UNIVERSIDADE FEDERAL DO PARANÁ

GABRIELLE ARAUJO DO NASCIMENTO

AVALIAÇÃO DO EFEITO DE POLIMORFISMOS NOS GENES *FTO, ABCA1*, *ABCA7* E *ABCG1* SOBRE INDICADORES DE OBESIDADE E DISLIPIDEMIAS EM CRIANÇAS E ADOLESCENTES SUBMETIDOS A TREINAMENTO FÍSICO

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Dissertação apresentada ao Programa de Pós-Graduação em Genética, Departamento de Genética, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientadora: Prof. Dra. Luciane Viater Tureck

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PARECER

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Genética da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de mestrado de GABRIELLE ARAÚJO DO NASCIMENTO, intitulada: "Avaliação do efeito e da associação entre polimorfismos dos genes FTO, ABCA1, ABCA7 e ABCG1 e indicadores de obesidade e dislipidemias em crianças e adolescentes submetidos a treinamento físico", após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua aprovação.

Curitiba, 27 de março de 2017

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Doutora Luciane Viater Tureck
Presidente

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RESUMO

A obesidade e as dislipidemias geralmente estão associadas, e na maior parte dos casos possuem origem complexa, sendo decorrentes da interação entre os fatores ambientais e fatores genéticos. Dentre os fatores genéticos já conhecidos encontram-se genes relacionados ao metabolismo, como o gene *FTO* (*Fat Mass and Obesity Associated*) e os genes dos transportadores ABC. Polimorfismos de nucleotídeo único (SNPs) no gene *FTO* foram associados com o ganho de peso, enquanto os transportadores ABC estão relacionados com o efluxo de colesterol, e, nesse trabalho, foram analisados SNPs dos genes *ABCA1*, *ABCA7* e *ABCG1*. Visto isso, o objetivo desse estudo é avaliar se há influência de polimorfismos nesses genes sobre variáveis antropométricas (índice de massa corporal ajustado para idade e sexo (IMC escore-Z), circunferência abdominal (CA), circunferência da cintura (CC), gordura corporal (GC) e massa magra (MM)) e bioquímicas (glicose em jejum, glicose 120, insulina em jejum, insulina 120, HOMA-IR (do inglês *homeostasis model assessment of insulin resistance*), QUICKI (do inglês *quantitative insulin sensitivity check index*) e perfil lipídico) de 557 crianças e adolescentes (eutróficos, sobrepeso e obesos) estudantes de escolas de Curitiba (PR), além de verificar o efeito de tais polimorfismos nas mudanças desses marcadores em resposta a um programa de exercícios físicos. A genotipagem foi realizada por ensaio de discriminação alélica. As análises estatísticas realizadas foram contagem direta dos genótipos, cálculo de frequência alélica, comparação de médias (teste T e teste Mann Whitney), análise de regressão múltipla e predição de risco. Todos os SNPs analisados promoveram variação significativa em alguma das variáveis analisadas. Com relação ao gene *FTO*, o alelo A do SNP rs9939609 foi associado a um aumento da insulina e HOMA-IR, e diminuição de QUICKI. Em relação aos genes dos transportadores ABC, o alelo C do SNP rs1800977 (*ABCA1*) foi associado a aumento no IMC escore-Z, CA, GC, insulina 120 e redução em QUICKI; o alelo A do SNP rs2230806 (*ABCA1*) foi associado a aumento no IMC escore-Z, CA e redução em %MM; o alelo C do SNP rs2279796 (*ABCA7)* foi associado à maior IMC escore-Z; o SNP rs692383 (*ABCG1)* foi associado à maior IMC escore-Z, CA, HDL-C, glicose, insulina e HOMA-IR e o alelo G do SNP rs3827225 (*ABCG1)* foi associado à maior VLDL-C e glicose. Com relação ao efeito na resposta aos exercícios físicos, os genes *FTO, ABCA7* e *ABCG1* não apresentaram interação, enquanto o alelo C do SNP rs1800977 (*ABCA1*) foi associado à maior redução de IMC escore-Z e maior aumento de QUICKI em resposta ao exercício e o alelo A do SNP rs2230806 (*ABCA1)* foi associado à maior ganho de MM. Nesse trabalho nós verificamos os efeitos dos polimorfismos analisados em variáveis relacionadas ao metabolismo (adiposidade, metabolismo da glicose e de lipídeos), sendo que alguns desses polimorfismos também interagiram com os programas de exercícios físicos aplicados. Os resultados obtidos corroboram e abrem novas perspectivas de estudo quanto ao papel da interação entre fatores ambientais e genéticos na prevenção e tratamento de patologias complexas, como a obesidade e as dislipidemias, no sentido de tornar tais medidas cada vez mais individualizadas.

Palavras chave: Obesidade, dislipidemias, exercício físico, *FTO, ABCA1, ABCA7, ABCG1,* rs9939609, rs1800977, rs2230806, rs2279796, rs692383, rs3827225.

ABSTRACT

Obesity and dyslipidemias are usually associated, and in most cases have complex origin, resulting from interaction between environmental and genetic factors. Among these already know genetic factors there are genes related to metabolism, such as *FTO* (Fat Mass and Obesity Associated) and the ABC transporters genes. Single nucleotide polymorphisms (SNPs) in *FTO* gene are associated to weight gain, while ABC transporters are related to cholesterol efflux, and SNPs in *ABCA1, ABCA7* and *ABCG1* genes were analyzed in this work. The objective of this study is to evaluate the influence of polymorphisms in these genes on anthropometric (body mass index adjusted for age and sex (BMI Z-score), abdominal circumference (AC), waist circumference (WC), fat mass (FM) and lean body mass (LBM)) and biochemical variables (fasting glucose, glucose 120, fasting insulin, insulin 120, HOMA-IR (homeostasis model assessment of insulin resistance), QUICKI (quantitative insulin sensitivity check index) and lipid profile) of 557 children and adolescents (normal weight, overweight and obese) in Curitiba (PR), and verify these polymorphisms effects in the changes of these markers in response to a physical exercise program. Genotyping was carried out by allelic discrimination assay. The statistical analyzes made were direct counting of genotypes, allelic frequency calculation, comparison of means (T test and Mann-Whitney test), multiple regression analysis and risk prediction. All the analyzed SNPs promoted significant variation in some of the variables. Regarding *FTO* gene, the rs9939609 SNP A-allele was associated to higher insulin and HOMA-IR, and reduced QUICKI. In relation to the ABC transporter genes, SNP rs1800977 C-allele (*ABCA1*) was associated to higher BMI-Z score, AC, FM and insulin 120 increase and QUICKI reduction; SNP rs2230806 (*ABCA1*) A-allele was associated to higher BMI-Z score and AC and %LBM reduction; SNP rs2279796 (*ABCA7*) C-allele was associated to higher BMI Z-score; SNP rs692383 (*ABCG1*) was associated to higher BMI Z-score, AC, HDL-C, glucose, insulin and HOMA-IR, and SNP rs3827225 (*ABCG1*) G-allele was associated to higher VLDL-C and glucose. Regarding the effect on physical exercise response, *FTO, ABCA7* and *ABCG1* genes did not shown interaction, whereas rs1800977 (*ABCAI*) C-allele was associated to higher reduction of BMI Z-score and increase in QUICKI in response to physical exercise and rs2230806 SNP (*ABCA1*) A-allele was associated to higher gain of LBM. In this study, we verified the effects of the polymorphisms analyzed on variables related to metabolism (adiposity, glucose metabolism and lipid metabolism), and some of these polymorphisms also interacted with the applied physical exercise programs. The results obtained corroborate and open new perspectives on the role of the interaction between environmental and genetic factors in the prevention and treatment of complex pathologies, such as obesity and dyslipidemias, in order to make these measures more individualized.

Key-words: Obesity, dyslipidemia, physical exercise, *FTO, ABCA1, ABCA7, ABCG1,* rs9939609, rs1800977, rs2230806, rs2279796, rs692383, rs3827225.

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ABC – *ATP-binding cassette*

AGs – Ácidos Graxos

AMPK – *AMP-activated protein kinase*

Apo - Apolipoproteína

CA – Circunferência Abdominal

CC – Circunferência da Cintura

CETP - C*holesteryl Ester Transfer Protein*

CT – Colesterol Total

DT2 - Diabetes Mellitus Tipo 2

EC – Ésteres de Colesterol

FCM – Frequência Cardíaca Máxima

FCR – Frequência Cardíaca de Reserva

FTO – *Fat mass and Obesity Associated*

GC – Gordura Corporal

GWAS - *Genome-wide Association Studies*

HDL – *High Density Lipoprotein*

HDL-C – *High Density Lipoprotein Cholesterol*

HIIT - *High-Intensity Interval Training*

HOMA-IR - *Homeostasis Model Assessment of Insulin Resistance*

IDL – *Intermediate Density Lipoprotein*

IGF-1 - *Insulin Growth Factor 1*

IMC – Índice de Massa Corporal

LCAT - L*ecithin Cholesterol Acyltransferase*

Lp (a) – Lipoproteína A

LDL – *Low Density Lipoprotein*

LDL-C – *Low Density Lipoprotein Cholesterol*

MM – Massa Magra

NBD – *Nucleotide-Binding Domains*

OMS – Organização Mundial da Saúde

PAD – Pressão Arterial Diastólica

PAS – Pressão Arterial Sistólica

PPARγ - *Peroxisome Proliferator-Activated Receptor Gamma*

QUICKI - Q*uantitative Insulin Sensitivity Check Index*

RM – Repetição Máxima

SIRT1 – Sirtuína 1

SNP – *Single Nucleotide Polymorphism*

TG – Triglicerídeos

TMD – *Transmembrane Domains*

TNF-α - *Tumor Necrosis Factor-α*

VLDL – *Very Low Density Lipoprotein*

VLDL-C – *Very Low Density Lipoprotein Cholesterol*

$VO_{2máx}$ - Volume Máximo de Oxigênio

SUMÁRIO

1 INTRODUÇÃO

A obesidade tornou-se um problema de saúde pública, visto que a sua prevalência vem aumentando de maneira preocupante nos últimos anos, devido à combinação de ambiente favorável ao seu desenvolvimento e fatores de predisposição genética (NETO *et al.*, 2012; WHO, 2015). É preciso destinar atenção especial ao aumento da prevalência dessa enfermidade em crianças e adolescentes, já que poderá trazer consequências também na vida adulta desses indivíduos (LEITE *et al*., 2009; WHO, 2015).

