.8
Liver (%)	2.6±0.2	2.5±0.3	2.5±0.4	2.5±0.3
Cholesterol (mg/g tissue)	2.4±1.9	13.8±2.0$^{\#\#\#}$	12.2±1.9	11.6±2.7
Triglyceride (mg/g tissue)	14.8±7.6	42.7±5.7$^{\#\#\#}$	41.3±5.0	37.8±2.6

Control, normal diet with CMC; HE+CMC, high energy diet with CMC; HE+GC-L, high energy diet with 305 mg/kg/day of GC; HE+GC-H, high energy diet with 1220 mg/kg/day of GC. Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared with the control group.

Table 5. *Effects of Supplements On Plasma Biochemical Values in Rats Fed with HE Diet*

Parameters	Control	HE+CMC	HE+GC-L	HE+GC-H
AST(U/L)	138.8±32.6	204.3±41.8$^{\#\#\#}$	177.2±32.0	157.3±38.6*
ALT(U/L)	40.8±7.3	41.1±7.0	38.3±4.9	41.2±7.3
TG (mg/dL)	39.4±14.2	59.7±24.0$^{\#}$	50.0±7.8	35.5±7.9**
T-Cho (mg/dL)	42.4±8.5	55.1±5.7$^{\#\#\#}$	47.8±6.9*	44.0±5.1***
LDL-C (mg/dL)	8.8±2.5	17.0±8.2$^{\#\#\#}$	13.3±3.3	10.6±3.7**
HDL-C (mg/dL)	25.3±5.7	26.8±3.9	24.4±4.7	25.4±3.9
FFA (mmol/L)	0.45±0.09	0.68±0.17$^{\#\#\#}$	0.58±0.19	0.48±0.11**
KB (nmol/mL)	471.1±165.1	780.8±167.6$^{\#\#\#}$	779.0±159.4	735.2±162.2
Crea (mg/dL)	0.28±0.06	0.37±0.05$^{\#\#}$	0.33±0.06	0.33±0.05
UA (mg/dL)	1.54±0.31	1.86±0.50	1.90±0.33	1.75±0.29
Na(mEq/L)	143.6±1.4	144.2±0.7	143.8±1.5	143.3±1.1
K(mEq/L)	4.7±0.3	4.6±0.4	4.7±0.2	4.5±0.3
Glu (mg/dL)	124.4±15.4	154.7±25.3$^{\#\#\#}$	140.0±14.9	133.5±11.5*

Control, normal diet with CMC; HE+CMC, high energy diet with CMC; HE+GC-L, high energy diet with 305 mg/kg/day of GC; HE+GC-H, high energy diet with 1220 mg/kg/day of GC. Values were expressed as means±SD, n=12 in each group. Data were analyzed by one-way analysis of variance using the Dunnett's test. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ as compared with the control group. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ as compared with the HE+CMC group. AST, aspartate aminotransferase; ALT, alanine aminotransferase; TG, triglyceride; T-Cho, total cholesterol; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; FFA, free fatty acid; KB, ketone body; Crea, creatinine; UA, uric acid; Na, sodium; K, potassium; Glu, glucose.

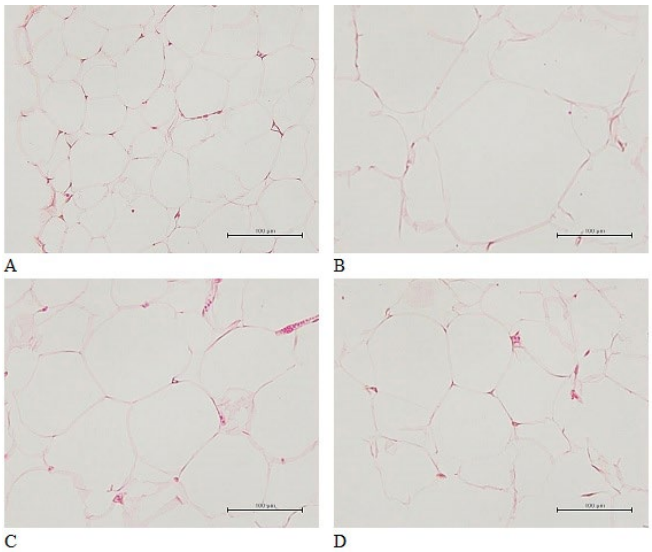
Serum Biochemical Tests

According to the 13 serum biochemical indicators in Table 5, the HE+CMC group and the control group did not differ in terms of ALT, HDL-C, UA, Na, or K; the remaining 8 indicators were higher in the HE+CMC group than the control group. The GC groups exhibited significantly lower serum T-Cho concentrations; the HE+GC-H groups had significantly lower serum concentrations of LDL-C and FFA, and all 2 dosages of GC demonstrated trends of lowering blood sugar, with the HE+GC-H dosage achieving significant decreases ($p<0.05$). No adverse effects were observed in this experiment.

Tissue Sections

The diameters of fat cells around the epididymis in the HE+CMC group were significantly greater than those of the control group (Figure 2). The fat cell diameters of rats in the 2 GC groups were significantly smaller than those of the rats in the HE+CMC group.

Figure 2. *Histological Observations of Epididymal Adipose Tissue (400x)*



(A) Control, normal diet with CMC; (B) HE+CMC, high energy diet with CMC; (C) HE+GC-L, high energy diet with 305 mg/kg/day of GC; (D) HE+GC-H, high energy diet with 1220 mg/kg/day of GC.

Obes Res Open J. 2021; 8(1): 18-25. doi: 10.17140/OROJ-8-147

DISCUSSION

The Sprague–Dawley rats used in this experiment had starting weights of approximately 188 g and were fed an HE diet (5.24 Kcal/g) for 9-weeks. The results in Tables 1 and 3 demonstrate that compared with the control group, the HE+CMC group gained 29.4% more weight and 256% more body fat (i.e., epididymal, perirenal, and mesenteric adipose tissue). The HE diet significantly increased the rats' body fat accumulation, and after 5-weeks on the HE diet, the rats were administered GC every day for the next 4-weeks. In the HE+GC-L group, body weight decreased by 7.9%, and body fat decreased by 20.9%. In the HE+GC-H group, body weight decreased by 9.2%, and body fat decreased by 24.6%. These results indicate that GC can considerably reduce body fat accumulation in rats.

Food efficiency refers to the degree that food is digested, absorbed, and utilized after entering the body. Higher percentages indicate that the food is more fully utilized in the body. During the period that the rats were administered GC, the rats' food and caloric efficiencies were calculated according to their total weight gain, total food intake, and total caloric intake during the fifth to ninth-week. The GC groups exhibited significantly lower food efficiency and caloric efficiency than the HE+CMC group. These results indicate that GC can lower food efficiency and caloric efficiency to reduce body weight and body fat accumulation.

An HE diet activates liver 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) and Sterol regulatory element binding protein-1c (SREBP1c), increasing the synthesis of cholesterol and TG.[19,20] Therefore, the rats in the HE+CMC group had significantly higher concentrations of liver cholesterol and liver TG than the control group rats, but the administration of GC had no effects on the synthesis of liver cholesterol and liver TG. HE feed caused the rats' absolute liver weight to increase, and studies have indicated that rats' liver weight and body weight are correlated[21]; therefore, analyzing weight gain on the basis of relative weight is more objective. No difference was observed in relative liver weights among the 2 GC groups and the HE+CMC group. The HE diet was associated with an increase in TG, T-Cho, LDL-C, and FFA in the blood, and the consumption of GC was associated with lower concentrations of TG, T-Cho, LDL-C, and FFA. The HE diet resulted in body fat accumulation in the rats and may have led to insulin resistance and increased blood sugar; the consumption of GC in the 2 treatment groups was significantly associated with lower blood sugar. Fat accumulation increases the release of FFA into the blood and reduces the clearance rate of FFA in the body.[22] As such, feeding rats with HE diet will cause obesity and an increase in FFA in the blood; however, GC can reduce the accumulation of fat and the concentration of FFA in the blood. The 2 dosages of GC overall demonstrated no effect on renal indicators, namely UA, creatinine, and electrolytes (Na and K concentrations in the blood). The H&E staining of the fat cells revealed that the HE+CMC group's fat cells were significantly larger than those of the control group. In the GC groups, the increase in the size of the fat cells was inhibited. In a meta-analysis of randomized controlled trials in humans, *Garcinia cambogia* supplements were found to significantly reduce body weight by 1.34 kg, body mass index (BMI) by 0.99 kg/m^2, and waist circumferences by 4.16 cm compared with the placebo group.[23] Moderately obese participants (aged 21-50-years, BMI>26 kg/m^2) were administered a compound of 4667 mg of *Garcinia cambogia* extract (containing 60% HCA, which is equivalent to 2800 mg of HCA each day), 4 mg of chromium nicotinate, and 400 mg of *Gymnema sylvestre* extract; this compound was able to significantly lower levels of T-Cho, LDL-C, TG, and serum leptin [24] which indicates that compound *Garcinia cambogia* and chrome supplements are effective and do not adversely affect the human body in this test. However, in 2019, there was an adverse event related to *Garcinia cambogia*. A woman overdose consumed more than 1500 mg of HCA per day, which led to visual disturbance.[25] In another literature review, some experiments related to *Garcinia cambogia* did not have a significant effect, but with exercise and divided consumption of *Garcinia cambogia* extract was more effective.[26] Considering related cases and complying with Taiwan's food regulations, the daily limit of *Garcinia cambogia* is 1500 mg HCA.[9] Therefore, it is designed to be the content of GC and can be used in human experiments with diet and exercise planning in the future, and observe the overall effect and influence.