Um dos principais fatores desencadeantes da obesidade é a falta de equilíbrio entre a ingestão e o gasto de calorias (WHO, 2015). Em grande parte dos casos, encontra-se associada às dislipidemias (NETO *et al.*, 2012), que são caracterizadas por uma quantidade anormal de lipídeos no sangue (como colesterol e triglicerídeos (TG)) (TONKIN; BYRNES, 2014).

Tanto a obesidade quanto as dislipidemias são fatores de risco para as doenças cardiovasculares, que, por sua vez, são as causas mais frequentes de morbidade e mortalidade (LEITE *et al*., 2009). A obesidade e as dislipidemias comuns (que não são monogênicas) possuem etiologia complexa, sendo que a interação entre fatores ambientais e componentes genéticos resulta em perfis mais ou menos susceptíveis a essas doenças (UUSITUPA, 2005; XAVIER *et al*., 2013). Por isso, é importante o estudo de polimorfismos em genes relacionados ao metabolismo, já que os mesmos podem levar a modificações na quantidade e funcionalidade do produto gênico, ocasionando dessa forma impacto nas vias das quais estes produtos participam. Nesse sentido, emergem como genes candidatos importantes: gene *FTO* (*Fat Mass and Obesity Associated*), que já foi associado ao índice de massa corporal (IMC) (FRAYLING *et al*., 2007), e genes dos transportadores ABC (*ABCA1, ABCA7* e *ABCG1)*, que estão envolvidos com o efluxo de colesterol (TARLING; DE AGUIAR VALLIM; EDWARDS, 2013).

Considerando esse contexto, o presente estudo tem por objetivo verificar o efeito de polimorfismos de nucleotídeo único (*single nucleotide polymorphisms* – SNPs) nos genes *FTO, ABCA1, ABCA7* e *ABCG1* na variação de medidas antropométricas (IMC, circunferência abdominal (CA), circunferência da cintura (CC), porcentagem de gordura corporal (%GC), gordura corporal (GC), porcentagem de massa magra (%MM) e massa magra (MM)) e bioquímicas (glicemia em jejum,

glicemia 120, insulina em jejum, insulina 120, HOMA-IR (do inglês *homeostasis model assessment of insulin resistance*), QUICKI (do inglês *quantitative insulin sensitivity check índex*) e perfil lipídico) de 557 crianças e adolescentes (obesos, sobrepeso e eutróficos) do estado do Paraná, submetidos a diferentes programas de exercícios físicos supervisionados.

O tópico de “resultados e discussão” dessa dissertação foi apresentado na forma de capítulos, sendo que cada capítulo contém um artigo. O primeiro capítulo é um artigo conjunto, e, além da amostra de crianças e adolescentes analisada em toda a dissertação, foi incluída também uma amostra de mulheres obesas submetidas a uma intervenção dietética.

2 REVISÃO DE LITERATURA

2.1 OBESIDADE

A obesidade já é considerada uma epidemia pela Organização Mundial da Saúde (OMS) (SHAWKY; SADIK, 2012), visto que o número de indivíduos obesos vem atingindo proporções alarmantes tanto nos países de alta renda quanto nos países de baixa renda (BULBUL; HOQUE, 2014). Desde 1980, a prevalência global de obesidade quase dobrou, sendo que em 2014 mais de 1,9 bilhões de adultos estavam acima do peso. Desses, cerca de 600 milhões eram obesos (WHO, 2015). Dados de 2015 revelam que 53,9% dos brasileiros estão acima do peso, sendo que, destes, 18,9% são obesos (MINISTÉRIO DA SAÚDE, 2016). Devido às diversas implicações para a saúde associadas à obesidade, ela é estimada como a segunda principal causa de morte evitável (JAHANGIR; SCHUTTER; LAVIE, 2014).

A obesidade e o excesso de peso são definidos como um acúmulo de gordura anormal ou excessivo que representa risco para a saúde. Um indivíduo é considerado obeso quando seu IMC, que é calculado pelo peso (em quilogramas) dividido pelo quadrado da altura (em metros), é maior do que 30. Quando o indivíduo possui um IMC entre 25 e 30, é considerado acima do peso (WHO, 2015). Também existem outros critérios, menos utilizados, como peso corporal, medida da cintura, relação cintura-quadril, percentual de gordura e quantidade de gordura visceral e subcutânea (UUSITUPA, 2005).

Para as crianças e adolescentes usa-se o IMC escore-z, uma medida que considera a idade e o sexo, conforme mostram os GRÁFICOS 1 e 2. Dados o IMC, a idade e o sexo da criança, é possível determinar o IMC escore-Z através de tabelas (disponíveis em www.cdc.gov/growthcharts) (MUST; ANDERSON, 2006). De acordo com a OMS, a criança ou adolescente é considerado com peso normal (eutrófico) quando seu IMC escore-Z encontra-se entre -2 e +1, acima do peso quando está entre +1 e +2, e obeso quando se encontra acima de +2 (WHO, 2007).

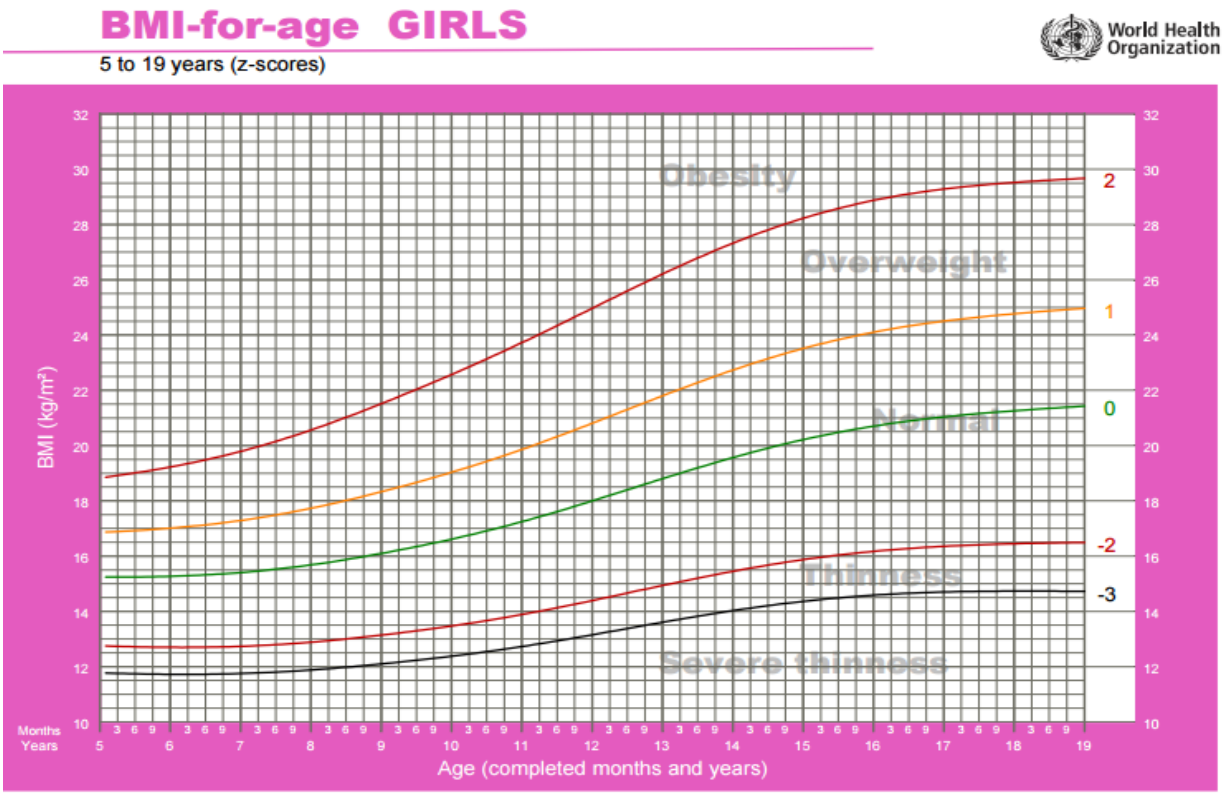

GRÁFICO 1 – IMC DE ACORDO COM A IDADE PARA MENINAS
Fonte: WHO, 2007.

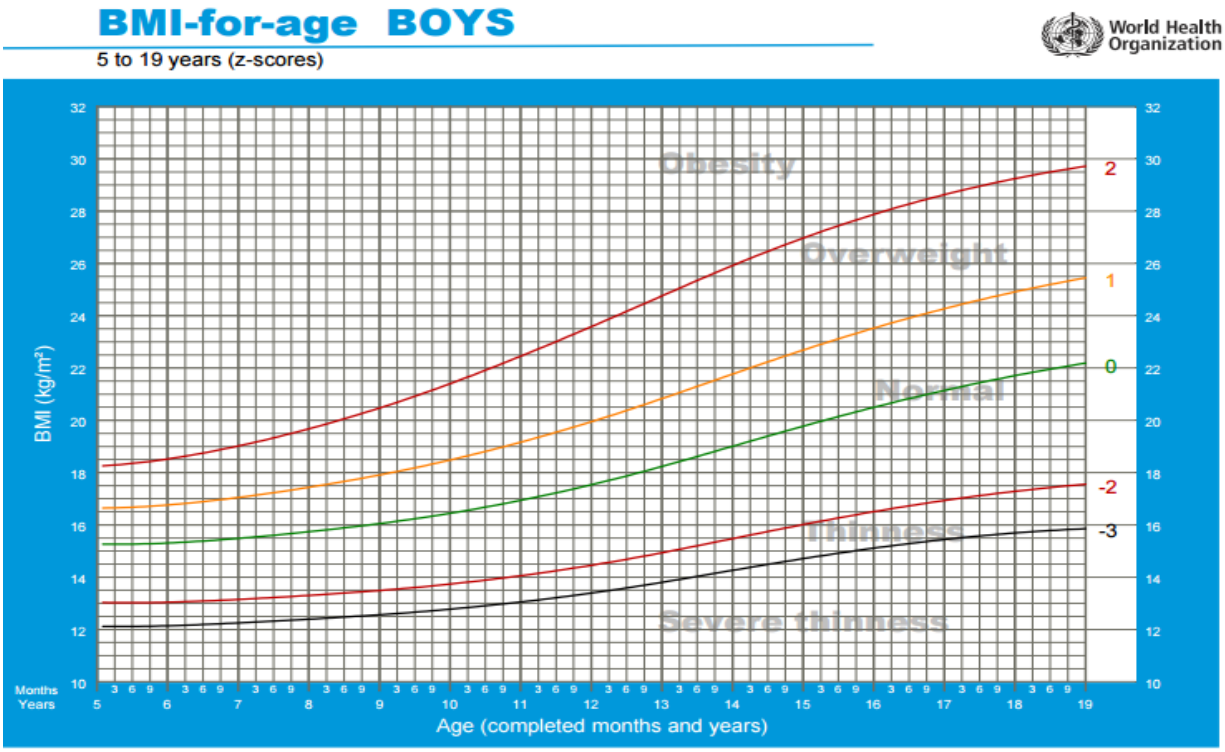

GRÁFICO 2 – IMC DE ACORDO COM A IDADE PARA MENINOS
Fonte: WHO, 2007.

A falta de equilíbrio entre a ingestão e o gasto de calorias é considerada o principal fator para o aumento da prevalência da obesidade, sendo que polimorfismos em genes que participam dessas vias metabólicas podem contribuir para esse desequilíbrio, ou de alguma forma compensá-lo, dependendo do perfil individual de susceptibilidade ou proteção que configurarem. Além de a população estar consumindo mais alimentos ricos em gordura, há uma diminuição na prática de atividades físicas devido ao aumento de formas de trabalho sedentárias, mudanças nos meios de transporte e urbanização (WHO, 2015). Nos países em desenvolvimento, a quantidade de indivíduos obesos ou acima do peso está crescendo rapidamente, apesar de ainda haver problemas como doenças infecciosas e desnutrição (WHO, 2015).

O que faz da obesidade um problema de saúde pública é o distúrbio metabólico que ela por si só desencadeia, e secundariamente o risco que ela representa para o desenvolvimento de outras doenças, como doenças cardiovasculares, asma, diabetes tipo 2 (DT2), dislipidemias, e alguns tipos de câncer (BIENERTOVÁ-VASKŮ *et al*., 2010; JUNG; CHOI, 2014). Parte dessas doenças impossibilita o indivíduo de realizar exercícios físicos, o que contribui ainda mais para o ganho de peso (BAIRDAIN *et al*., 2014).

2.1.1 Obesidade infantil

As crianças requerem uma atenção especial, pois, segundo a OMS, mais de 42 milhões de crianças com menos de cinco anos de idade estavam acima do peso em 2013 (WHO, 2015). A tecnologia presente no nosso dia-a-dia é um dos principais motivos pelos quais as crianças e adolescentes dedicam menos tempo a exercícios físicos, já que geralmente preferem um lazer passivo a um lazer ativo (MILANO, 2008; RIBAS; SILVA, 2014). Quando a criança é obesa, haverá maior risco de sofrer morte prematura e incapacidade na sua vida adulta, sendo que aproximadamente 70% das crianças e adolescentes obesos tornam-se adultos obesos (REILLY, 2007). Além disso, outros problemas podem ocorrer ainda na infância, como dificuldades respiratórias, aumento no risco de fraturas, hipertensão, marcadores precoces da doença cardiovascular, resistência à insulina e efeitos psicológicos (WHO, 2015).

Com relação ao Brasil, os dados mais recentes são de 2009, e mostram que um terço das crianças de 5 a 9 anos estava acima do peso (mais de oito vezes a

frequência de déficit de peso) e 14,3% estavam obesas, sendo que os índices foram maiores para meninos. Dentre os adolescentes (10 a 19 anos), um quinto estava com excesso de peso (seis vezes maior do que a frequência de déficit de peso) e 4,9% estavam obesos. Os maiores índices foram encontrados na população masculina e no grupo de 10 a 11 anos (IBGE, 2010).

Além dos problemas físicos causados pela obesidade, podem também estar presentes problemas psicológicos, como baixa autoestima, autoavaliação negativa, ansiedade e depressão (ABDEL-AZIZ *et al*., 2014).

2.1.2 Obesidade e dislipidemias

Há uma associação positiva entre excesso de peso e as dislipidemias, de forma que o sobrepeso reflete em alterações lipídicas (NETO *et al*., 2012). As dislipidemias são caracterizadas por um distúrbio no metabolismo lipídico e são uma das maiores responsáveis pelas doenças cardiovasculares, como aterosclerose (TONKIN; BYRNES, 2014).

Os lipídeos são transportados do tecido de origem, através de lipoproteínas, para os tecidos nos quais serão armazenados ou consumidos. As lipoproteínas são formadas por um núcleo central de lipídeos hidrofóbicos (como os TG e os ésteres de colesterol) que é envolvido por fosfolipídeos polares, colesterol livre e apolipoproteínas, conforme mostra a FIGURA 1. As lipoproteínas são classificadas, de acordo com sua densidade, em: lipoproteína de alta densidade (HDL, do inglês *high density lipoprotein*); lipoproteína de baixa densidade (LDL, do inglês *low density lipoprotein*); lipoproteína de muito baixa densidade (VLDL, do inglês *very low density lipoprotein*) e quilomícrons, que estão representadas na FIGURA 2. HDL e LDL são ricas em colesterol, enquanto VLDL e quilomícrons são ricas em TG (NELSON; COX, 2011; RANG *et al*., 2012; XAVIER *et al*., 2013). Há ainda a lipoproteína de densidade intermediária (IDL, do inglês *intermediary density lipoprotein*) e a lipoproteína (a) - Lp(a) -, que é formada pela ligação de LDL com a apolipoproteína A (apoA). A função fisiológica da Lp(a) ainda não é conhecida, mas estudos mostram associação com a aterosclerose (NELSON; COX, 2011; RANG *et al*., 2012; XAVIER *et al*., 2013).

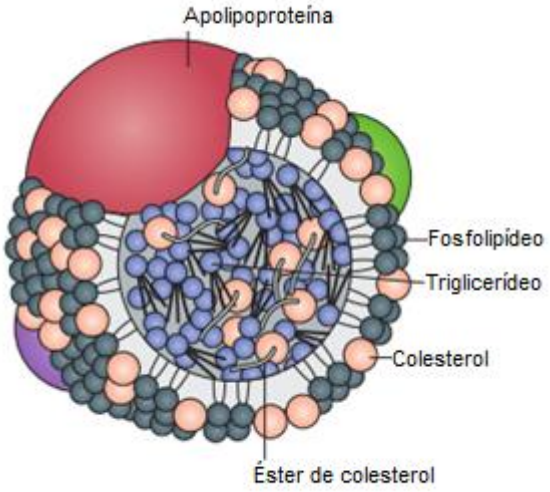

FIGURA 1 – COMPONENTES ESTRUTURAIS DAS LIPOPROTEÍNAS
FONTE: Adaptado de RIDKER, 2014.

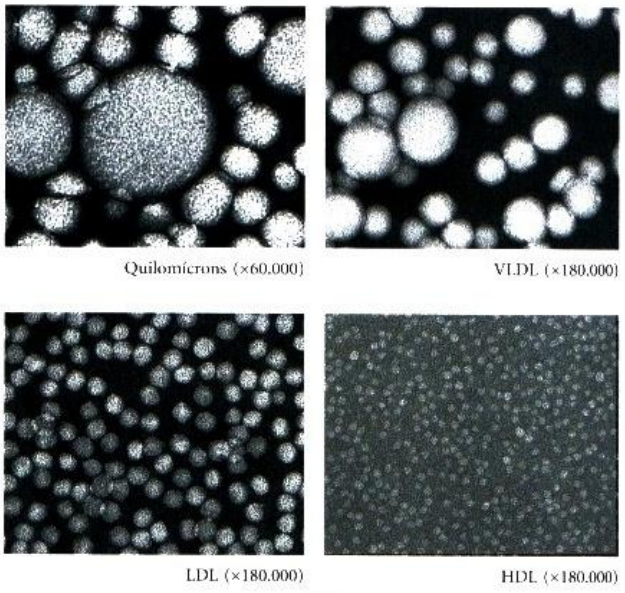

FIGURA 2 - QUATRO CLASSES DE LIPOPROTEÍNAS
FONTE: Adaptado de NELSON; COX, 2011.
NOTA: Visualização ao microscópio eletrônico após coloração negativa. No sentido horário, a partir da parte superior à esquerda: quilomícron, 50 a 200 nm de diâmetro; VLDL, 28 a 70 nm; HDL, 8 a 11 nm e LDL, 20 a 25 nm.