CGA in green coffee beans can improve obesity-related hormone levels, inhibit the absorption of cholesterol in the intestines, and inhibit cholesterol synthesis in the liver.[13] Its mechanism of action likely involves activating AMP-activated protein kinase (AMPK) pathways to increase the transport of glucose among the muscles, which then inhibits G6Pase expression in the liver, reduces hepatic steatosis, improves glucose intake in the skeletal muscles, improves fasting tolerances of blood sugar and glucose, increases insulin sensitivity, and lowers fatty acid production.[27] Some clinical studies have revealed that obese people can lower their average weight by 5.4 kg by consuming coffee enriched with 500 mg of CGA every day. Coffee rich in CGA has significant effects on the absorption and utilization of glucose.[28] Animal experiments that involved rats on a high-cholesterol diet found that ingesting 1 to 10 mg/kg body weight/day of CGA can reduce T-Cho and LDL-C.[29] In the current study, each GC tablet contained 20 to 30 mg of CGA; the daily dosage of 4 tablets for adults thus contains 80 to 120 mg of CGA in total, which is the equivalent of 8.3 to 12.4 mg/kg body weight/day in rats, and is expected to achieve this cholesterol-lowering effect.

In a double-blind placebo-controlled study in Japan, obese adults (BMI>25 kg/m^2) were given capsules containing 1 mg or 3 mg of fucoxanthin or placebos for 4-weeks. The group that was given 3 mg of fucoxanthin exhibited significant reductions in their relative weight, BMI, and visceral fat area; total body fat, subcutaneous fat area, waist circumference, and leg circumference were also significantly lower in the group given 1 mg of fucoxanthin than in the placebo group.[30] Each GC tablet contained approximately 2 mg of fucoxanthin, which is expected to lower obesity parameters in obese people with long-term use. Prior studies have indicated that fucoxanthin, through the downregulation of lipoprotein receptors and scavenger receptor class B member 1, can significantly reduce the concentrations of plasma and TG in the liver. Fucoxanthin supplements can also lower the messenger ribonucleic acid (mRNA) expression of fatty acid synthase, an

enzyme that catalyzes fatty acid synthesis, and reduces the phosphorylation of insulin receptor substrate 1 to inhibit the intake of glucose by mature fat cells.[31]

Furthermore, among mice in which obesity was induced by a HE diet, consumption of mulberry leaf extract for 13-weeks was associated with a reduction in body weight, fat accumulation, and blood sugar as well as improved insulin sensitivity. Mulberry leaf extract can help manage obesity by activating brown adipose tissue, increasing gene expressions of UCP 1, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), peroxisome proliferator-activated receptor-gamma coactivator-1β (PGC-1β), peroxisome proliferator-activated receptor gamma (PPARγ), and regulating gut flora.[32] In another study, diabetic mice were given DNJ (50 and 100 mg/kg b.w./day), which is found in mulberry leaf extract. The results indicated that DNJ can be absorbed through the gastrointestinal mucosa and rapidly diffused into the liver to lower postprandial blood sugar and alleviate the toxicity of supraphysiological glucose to beta cells. This significantly reduces glycated hemoglobin.[15]

The GC supplement also contains chromium yeast, which use *Saccharomyces cerevisiae* as a carrier. Minerals are added to a fermentation medium, where it is absorbed by the live yeast and converted into a natural form. Chromium, possibly by bolstering the kinase activity of insulin receptor β (IR-β), increases the activity of the downstream effectors of insulin signals phosphatidylinositol 3-kinase (PI3K) and protein kinase B (Akt) and reinforces the transport of glucose transporter type 4 (Glut4) toward the surface of the cells. Chromium can also upregulate AMPK to increase glucose intake.[33]

LIMITATION

The current animal experiments revealed preliminary but significant results. In the future, the long-term effects of the GC supplement in the human body can be examined. We will further discuss simple plant extracts (e.g. green coffee bean extract, wakame extract) and GC supplement, whether there is a significant difference between them. The ability of GC compounds to improve obesity in humans merits further discussion.