2.1.2.1 Metabolismo dos lipídeos

2.1.2.1.1 Via exógena ou intestinal

Os TGs obtidos através da dieta são hidrolisados pelas lipases pancreáticas em diglicerídeos, monoglicerídeos, ácidos graxos (AGs) livres e glicerol. Sais biliares emulsificam esses lipídeos formando micelas, o que facilita a absorção intestinal. Após a absorção, os AGs são utilizados na produção de quilomícrons, juntamente com o colesterol da dieta e apolipoproteínas, como a apolipoproteína B-48 (apoB48) (exclusiva dessa classe de lipoproteínas), apolipoproteína C-II (apoC2) e apolipoproteína E (apoE). Os quilomícrons seguem para grande parte dos tecidos, onde sofrem hidrólise pela lípase lipoproteica, liberando AGs livres e glicerol que podem ser armazenados, no caso do tecido adiposo, ou oxidados para obtenção de energia, a exemplo do que ocorre no músculo esquelético. Os remanescentes de quilomícrons, desprovidos da maioria dos seus TG, mas ainda contendo colesterol e apolipoproteínas, vão para o fígado. Há liberação de colesterol que pode ser armazenado, oxidado a ácidos biliares, secretado inalterado na bile ou ingressar na via endógena (MURRAY; GRANNER; RODWELL, 2007; NELSON; COX, 2011; RANG *et al*., 2012; XAVIER *et al*., 2013).

2.1.2.1.2 Via endógena ou hepática

No fígado, os TG oriundos da lipogênese (síntese de AGs a partir dos carboidratos), de AGs livres e de remanescentes dos quilomícrons juntam-se ao colesterol e apolipoproteínas para serem exportados na forma de VLDL (MURRAY; GRANNER; RODWELL, 2007). As partículas dessa lipoproteína seguem para a maior parte dos tecidos (como tecido adiposo e músculo esquelético), onde os TG são hidrolisados, dando origem a AGs e glicerol que são absorvidos. Devido à hidrólise, as partículas lipoproteicas ficam menores e tornam-se remanescentes de VLDL, também chamados de IDL. Estas são rapidamente removidas do plasma, e contém duas apolipoproteínas: apoB100 e apoE. A remoção adicional de TG das IDLs produz LDL (que ainda contém o complemento total de ésteres de colesterol e possui como única apolipoproteína a apoB100) (NELSON; COX, 2011). A LDL tem grande importância no processo aterogênico, mas, fisiologicamente, fornece o

colesterol para incorporação em membranas celulares e para a formação de esteróides, sendo captada por todos os tecidos. O colesterol pode retornar dos tecidos ao fígado por meio da HDL (que contém apoA1 e apoA2, entre outras apolipoproteínas), o que é chamado de transporte reverso do colesterol, ação que protege o leito vascular contra a aterogênese (NELSON; COX, 2011; RANG *et al*., 2012; XAVIER *et al*., 2013) (um esquema do metabolismo lipídico encontra-se na FIGURA 3). Estudos epidemiológicos mostram também que há uma relação inversa entre a concentração plasmática de HDL e o risco de doenças cardiovasculares (DI ANGELANTONIO *et al*., 2009; RANG *et al*., 2012;).

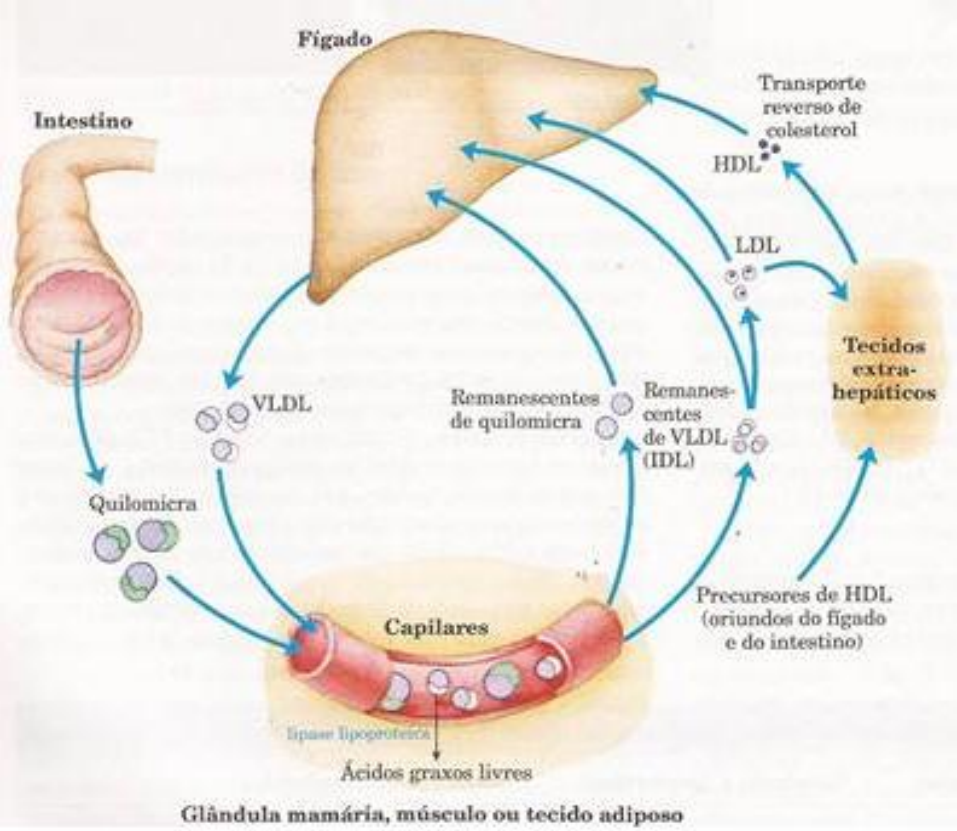

FIGURA 3 – METABOLISMO LIPÍDICO
FONTE: NELSON; COX, 2011.
NOTA: Os lipídeos provenientes da dieta são transportados na forma de quilomícrons até os capilares de grande parte dos tecidos (principalmente músculo e tecido adiposo), onde sofrem hidrólise pela lipase, liberando AGs livres e glicerol. Os remanescentes de quilomícrons (contendo na maior parte colesterol e apolipoproteínas) são captados pelo fígado. No fígado, os lipídeos são exportados na forma de VLDL. Essas lipoproteínas seguem para a maioria dos tecidos onde são hidrolisadas pela lipase, tornando-se remanescentes de VLDL (IDL). Estas podem ir direto para o fígado ou, em caso de perda adicional de TG, transformarem-se em LDL. A LDL pode ir para o fígado ou ser captada pelos tecidos extra-hepáticos. O colesterol pode retornar desses tecidos para o fígado pela HDL (transporte reverso do colesterol).

A HDL possui um metabolismo complexo, pois passa por várias etapas. A apoA1 é sintetizada principalmente no fígado e, para que não seja rapidamente degradada, recebe colesterol através de um transportador de membrana da família ABC (ABCA1), tornando-se HDL discóide ou nascente (LEWIS; RADER, 2005). A HDL nascente é constituída de apoA1, colesterol livre e fosfolipídios, e recebe colesterol de outras lipoproteínas e das membranas celulares através de outro transportador da família ABC (ABCG1), tornando-se HDL_3 (UEHARA; SAKU, 2014). A HDL_3 sofre ação da lecitina-colesterol aciltransferase (LCAT, do inglês *lecithin cholesterol acyltransferase*), enzima que catalisa a esterificação do colesterol livre, transformando a HDL_3 em HDL_2, rica em ésteres de colesterol (EC) (LEWIS; RADER, 2005; LIMA; COUTO, 2006; SUPERKO *et al*., 2012; UEHARA; SAKU, 2014).

A HDL_2 pode sofrer ação da proteína de transferência de colesteril éster (CETP, do inglês, *cholesteryl ester transfer protein*), que promove trocas de lipídeos entre HDL e VLDL, sendo que a HDL sofre depleção de EC e é enriquecida com TG vindos da VLDL (XAVIER *et al*., 2013).

2.1.2.2 Classificação, diagnóstico e terapia das dislipidemias

As dislipidemias são classificadas de acordo com o tipo de lipídeo alterado em: hipertrigliceridemia isolada (valores aumentados de TG), hipercolesterolemia isolada (valores aumentados de colesterol), hiperlipidemia mista (valores aumentados de colesterol e TG) e colesterol da HDL (HDL-C) baixo (que pode estar associada a aumento do colesterol e/ou dos TG) (XAVIER *et al*., 2013). Os valores de referência de colesterol total (CT), colesterol da LDL (LDL-C), HDL-C e TG para crianças e adolescentes estão na TABELA 1.

TABELA 1 – VALORES DE REFERÊNCIA DE CT, LDL-C, HDL-C E TG EM CRIANÇAS E ADOLESCENTES (ENTRE 2 E 19 ANOS DE IDADE)

Lipídeos	Desejáveis (mg/dL)	Limítrofes (mg/dL)	Aumentados (mg/dL)
CT	< 150	150 - 169	≥ 170
LDL-C	< 100	100 – 129	≥ 130
HDL-C	≥ 45		
TG	< 100	100 - 129	≥ 130

FONTE: GIULIANO *et al*., 2005.

O diagnóstico de dislipidemias na população pediátrica é especialmente importante, pois há um grande risco de complicações cardiovasculares em idade precoce. Com o diagnóstico precoce, podem ser tomadas medidas preventivas e terapêuticas para que o risco cardiovascular seja reduzido (LEITE *et al*., 2009).

A princípio, a recomendação às crianças dislipidêmicas é uma alimentação mais saudável e realização de exercícios físicos. Caso essa mudança no estilo de vida não seja suficiente para que os níveis lipídicos alcancem os valores recomendados, pode ser indicado o uso de drogas hipolipemiantes quando houver: a) dislipidemia familiar com níveis de LDL-C > 190 mg/dL; b) antecedentes familiares de aterosclerose prematura ou, no mínimo dois ou mais fatores de risco, com LDL-C > 160 mg/dL; c) manifestação de aterosclerose, com LDL-C > 130 mg/dL (SOCIEDADE BRASILEIRA DE CARDIOLOGIA, 1996).

2.1.3 Obesidade, dislipidemias e exercício físico

O incentivo à realização de exercícios físicos é de extrema importância, visto que quanto maior a prática de exercícios físicos, menor o risco de mortalidade cardiovascular, devido à melhora promovida nos níveis de lipídeos e de lipoproteínas no sangue (GORDON; CHEN; DURSTINE, 2014). Além disso, a realização de exercícios físicos ajuda no combate à obesidade, pois é efetiva em promover perda de peso (LOPES *et al*., 2016).

De acordo com a OMS, crianças e adolescentes devem realizar no mínimo 60 minutos de exercícios físicos por dia, principalmente aeróbicos e de intensidade moderada a vigorosa. Também devem realizar exercícios que fortaleçam os músculos e ossos pelo menos três vezes por semana (WHO, 2016).

Os parâmetros metabólicos podem responder de diferentes maneiras de acordo com os tipos de exercícios físicos, como é visto a seguir.

Dentre as lipoproteínas, os níveis de HDL-C são os mais prováveis de apresentarem melhorias em resposta a exercícios físicos, já que os estudos envolvendo essa lipoproteína apresentam resultados mais consistentes (MANN; BEEDIE; JIMENEZ, 2014) - apesar de nem todos os estudos envolvendo exercícios físicos demonstrarem tal melhora (KANG *et al*., 2002; LOPES *et al*., 2016). Indivíduos que realizam mais exercícios físicos possuem maiores níveis de HDL-C (KRAUS *et al*., 2002), sendo que a prática de exercícios aeróbios regulares promove, em média, um aumento de 4,3% nos níveis de HDL-C (GORDON; CHEN; DURSTINE, 2014). Possíveis explicações para o aumento de HDL-C devido ao exercício físico são a redução do catabolismo de HDL-C no fígado e o aumento da síntese de apoA1 (principal apolipoproteína da HDL) (GORDON; CHEN; DURSTINE, 2014).

Com relação ao LDL-C, estudos mostram que exercícios aeróbios não geram uma redução significativa de seus níveis a não ser que ocorra também uma redução no peso corporal (KELLEY; KELLEY; TRAN, 2005). Entretanto, apesar de não se observar redução nos valores de LDL-C, pode ocorrer uma alteração nas subfrações de LDL-C (KRAUS *et al*., 2002). Com relação aos treinamentos resistidos, alguns estudos observaram uma redução nos valores de LDL-C em programas de treinamento com duração maior de 12 semanas (GORDON; CHEN; DURSTINE, 2014).

Os níveis de TG são reduzidos tanto com a prática de exercícios aeróbios (redução de aproximadamente 6%) quanto de exercícios resistidos (redução de aproximadamente 11%) (GORDON; CHEN; DURSTINE, 2014). Em populações anteriormente sedentárias, quanto maior o tempo destinado à prática de exercícios, maior a redução de TG (AADAHL; KJÆR; JØRGENSEN, 2007; MANN; BEEDIE; JIMENEZ, 2014).

Com relação aos níveis de CT, exercícios resistidos parecem não exercer efeito em seus níveis, enquanto exercícios aeróbios promovem uma redução de aproximadamente 3% (MANN; BEEDIE; JIMENEZ, 2014).

O metabolismo da glicose também é alterado pela realização de exercícios aeróbicos. Estudos em jovens obesos mostraram uma redução nos níveis de glicose e insulina em jejum, e nos marcadores de resistência insulínica. Os treinamentos realizados por mais de 12 semanas, em uma frequência de três vezes por semana e

60 minutos de exercício aeróbico por sessão mostraram melhores resultados (apenas para a insulina) (GARCÍA-HERMOSO *et al*., 2014).

Embora não se saiba exatamente quais são os mecanismos pelos quais o exercício físico altera o perfil lipídico, parece que a realização de exercícios físicos estimula o músculo a utilizar lipídeos (vindos do plasma, VLDL e TG) ao invés de glicogênio (MANN; BEEDIE; JIMENEZ, 2014). Isso pode ocorrer através do aumento da atividade (promovido pelo treinamento físico) da LCAT, que transfere colesterol para HDL (CALABRESI; FRANCESCHINI, 2010; RIEDL *et al*., 2010). Após a prática de exercícios físicos também pode ocorrer aumento da lipase (FERGUSON *et al*., 1998) e redução da atividade da CETP (que transfere colesterol da HDL para outras lipoproteínas) (MANN; BEEDIE; JIMENEZ, 2014).

Com relação ao treinamento resistido, algumas possíveis razões para a melhora dos níveis de lipídeos e lipoproteínas gerados seriam: manutenção da massa magra, taxa metabólica de repouso mais alta, melhor controle da insulina e aumento do metabolismo de gordura (GORDON; CHEN; DURSTINE, 2014).

De uma maneira geral, a prática de exercícios físicos gera benefícios em todo o organismo: no pâncreas, promove aumento da produção de insulina (via atividade da HDL ou expressão de sirtuína 1 (SIRT1)); no fígado, aumenta a atividade e a quantidade de enzimas hepáticas; nos músculos, aumenta a lipoproteína lipase (LPL), a expressão de proteína quinase ativada por AMP (AMPK, do inglês *AMP-activated protein kinase*), a sensibilidade à insulina e os níveis de fator de crescimento semelhante à insulina tipo 1 (IGF-1, do inglês *insulin growth factor 1*); no tecido adiposo, diminui a quantidade de gordura, o fator de necrose tumoral-α (TNF-α, do inglês *tumor necrosis factor-α*), a adipogênese (através da expressão de SIRT1), e aumenta a lipólise (através da inibição de receptor ativado por proliferadores de peroxissoma gama - PPARγ, do inglês *peroxisome proliferator-activated receptor gamma*); e no sangue, aumenta os níveis de HDL-C (apenas exercício aeróbico), diminui os níveis de LDL-C (apenas exercício resistido), proteína C reativa, interleucina-1β, pressão sanguínea sistólica e diastólica (apenas aeróbico) (GORDON; CHEN; DURSTINE, 2014).

Dentre os diversos tipos de exercícios físicos, algumas modalidades serão abordadas nesse trabalho: exercícios aeróbios terrestre, treinamento combinado, treinamento intervalado de alta intensidade (HIIT, do inglês *high-intensity interval*

training) e exercícios aquáticos (programa de aprendizagem de técnicas de natação e caminhada aquática em suspensão).

O exercício aeróbico envolve exercícios de resistência cardiorrespiratória, como corrida e ciclismo. Para aumentar os níveis de HDL-C, exercícios aeróbicos moderados já são suficientes. Entretanto, para melhorar os níveis de LDL-C e TG, a intensidade dos exercícios deve ser maior (MANN; BEEDIE; JIMENEZ, 2014).

O treinamento resistido é definido como um exercício que desenvolve a força utilizando resistência externa ou o peso do próprio corpo (MANN; BEEDIE; JIMENEZ, 2014). O treinamento resistido não foi aplicado isoladamente nesse trabalho, mas sim em conjunto com exercícios aeróbicos (treinamento combinado).

O treinamento combinado (combinação de treinamento aeróbico e de resistência) promove perda de peso, aumento da massa livre de gordura e aumento da sensibilidade à insulina, mesmo que não haja perda de peso (MANN; BEEDIE; JIMENEZ, 2014; LOPES *et al*., 2016). O treinamento combinado pode ter efeitos melhores do que os exercícios aeróbicos ou resistidos feitos isoladamente com relação à resistência insulínica (JORGE *et al*., 2011), redução da gordura corporal total (SIGAL *et al*., 2014) e da gordura visceral (DÂMASO *et al*., 2014; LOPES *et al*., 2016).

Outro tipo de exercício é o HIIT, definido como exercícios de alta intensidade intercalados por períodos de repouso (GIBALA *et al*., 2012). Esse tipo de treinamento é muito interessante para crianças, pois reflete seus padrões de atividade espontâneos (RACIL *et al*., 2016). Em uma meta-análise, García-Hermoso e colaboradores (2016) compararam os resultados obtidos com HIIT (77-95% da frequência cardíaca máxima (FCM) e 80-90% da capacidade aeróbica máxima ($VO_{2máx}$)) e com outros exercícios, como caminhada, corrida e ciclismo, realizados em uma intensidade mais baixa (60-80% da $VO_{2máx}$), e verificaram que HIIT promoveu melhores resultados em relação à pressão arterial sistólica (PAS) e $VO_{2máx}$. Nas outras variáveis analisadas (IMC, CC, % e quantidade de gordura, CT, HDL-C, LDL-C, TG, glicose, insulina, HOMA-IR e pressão arterial diastólica (PAD)), os resultados gerados por HIIT foram tão bons quanto os gerados pelos outros exercícios. Além disso, HIIT demanda uma menor quantidade de tempo para realização dos exercícios, o que o torna uma escolha interessante (GARCÍA-HERMOSO *et al*., 2016).

Exercícios aquáticos apresentam alguns benefícios em relação aos exercícios aeróbicos terrestres, pois reduzem as dores, superaquecimento, transpiração e sensação de exaustão (LEITE *et al*., 2010). Entre os benefícios proporcionados estão perda de gordura corporal e melhora da aptidão cardiorrespiratória (LEITE *et al*., 2010).

2.1.4 Fatores genéticos na obesidade e dislipidemias

A obesidade pode ser monogênica ou comum (multifatorial), estando, nesse último caso, associada tanto a hábitos alimentares e ao estilo de vida quanto a componentes genéticos (UUSITUPA, 2005; SHAWKY; SADIK, 2012). Já se sabe que há um fator genético envolvido no comportamento alimentar, mas os mecanismos responsáveis por ele ainda não foram bem elucidados (BIENERTOVÁ-VAŠKŮ *et al*., 2010).

Os fatores genéticos têm grande influência no ganho de peso, visto que estudos com gêmeos sugerem uma herdabilidade da massa corporal entre 40% a 70% com uma concordância de 0,7 - 0,9 entre gêmeos monozigóticos e 0,35 - 0,45 entre gêmeos dizigóticos (STUNKARD *et al*., 1990).

Há uma grande quantidade de genes envolvidos no metabolismo e na gênese da obesidade, entre eles, encontra-se o *FTO*, que será abordado mais adiante nesse trabalho.