CONCLUSION

The use of the recommended dosage of GC for the human body in HE diet-induced obese rats can evidently lower fat accumulation and body fat (epididymal, perirenal, and mesenteric adipose tissue), food efficiency and caloric efficiency, reduce the food absorption and utilization in these rats. As such, GC has potential as a novel weight-loss dietary supplement. In the future, GC supplements can be examined in human experiments, with in-depth research on the lasting effects and possible variable improvements on the human body.

ACKNOWLEDGEMENTS

This study was sponsored by HealthTake Corporation, who provided the GC used in this study.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE PERMISSION

Yes.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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ISSN 2377-8385

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Case Study

A Case Study of Inositol and Soluble Fiber Supplementation on Glycemic Control in an Overweight Subject

Haley Serra, MS, RDN;Yi Li, PhD*

Department of Nutrition and Dietetics, Saint Louis University, MO 63103, USA

Corresponding author
Yi Li, PhD
Assistant Professor, Department of Nutrition and Dietetics, Doisy College of Health Sciences, Saint Louis University, MO 63103, USA; Tel. 314-977-8675;
E-mail: yi.li@health.slu.edu

Article information
Received: November 22nd, 2021; **Revised:** December 8th, 2021; **Accepted:** December 9th, 2021; **Published:** December 16th, 2021

Cite this article
Serra H, Li Y. A case study of inositol and soluble fiber supplementation on glycemic control in an overweight subject. *Obes Res Open J.* 2021; 8(1): 26-31. doi: 10.17140/OROJ-8-148

ABSTRACT

Background
Soluble fiber has been shown to improve glycemic control by slowing the absorptions of glucose. And inositol has been shown to improve glycemic control in type 2 diabetes (T2D) and gestational diabetes via recruiting glucose transporter type 4 (GLUT4) to cell surface. However, neither inositol supplementation nor combination of inositol and soluble fiber supplementation has been studied in overweight.

Objective
To investigate if supplementation of inositol improves biological markers of glycemic control overweight and obesity, and that supplementation of inositol in combination with soluble fiber have synergistic effects to further improve these markers.

Design
A single cohort, uncontrolled, test-retest design was planned to be implemented over 5-weeks in which the participants supplemented 2 grams of myo-inositol twice daily for 4-weeks and then 2 grams of myo-inositol plus 2 grams of soluble fiber each twice daily for 1-week in overweight and obese subjects. Only one overweight subject was able to complete both phases of supplementation due to coronavirus disease-2019 (COVID-19), therefore the study is reported as a case study.

Results
Supplementation of 4 grams of myo-inositol daily for 4-weeks resulted in improved glucose parameters and lipid parameters including fasting blood glucose, post-prandial blood glucose, total blood cholesterol level, blood high-density lipoprotein (HDL) cholesterol level, and blood triglyceride level. The combination of inositol and soluble fiber supplementation further improved the total blood cholesterol level.

Conclusion
These results indicate there is potential benefit of inositol supplementation for sub-clinical hyperglycemic, overweight subjects on glycemic control.

Keywords
Dietary supplementation; Overweight; Glycemic control; Inositol; Soluble fiber; Type 2 diabetes (T2D); COVID-19.

INTRODUCTION

It is well-recognized that overweight and obesity are associated with impaired glucose control.[1] For healthy individuals, a normal fasting blood glucose level is between 70 and 100 mg/dL, and a normal post-prandial blood glucose level is between 100 and 140 mg/dL. In people with high body weight, insulin resistance develops due to excess adipose tissue resulting in the dysregulation of blood glucose levels.[2,3] Glucose is the primary fuel in the body for energy production in the cells, especially in the brain and red blood cells.[4,5] Glucose molecules circulate in the bloodstream supplying energy to the cells until extra glucose molecules are taken up by the tissues for storage.[5] Glycemic control is defined as the overall adequacy of which the body regulates blood glucose within a normal