Com relação às dislipidemias, estas podem ser classificadas como primárias ou secundárias. Na forma primária a causa é genética, sendo que na maioria das vezes só se manifesta frente a hábitos alimentares inadequados e sedentarismo (RANG *et al*., 2012), enquanto a dislipidemia secundária pode ocorrer como consequência de outras condições, como obesidade, alcoolismo e administração de fármacos, com variação na susceptibilidade, decorrente da variabilidade genética interindividual (RANG *et al*., 2012; MEDEIROS *et al*., 2014).

Dentre os diversos genes que podem alterar o metabolismo lipídico, encontram-se os genes da família de transportadores ABC, que também serão abordados nesse trabalho.

2.2 GENES RELACIONADOS À OBESIDADE E DISLIPIDEMIAS CONTEMPLADOS NESSE ESTUDO

2.2.1 *FTO* (*Fat Mass and Obesity Associated*)

O gene *FTO* (*Fat Mass and Obesity Associated*) foi primeiramente identificado em 1999, em uma deleção de 1,6Mb no cromossomo 8 em modelos de camundongo conhecidos como "***fused toes"*** (dedos fundidos) (PETERS; AUSMEIER; RUTHER, 1999).

O primeiro estudo a associar fortemente o *FTO* com obesidade foi um estudo de associação de todo o genoma (GWAS, do inglês *Genome-wide Association Studies*) para DT2 realizado por Frayling e colaboradores em 2007. Nesse estudo, foram encontrados SNPs em 16q12 (onde se encontra o *FTO*) associados com DT2. Entretanto, após ajustar para IMC, a associação com DT2 desapareceu, o que sugere que a associação dos SNPs do *FTO* era na verdade com o IMC, e não necessariamente com a doença. Nesse estudo, os SNPs que demonstraram efeito estavam localizados no íntron 1 do gene (FRAYLING *et al*., 2007).

Estudos posteriores confirmaram a associação dos SNPs com o IMC e o risco de obesidade em diferentes populações além da europeia (FRAYLING *et al*., 2007; YEO, 2014), como em asiáticos (CHO *et al*., 2009) e hispano-americanos (SCUTERI *et al*., 2007). Essa associação foi verificada também em crianças (FRAYLING *et al*., 2007), sendo que os efeitos dos SNPs do *FTO* podem ser observados a partir dos três anos de idade, e sua ação máxima é na maioridade (HARDY *et al*., 2009; SOVIO *et al*., 2011).

No primeiro estudo GWA realizado, o SNP que teve associação mais significativa com IMC foi o rs9939609 (FRAYLING *et al*., 2007), sendo que outras pesquisas identificaram diferentes polimorfismos (LARDER *et al*., 2011). Já foram publicados diversos trabalhos associando outros loci à obesidade (31 loci já foram encontrados), mas os SNPs do *FTO* continuam sendo os SNPs com associação mais forte com a obesidade (YEO; O'RAHILLY, 2012), com maior efeito, e com maior frequência alélica (YEO, 2014).

Dentre os diversos polimorfismos já descritos do gene *FTO,* o mais investigado e mais fortemente associado com a obesidade é o rs9939609 (T>A), que é caracterizado pela substituição de T para A no íntron 1. Diversos estudos já

verificaram um efeito aditivo do alelo A (DA SILVA *et al*., 2013). Os indivíduos que são homozigotos para o alelo de risco (alelo A) possuem cerca de 3 kg a mais e um risco 1,7 vezes maior de obesidade do que os indivíduos homozigotos para o alelo T (FRAYLING *et al*., 2007). Cerca de 60% dos europeus possuem pelo menos um alelo A, sendo que 16% são homozigotos A/A (LOOS; BOUCHARD, 2008).

Já foram realizados diversos estudos associando o alelo A do polimorfismo rs9939609 do gene *FTO* com sobrepeso/obesidade em crianças e adolescentes, sendo que essa associação foi verificada em crianças das populações europeia (FRAYLING *et al*., 2007), asiática (LEE *et al*., 2010) e ameríndia (RIFFO *et al*., 2012), mas não na africana (GRANT *et al*., 2008). No Brasil, foram realizados dois estudos associando o alelo A do SNP rs9939609 com IMC em crianças e adolescentes. Da silva e colaboradores (2013) analisaram o efeito deste SNP em 348 crianças que foram acompanhadas do nascimento até os oito anos de idade, e também em outra amostra independente, composta por 615 crianças e adolescentes de quatro a 18 anos de idade, ambas compostas por indivíduos do Rio Grande do Sul. Foram estudadas as seguintes variáveis: IMC, circunferência abdominal e dobras cutâneas tricipital e subescapular. Os autores concluíram que os indivíduos com genótipo A/A possuem maior IMC escore-Z, circunferência abdominal e dobras cutâneas. Já o estudo realizado por Lourenço e colaboradores (2014) analisou 1225 crianças da Amazônia brasileira com menos de 10 anos, sendo que as análises foram repetidas cinco anos depois em 436 crianças, e foi verificado que os indivíduos portadores do alelo A possuem um IMC maior. Em ambos os estudos, não foi analisado o efeito do polimorfismo na resposta a exercícios físicos, e também não foram analisadas as diversas variáveis bioquímicas que foram analisadas nesse trabalho, como a glicemia e o perfil lipídico.

O *FTO* é expresso no organismo inteiro, principalmente no hipotálamo, que está envolvido com a regulação do balanço energético (FAWCETT; BARROSO, 2010). Indivíduos que possuem o alelo de risco do SNP rs9939609 têm um aumento na ingestão de comida e diminuição da saciedade (WARDLE *et al*., 2009; YEO, 2014), e ainda não foi definido se há uma relação do *FTO* com o gasto de energia, visto que os resultados dos estudos são contraditórios (LARDER *et al*., 2011).

O aumento do peso corporal gerado pelo alelo A do polimorfismo rs9939609 parece estar associado com um aumento da expressão de *FTO*, visto que em indivíduos portadores do alelo de risco os transcritos são mais abundantes

(BERULAVA; HORSTHEMKE, 2010). Além disso, outras observações corroboram essa hipótese, como: ratos com superexpressão de *FTO* possuem fenótipo obeso, assim como os indivíduos portadores do alelo de risco, e ratos *knockout* para *FTO* possuem fenótipo magro (FISCHER *et al*., 2009; CHURCH *et al*., 2010; MERKESTEIN *et al*., 2014).

Análises *in silico* mostraram que a sequência da Fto humana é semelhante à de membros da família de dioxigenases dependentes de Fe^{2+} e 2-oxoglutarato (2-OG) (GERKEN *et al*., 2007). *In vitro*, foi verificado que a Fto é capaz de realizar a demetilação de 3-metiltimina na fita simples de DNA (GERKEN *et al*., 2007), e de 3-metiluracila (JIA *et al*., 2008) e 6-metiladenosina na fita simples de RNA (JIA *et al*., 2011), conforme mostra a FIGURA 4. Ainda não se sabe exatamente qual a relação entre a função de demetilase da Fto e a obesidade, mas suspeita-se que essa enzima realize a demetilação de genes envolvidos com o metabolismo, sendo que alterações do processo normal poderiam levar à obesidade (FAWCETT; BARROSO, 2010).

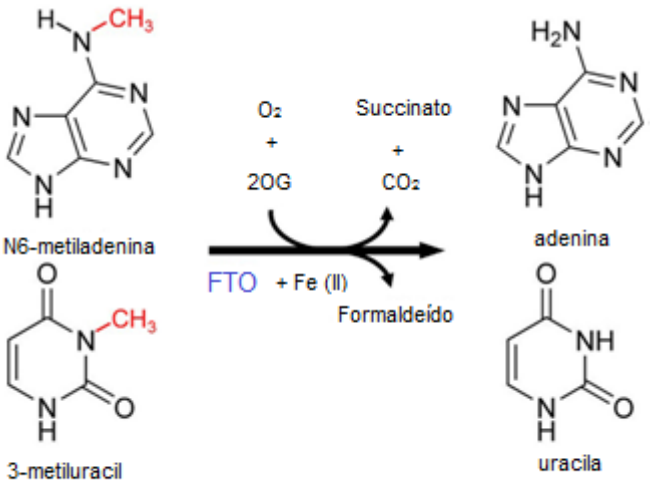

FIGURA 4 - PAPEL BIOQUÍMICO DA FTO
FONTE: Adaptado de YEO, 2014.
NOTA: Fto catalisa a demetilação dependente de Fe^{2+} e 2OG de N6-metiladenina e 3-metiluracila, com produção de succinato, CO_2 e formaldeído.

Diversos estudos encontraram possíveis alvos para a Fto, entre eles, a grelina (MERKESTEIN et al., 2014). Em ratos *knockout* para *FTO* há uma redução da acil-grelina, e em células com superexpressão de *FTO* observa-se um aumento da expressão da grelina. Esse fato poderia explicar o aumento da ingestão alimentar

observado nos indivíduos portadores do alelo de risco, visto que a grelina é um hormônio orexigênico (KARRA *et al*., 2013; MIHALACHE *et al*., 2016).

Outro possível alvo da Fto é a adiponectina: um estudo realizado por Merkestein e colaboradores (2014) mostrou que em ratos com superexpressão de *FTO* podem ser observados níveis reduzidos de adiponectina a partir de 20 semanas. A adiponectina é um hormônio produzido pelo tecido adiposo branco que possui diversas funções, como diminuição da glicose, de TG e de AG, entre outros (SHEHZAD *et al*., 2012).

2.2.2 Transportadores ABC

Os transportadores ABC (do inglês *ATP binding cassette*) são uma família de proteínas transmembrana que utilizam a energia da hidrólise do ATP para realizar o transporte de substâncias através de membranas extracelulares e intracelulares (QUAZI; MOLDAY, 2011; TARLING; DE AGUIAR VALLIM; EDWARDS, 2013), sendo que, nos eucariotos, a maioria dos transportadores é exportadora (WILKENS, 2015). Dentre as substâncias transportadas estão aminoácidos, açúcares, nucleosídeos, vitaminas, metais, peptídeos, lipídeos, oligonucleotídeos, polissacarídeos e drogas (QUAZI; MOLDAY, 2011; WILKENS, 2015).