range. Glucose homeostasis is regulated primarily by glucoregulatory hormones insulin and glucagon.[6] In muscle, insulin promotes glucose uptake and protein synthesis, in adipose tissue insulin promotes glucose and fatty acid uptake and inhibition of lipolysis, and in the liver insulin promotes glucose utilization and suppresses glucose production.[7] Glucagon facilitates the release of glucose from stored glycogen in the body to increase glucose concentration in the blood.[5,8] These hormones act in a negative feedback loop regulating one another. Disruptions in the signalling of these hormones, physical damage to the cells involved, and decreased effect of these mechanisms can all lead to glucose dysregulation. In overweight and obesity, body tissues become less sensitive to the effects of insulin resulting in decreased uptake of glucose from the bloodstream.[1] Furthermore, the presence of insulin does not properly inhibit glucagon action on the liver resulting in continued hepatic glucose output despite adequate glucose already present in the blood.[9] Impaired glycemic control is associated with disturbances in the metabolism of all three macronutrients, damage to vascular tissue including blood vessels, and disruption of organ function, all of which can then lead to chronic health conditions, such as cardiovascular disease, stroke, and type 2 diabetes (T2D).[5,10,11] Glucose dysregulation results in aberrations of lipid metabolism, which increases risk of atherosclerosis and cardiovascular disease.[11,12] Studies indicate there is a direct relationship among blood glucose, lipid metabolism, and pancreatic beta cell dysfunction.[10] Soluble fiber has been shown to improve glycemic control by slowing the absorption of glucose in the gastrointestinal (GI) tract thereby decreasing the glycemic response.[13] The effect of psyllium as a soluble fiber on glycemic control has been widely researched and has shown significant positive results.[14-16] Inositol, an organic stereoisomer of glucose, is present in all mammalian tissues and is found naturally existing in fruits, grains, beans and nuts.[17] Inositol plays a role in various biological processes such as signal transduction, cell growth, phospholipid synthesis, osteogenesis, reproduction, etc.[18] It has been identified as a factor in the insulin signaling pathway that regulates blood glucose levels and has been implicated as beneficial to glycemic control when taken as a dietary supplement in T2D, gestational diabetes, polycystic ovary syndrome, and metabolic syndrome.[19,20] Currently there is little research on the effects of inositol supplementation on glycemic control in those who are euglycemic or have pre-clinical hyperglycemia, or the effects of combining inositol with other dietary supplements such as soluble fiber.

The purpose of this study was to investigate first, the efficacy of supplementation of inositol by itself on glycemic control in overweight and obese subjects with pre-clinical impaired glucose control and second, the effects of supplementation of inositol in combination with soluble fiber. Due to coronavirus disease-2019 (COVID-19), only one overweight subject completed both phases of supplementation. The results from the subject demonstrated that supplementation of 4 grams of myo-inositol daily resulted in improved glucose parameters and lipid parameters including fasting blood glucose, post-prandial blood glucose, total blood cholesterol level, blood high-density lipoprotein (HDL) cholesterol level, and blood triglyceride level in the overweight subject. The combination of inositol and soluble fiber supplementation further improved the total blood cholesterol level although the synergistic effects were not seen for other parameters. These results indicate there is potential benefit of inositol supplementation for sub-clinical hyperglycemic overweight subjects on glycemic control and on control of blood lipid levels.

MATERIALS AND METHODS

Participants and Screening

The study was performed from January through March 2020. Overweight and obese men and women of all ethnic and racial groups aged 18-years or older with body mass index (BMI) equal to or greater than 25 kg/m^2 were recruited from the St. Louis, Missouri, USA metropolitan area by way of verbal interactions, hardcopy flyer distribution, and social media recruitment on Facebook. Volunteers were pre-screened by email using a potential participant questionnaire to determine provisional eligibility. This questionnaire assessed age, height, weight, medical history and current medication(s)/supplement(s). If volunteers met the stated criteria, they were scheduled for a formal on-site screening in the Physiological Laboratory at Doisy College of Health Sciences, Saint Louis University. At this time, the participants signed an informed consent document and Health Insurance Portability and Accountability Act (HIPAA) form approved by the Institutional Review Board (IRB) of Saint Louis University (Protocol ID # 30729). After obtaining of informed consent, the official screening was conducted by measuring body height, weight and fasting blood glucose levels. Fasting blood glucose levels were measured using blood samples obtained *via* finger stick method.[21] Inclusion criteria included a calculated BMI equal or above 25 kg/m^2. A fasting blood sugar greater than or equal to 126 mg/dL, the presence of any significant medical condition including a medical diagnosis of diabetes mellitus or taking any medications and/or dietary supplements that may interfere with glucose or lipid regulation were cause for exclusion.

Study Design

The study followed a single cohort, uncontrolled, test-retest design. The study lasted 5-weeks and included two phases of supplementation. The first phase lasted 4-weeks and the participants supplemented 2 grams of myo-inositol twice daily. The second phase lasted 1-week, and the participants supplemented 2 grams of myo-inositol plus 2 grams of soluble fiber each twice daily. The participants underwent 3 data collections including baseline assessment, 4-week follow-up assessment, and 5-week follow-up assessment (Figure 1).

Body Mass Index Calculation

BMI was calculated by using the equation BMI=kg/m^2. Body weight of human subjects was measured in pounds using a standard balance scale and body height was measured in centimetres with the height rod attached to the scale. Height in centimetres were converted to meters and weight in pounds were converted to kilograms.

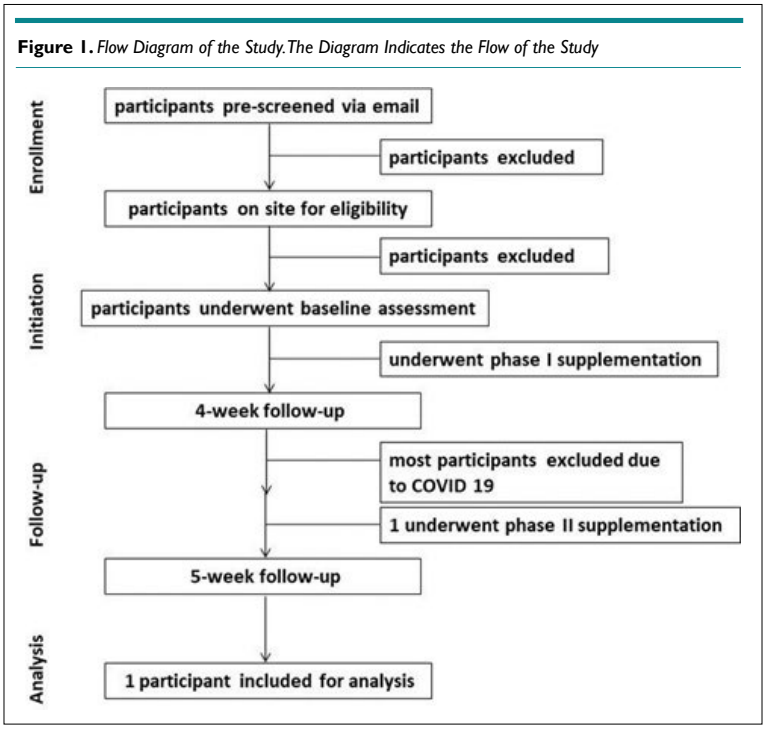


Figure 1. *Flow Diagram of the Study.The Diagram Indicates the Flow of the Study*

Glucose Tolerance Test

A modified glucose tolerance test was used to measure glycemic control of the participants.[22] The test was completed after overnight fasting. Participants drank a 10-ounce glucose drink containing 50 grams of dextrose (Thermo Fisher Scientific, Cat #: 401272P). The post-prandial measurements were taken exactly one hour after the drink was finished.

Analysis of Blood Samples

All blood samples were collected by finger stick method using disposable spring activated lancets. Approximately 15 μL of blood were used for each measurement. Analysis of all blood samples was conducted using a CardioChek (PTS Diagnostics, Cat #: 1708) to measure blood glucose concentration (mg/dL), total blood cholesterol concentration (mg/dL), blood HDL cholesterol concentration (mg/dL), and blood triglyceride concentration (mg/dL).

Nutrition Supplementation

It is considered very safe to supplement soluble fiber and inositol. Both soluble fiber and myo-inositol can be obtained without a prescription. It is recommended to orally take soluble fiber and myo-inositol up to 20 grams and 18 grams per day, respectively.[23] The supplement regime was broken into two phases. During Phase 1, lasting four-weeks, all participants were supplemented with 2 grams of myo-inositol orally twice daily. During phase 2, lasting one week, all participants were supplemented with 2 grams of soluble fiber orally twice daily in addition to the inositol regimen. Supplements were distributed at the beginning of each phase.

Participants were instructed verbally on the supplement regimen and given clearly written supplement instructions to take with them. To avoid reduction of inositol absorption by soluble fiber, inositol needed to be taken 1-hour before meals and soluble fiber needed to be taken after the meals. Supplements were counted and the exact number of intended supplements to take were distributed to each participant. Participants were instructed to return any remaining supplements at the end of each phase in order to account for missed doses. Supplements included Premium Supplements myo-inositol capsules (Norax Supplements, Newnan, GA, USA) and Equate Daily Fiber Capsules (Walmart, Cat #: 5570980260). A supplement log was distributed to all the participants in order to keep track of supplement intake and returned at the end of each phase.