Essas proteínas possuem dois domínios transmembrana (TMDs, do inglês *transmembrane domains*), cada um com seis domínios α-hélice transmembrana, que funcionam como um poro, e dois domínios de ligação a nucleotídeos (NBDs, do inglês *nucleotide-binding domains*), onde se liga o ATP (QUAZI; MOLDAY, 2011; WILKENS, 2015). A maioria dos transportadores é composta por um único polipeptídeo, mas alguns são compostos por duas “metades”, que podem ser iguais (homodímeros) ou diferentes (heterodímeros) (WILKENS, 2015).

Dos 48 transportadores ABC, 20 transportam lipídeos, como fosfolipídeos, esteróis, esfingolipídeos, ácidos biliares e compostos relacionados a lipídeos, e podem estar localizados na membrana plasmática ou na membrana de organelas internas, como complexo de Golgi, endossomo, retículo endoplasmático, peroxissomo e mitocôndria (KAMINSKI; PIEHLER; WENZEL, 2006; QUAZI; MOLDAY, 2011; TARLING; DE AGUIAR VALLIM; EDWARDS, 2013). De acordo com a estrutura do gene e a ordem dos domínios, essas proteínas são subdivididas em sete subfamílias: A a G (SINGARAJA *et al*., 2003). Nesse trabalho, serão

estudados os transportadores ABCA1, ABCA7 e ABCG1, cuja localização celular encontra-se na FIGURA 5.

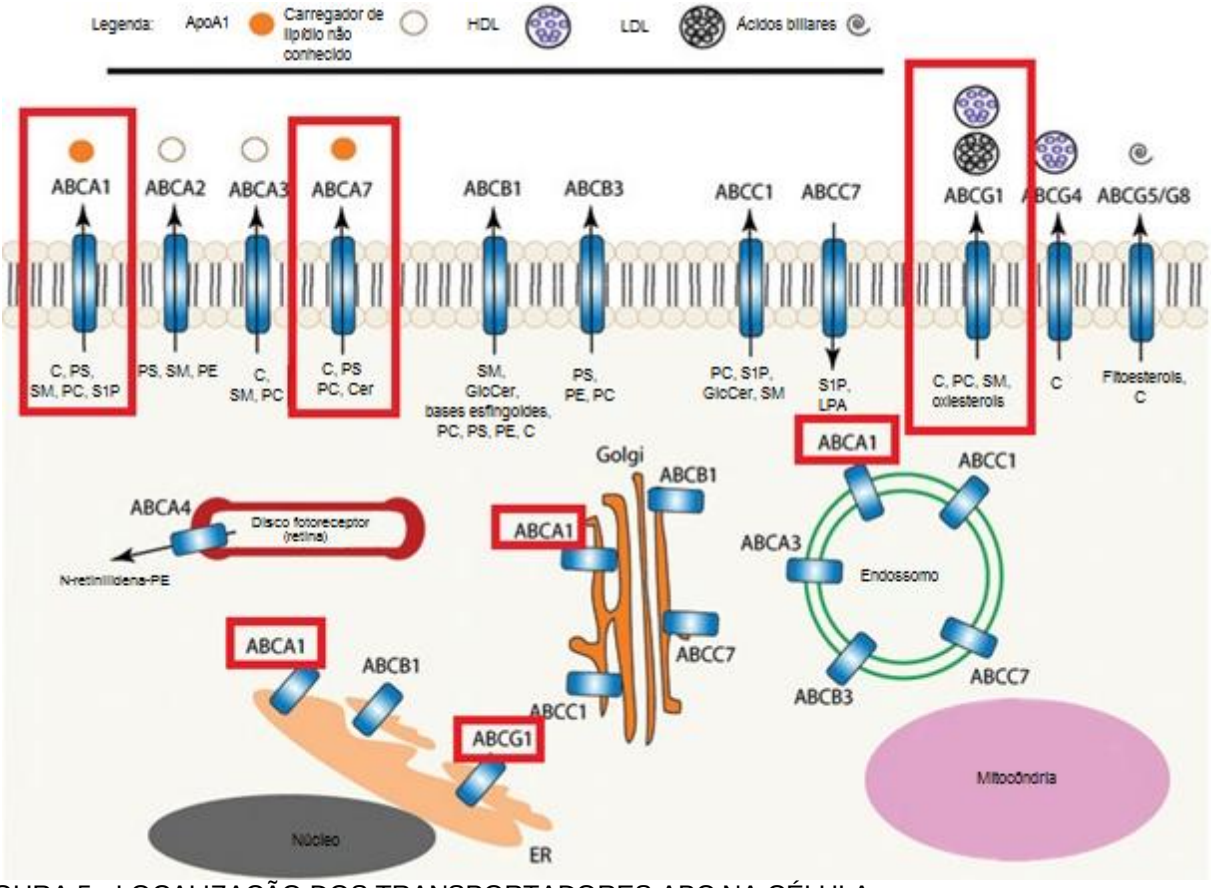

FIGURA 5 - LOCALIZAÇÃO DOS TRANSPORTADORES ABC NA CÉLULA
Nota: C = colesterol; PS = fosfatidilserina; SM = esfingomielina; PC = fosfatidilcolina; S1P = esfingolipídeo 1-fosfato; PE = fosfatidiletanolamina; Cer = ceramida; GlcCer = glicosilceramida; LPA = ácido lisofosfatídico; RE = retículo endoplasmático.
Fonte: Adaptado de QUAZI; MOLDAY, 2011.

2.2.2.1 *ABCA1*

A proteína ABCA1 possui 254 kDa, está localizada na membrana celular e na membrana de algumas organelas intracelulares (KAMINSKI; PIEHLER; WENZEL, 2006; QUAZI; MOLDAY, 2011) e é uma das principais proteínas envolvidas no metabolismo de colesterol, pois transfere fosfolipídeos e colesterol para apolipoproteínas (TARLING; DE AGUIAR VALLIM; EDWARDS, 2013). Está presente em todo o organismo, sendo que nos hepatócitos, enterócitos intestinais e adipócitos participa da formação da HDL (pois transfere colesterol em excesso para apoA1, a principal apolipoproteína da HDL); nos macrófagos, faz parte do transporte reverso do colesterol (QUAZI; MOLDAY, 2011; TARLING; DE AGUIAR VALLIM; EDWARDS, 2013). Interage principalmente com a apoA1 livre/pobre em lipídeos, tendo pouca

afinidade pela HDL_3 e nenhuma afinidade pela HDL_2 (WANG *et al*., 2000; UEHARA; SAKU, 2014). Possui 2 grandes loops extracelulares que ligam as α-hélices transmembrana, conforme mostra a FIGURA 6 (SINGARAJA *et al*., 2003; TARLING; DE AGUIAR VALLIM; EDWARDS, 2013).

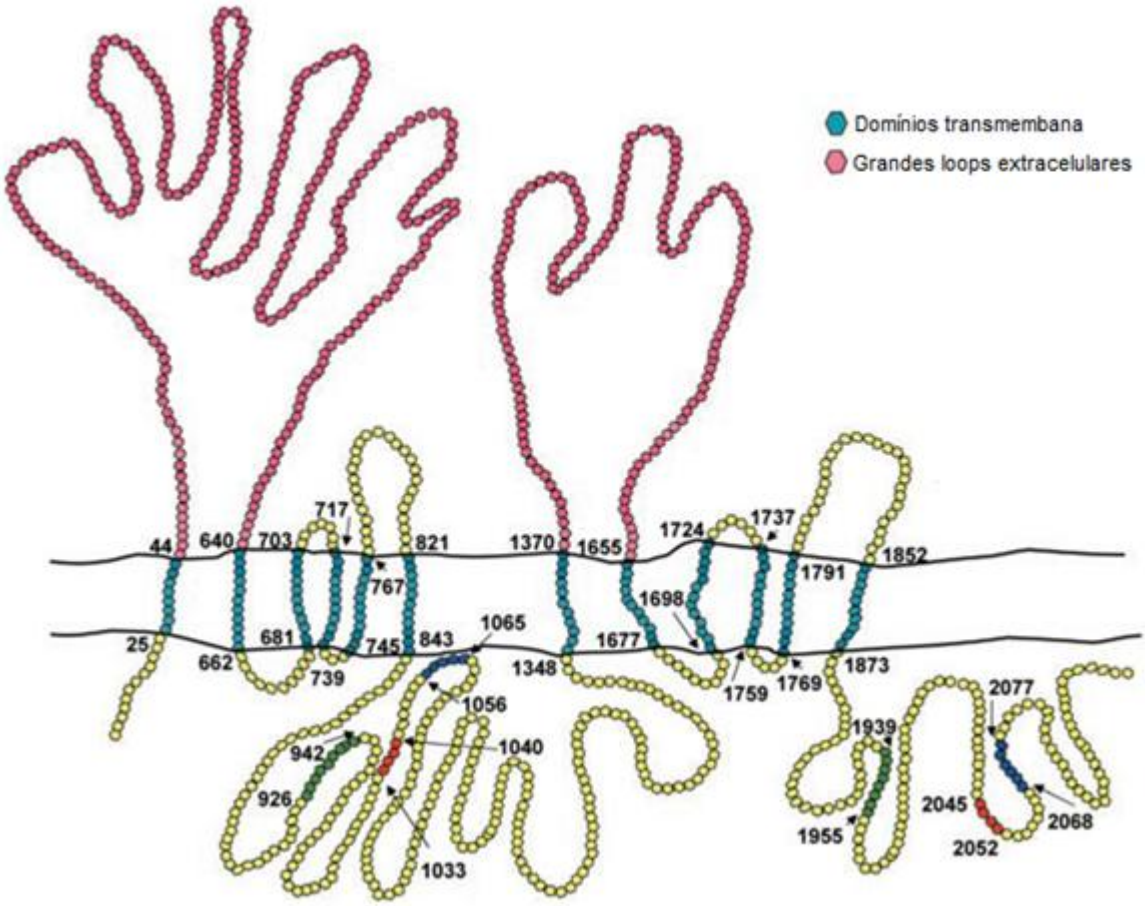

FIGURA 6 - REPRESENTAÇÃO ESQUEMÁTICA DO TRANSPORTADOR ABCA1
FONTE: Adaptado de SINGARAJA *et al*., 2003.