Statistical Analysis

A statistical power analysis using G*Power Software (version 3.1.9.4) to calculate the sample size was performed based on the following inputs: *t*-tests (Means: Difference between two dependent means (matched pairs), alpha=0.05, desired power=0.70.[24] The results indicated that a total sample size of 12 would be required to detect large effects of supplementation (d=0.8). However, since only one participant completed the whole study due to COVID-19, the study is presented as a case study.

RESULTS

Study Participants

The flow diagram demonstrates the flow of the study participants (Figure 1). Due to COVID-19, only an overweight subject was able to complete both phase 1 and 2 supplementation. This participant was a 49-year-old female with a height of 168 cm, a weight of 78.6 kg, and a calculated BMI of 27.9 kg/m^2 (Table 1). The participant also indicated no presence of any significant medical condition including a medical diagnosis of diabetes mellitus and did not take

medications and/or dietary supplements that may interfere with glucose or lipid regulation. In terms of number of doses throughout the study, the adherence to the supplements was 99% since the participant only missed one dose of 2 grams myo-inositol. Therefore, the results of this study from the only one participant are reported as case study to present the valuable data without inferential statistical analysis.

Table 1. *The Demographics of the Subject*

Participant	Gender	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)
#3	Female	49	168	78.6	27.9

BMI: body mass index

Glucose Parameters

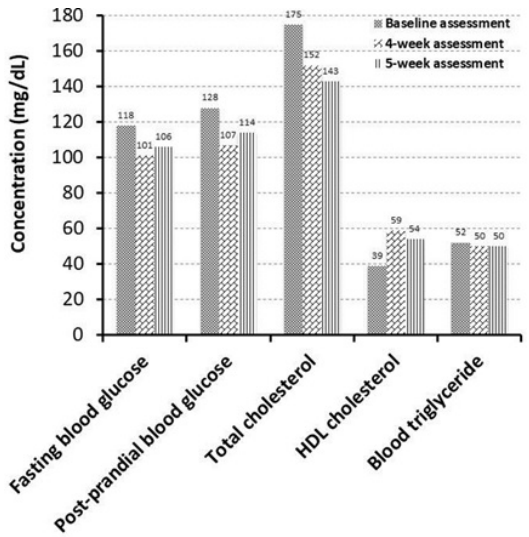
A decrease in both fasting blood glucose level (-14.4%) and post-prandial glucose level (-16.4%) were observed from baseline to the four-week point. Fasting blood glucose concentration decreased to 101 mg/dL from118 mg/dL after supplementing 2 grams of myo-inositol twice daily after phase 1 supplementation. Post-prandial blood glucose (after the 50 grams dextrose drink) decreased from 128 mg/dL at baseline to 107 mg/dL after phase 1 supplementation (Figure 2, Table 2). However, the further improvements were not observed after phase 2 supplementation. At the five-week follow-up, fasting and post-prandial blood glucose concentrations rose to 106 and 114 mg/dL respectively, (Figure 2, Table 2). These values were increased from the four-week values. However, they were still improved from the baseline numbers.

Table 2. *The Measurements of Blood Glucose Levels and Other Parameters*

	Baseline Assessment	4-week Assessment	5-week Assessment
Fasting blood glucose (mg/dL)	118	101	106
Post-prandial blood glucose (md/dL)	128	107	114
Total cholesterol (mg/dL)	175	152	143
HDL cholesterol (mg/dL)	39	59	54
Triglyceride (mg/dL)	52	<50	<50

BMI: body mass index

Figure 2. *The Effects of Inositol and Soluble Fiber Supplementation on Blood Glucose Levels and Other Parameters*



Fasting blood glucose, post-prandial blood glucose, total blood cholesterol, HDL cholesterol, and blood triglyceride levels were measured at the start of the supplementation (Baseline assessment), after supplementation of only inositol for 4 weeks (4- week assessment), and after supplementation of inositol plus soluble fiber for additional one week (5-week assessment).

Lipid Panel

Improvements in total cholesterol, HDL cholesterol, and triglyceride were seen from baseline to four weeks. Total cholesterol decreased from 175 mg/dL to 152 mg/dL after phase 1 supplementation, and then even further decreased to 143 mg/dL after phase 2 supplementation (Figure 2, Table 2). HDL cholesterol increased from 39 mg/dL at baseline to 59 mg/dL after phase 1 supplementation: however, a slight decrease was seen after phase 2 to 54 mg/dL (Figure 2, Table 2). A decrease in serum triglycerides was observed from baseline (52 mg/dL) to below 50 mg/dL after both phase 1 and phase 2.

DISCUSSION

Our results indicate that intervention with inositol to improve blood glucose regulation in the target population can be effective and therefore may prolong progression to prediabetes or T2D. Furthermore, improved glycemic parameters in this population can positively impact lipid parameters consequently. Glycemic control is one indicator of metabolic health. Overweight and obese are associated with the loss of glycemic control primarily through insulin resistance.[1] However, glucose regulation is a complex mechanism involving many different cell types, hormones, secondary compounds such as inositol, and lifestyle factors like diet and exercise. Because inositol is known to play a role in the insulin signaling pathway, and that dietary supplementation of inositol has been shown to improve glycemic control in conditions such as diabetes, we proposed that supplementing 4 grams of inositol daily for 4-weeks would result in improved glucose parameters. As soluble fiber decreases glycemic response by slowing absorption of exogenous glucose, supplementing 4 grams of soluble fiber daily in combination with the 4 grams of inositol would have even greater effects on these parameters due to a synergistic effect. Due to COVID-19, full data were only collected on one subject. The data collected from one subject is interpreted as a case study involving supplementation of inositol and soluble fiber on glucose and lipid parameters. If we interpret the results obtained from the only completed participant at face value, the correlation displayed between inositol supplementation and improved blood glucose parameters aligns with related studies as well as our hypothesis for this study. Inositol has shown benefits in glycemic control of T2D, and because overweight status is associated with pre-clinical hyperglycemia, we expected the same effects would occur in people of this population. As mentioned, previously, the subject studied was in the prediabetic range at baseline based on fasting blood glucose concentration. After phase 1 of supplementation the participant's

fasting blood glucose level was 101 mg/dL which is only 2 mg/dL above normal, indicating the participant was almost out of the prediabetic range. Furthermore, the subject had an HDL cholesterol level of 39 mg/dL at baseline, which was considered dyslipidemia.[5] At the 4-week point, HDL cholesterol increased to within normal range. Clinically, these results are very significant. These changes not only indicate improvement in glycemic control but decreased risk for developing T2D and cardiovascular diseases. If these results were replicated in a larger population of people who are prediabetic, then inositol supplementation may become one strategy for prevention of progression from prediabetes to diabetes. Combined supplementation of inositol and soluble fiber did not have the added benefit for most parameters that we expected. Inositol and soluble fiber supplements have different mechanisms of action on glycemic control. Inositol promotes glucose transporter type 4 (GLUT4) translocation to the cell membrane facilitating glucose uptake and soluble fiber binds to glucose in the GI tract slowing its absorption.[17] We hypothesized these mechanisms would have added benefit; however, the results did not reflect this hypothesis for most parameters. This could have been caused by reduced absorption of inositol by soluble fiber if the supplements were not taken far enough apart. That's why participants were instructed to take inositol one hour before meals and soluble fiber right after the meals. As a case study with one subject, one of the limitations is the great variance in dietary factors and physical activity. These factors may have a direct impact on blood glucose and lipid levels therefore could play a role in the change observed from baseline to follow-up measurements. And the purity and potency of nutritional supplements are not regulated by the Food and Drug Administration (FDA) as food and drugs are. Variations in the myo-inositol and soluble fiber capsules could be an unknown outlier in this study.

CONCLUSION

In conclusion, results from this study indicate inositol supplementation improves glycemic control and blood lipid profile, and supplementation of inositol plus soluble fiber further improved the total cholesterol level. These findings indicate that there is potential benefit of inositol supplementation for the sub-clinical hyperglycemic, overweight population on glycemic control, and that further research on these effects through future studies is warranted.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the participants who volunteered to this study, especially, the participant who completed the whole study. The authors also would like to thank Drs. Uthayashanker Ezekiel and Edward Weiss for their valuable suggestions regarding the study design and data analysis and thank Sabrina Hollar for assistance in editing the manuscript.

FUNDING

This work was supported by start-up funds from Saint Louis University to YL.

ETHICAL STATEMENT

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Board of Saint Louis University (Protocol ID # 30729). Written informed consent was obtained from all subjects.

AUTHOR'S CONTRIBUTION

HS and YL conceived and designed the study, interpreted the results, wrote the manuscript; HS conducted the study. All authors have critically reviewed the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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