O gene *ABCA1* possui 50 éxons, 147kb de tamanho e localiza-se na região 9q31.1 (KAMINSKI; PIEHLER; WENZEL, 2006). Já foram descritas pelo menos 50 mutações nesse gene (SINGARAJA *et al*., 2003), sendo que tais variantes podem alterar os níveis de HDL e, consequentemente, influenciar o risco de aterosclerose (MOKUNO *et al*., 2015). Nesse trabalho, serão estudados os polimorfismos rs1800977 e rs2230806.

O polimorfismo rs1800977 (T>C) corresponde a uma mudança de T para C no nucleotídeo 69, sendo que o alelo T tem como efeito aumento dos níveis de HDL-C. Esse polimorfismo localiza-se na região 5'UTR, e aumenta a atividade transcricional

(PORCHAY *et al*., 2006). A frequência do alelo T na população europeia é 0,36, de acordo com o HapMap (NCBI, 2015).

Já os indivíduos que possuem o polimorfismo rs2230806 (G>A) possuem A no lugar de G no nucleotídeo 1051, o que gera a substituição de uma Arginina por uma Lisina no aminoácido 219 (motivo pelo qual o polimorfismo também é chamado de R219K). Esse SNP localiza-se no éxon 7 (SINGARAJA *et al*., 2003) e promove mudanças no primeiro *loop* extracelular da ABCA1, que é importante para interação com apoA1 (PORCHAY *et al*., 2006). Dessa maneira, o alelo A está associado a maiores níveis de HDL-C, tendo um papel protetor em asiáticos e europeus (MA; LIU; SONG; 2011). Entretanto, esse efeito parece ser dependente do peso, visto que há aumento dos níveis de HDL em indivíduos magros, e diminuição nos indivíduos com excesso de peso (PORCHAY *et al*., 2006). Clee e colaboradores (2001) verificaram também uma diminuição dos níveis de TG. A frequência do alelo A é maior em asiáticos, sendo 0,424 em japoneses e 0,208 em descendentes de europeus (MOKUNO *et al*., 2015).

2.2.2.2 *ABCA7*

O transportador ABCA7 tem 235 kDa de tamanho (KAMINSKI; PIEHLER; WENZEL, 2006), possui 54% de homologia com o ABCA1 (KAMINSKI *et al*., 2000), e também participa da formação da HDL; entretanto, gera pequenas partículas de HDL pobres em colesterol, diferente do ABCA1 (QUAZI; MOLDAY, 2011). Está presente na membrana plasmática e em compartimentos intracelulares. É expresso no cérebro, pele, sistema mielolinfático (timo, baço, medula óssea), tecidos fetais e rim (onde participa do catabolismo da apoA1) (KAMINSKI *et al*., 2000; QUAZI; MOLDAY, 2011).

O gene *ABCA7* está localizado na região 19p13.3, e possui 24 kb e 46 éxons (KAMINSKI; PIEHLER; WENZEL, 2006). Dentre os diversos polimorfismos que já foram descritos nesse gene, encontra-se o SNP rs2279796 (C>T), uma variante intrônica que promove substituição de C por T. De acordo com o HapMap, a frequência do alelo T na população europeia é 0,580 (NCBI, 2015). Não foram encontrados estudos que buscassem associação entre esse SNP e obesidade e/ou dislipidemias.

2.2.2.3 *ABCG1*

O transportador ABCG1 é muito semelhante ao ABCA1 (QUAZI; MOLDAY, 2011) e realiza o efluxo de lipídeos, principalmente colesterol e fosfolipídeos, das células periféricas para HDL (CAVELIER *et al*., 2006; KOBAYASHI *et al*., 2006) e também para LDL (em menor quantidade do que HDL) (WANG *et al*., 2004). Com relação à HDL, não interage com a apoA1 livre de lipídeos, apenas com HDL_2 e HDL_3, sendo que possui um papel importante na lipidação inicial da HDL (WANG *et al*., 2004; UEHARA; SAKU, 2014). É um dos principais responsáveis pelo transporte de colesterol e é expresso em todo o organismo, com altos níveis de expressão nos macrófagos (CAVELIER *et al*., 2006; QUAZI; MOLDAY, 2011). É formado por homodímeros (CAVELIER *et al*., 2006) e está representado na FIGURA 7.

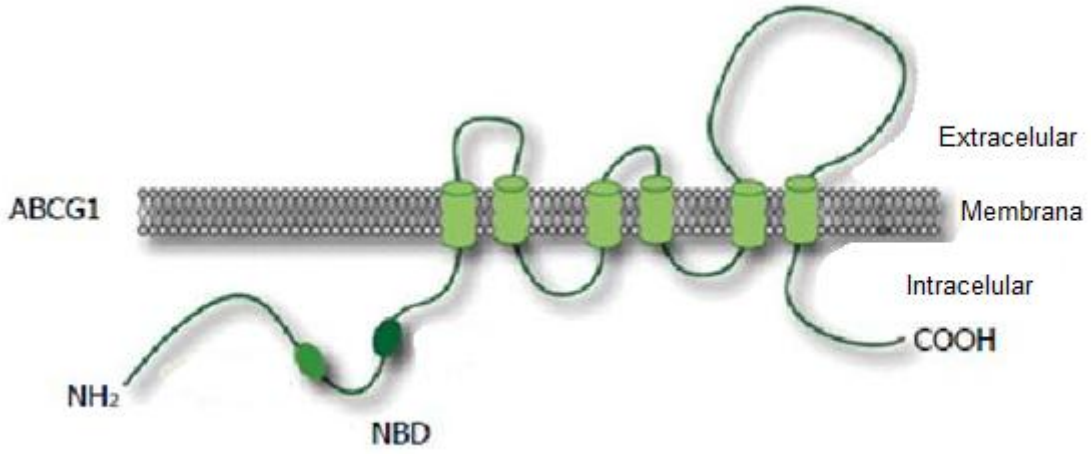

FIGURA 7 – REPRESENTAÇÃO ESQUEMÁTICA DO TRANSPORTADOR ABCG1
Fonte: Adaptado de UEHARA; SAKU, 2014.

O gene *ABCG1* está localizado em 21q22.3, possui 98kb e 23 éxons, gerando diversos transcritos (IIDA *et al*., 2002; CAVELIER *et al*., 2006). Já foram descritos 104 SNPs nesse gene (IIDA *et al*., 2002), dentre eles, o rs692383 e o rs3827225. No polimorfismo rs692383 (G>A), há uma mudança de G para A, em íntron, e a sua frequência na população europeia é 0,5, de acordo com HapMap. No polimorfismo rs3827225 (G>A) também ocorre uma substituição de G para A, em íntron, mas a frequência na comunidade europeia é de 0,265, segundo o HapMap (NCBI, 2015). Não foram encontrados estudos que verificassem o efeito desses SNPs em variáveis antropométricas e bioquímicas.

3 OBJETIVOS

3.1 OBJETIVO GERAL

- Avaliar se há influência de polimorfismos nos genes *FTO*, *ABCA1*, *ABCA7* e *ABCG1* na variação de medidas antropométricas e bioquímicas de crianças e adolescentes, bem como verificar o efeito de tais polimorfismos na resposta desses marcadores frente a um programa de exercícios físicos.

3.2 OBJETIVOS ESPECÍFICOS

- Comparar o efeito conjunto e individual de cada um dos programas de exercícios sobre as variáveis antropométricas e bioquímicas analisadas.
- Comparar as frequências alélicas estimadas dos SNPs investigados entre eutróficos, sobrepeso e obesos.
- Verificar se antes da aplicação dos exercícios físicos os SNPs foram responsáveis por variações significativas nas medidas antropométricas e bioquímicas (análise do momento pré).
- Verificar se os SNPs exerceram influência sobre a variação das medidas antropométricas e bioquímicas após a aplicação do exercício físico (análise do momento pós).
- Analisar se alelos ou genótipos específicos de cada um dos SNPs investigados impactaram em diferenças significativas quanto à resposta das variáveis antropométricas e bioquímicas frente à aplicação dos exercícios físicos nos indivíduos que compuseram a amostra.
- Investigar o efeito conjunto dos SNPs analisados dos genes ABC nas diferentes variáveis antropométricas e bioquímicas, visando compor uma predição de risco.

4 JUSTIFICATIVA

A prevalência das dislipidemias e da obesidade tem aumentado em grande parte dos países, devido ao atual estilo de vida (sedentarismo e preferência por alimentos práticos e hipercalóricos) (NETO *et al*, 2012; WHO, 2015). Essas patologias são responsáveis por aumentar significativamente a predisposição a várias outras comorbidades, tais como a aterosclerose, hipertensão, DT2, entre outras (JUNG; CHOI, 2014; MEDEIROS *et al*., 2014).

É preciso destinar atenção especial ao aumento da prevalência dessas enfermidades em crianças e adolescentes, já que irão trazer consequências também na vida adulta. Dessa forma, é de extrema importância o diagnóstico precoce, para que sejam tomadas medidas a fim de diminuir o risco de doença cardiovascular e outras complicações (WHO, 2015; LEITE *et al*., 2009; MEDEIROS *et al*., 2014).

Tanto as dislipidemias quanto a obesidade comum possuem, além de uma influência ambiental, um componente genético (UUSITUPA, 2005; RANG *et al*., 2012). A identificação desse componente é de grande auxílio porque possibilitaria tratamentos individualizados de acordo com as necessidades de cada paciente; já que se sabe que cada indivíduo responde de maneira diferente a determinada terapia de acordo com sua composição genética (SHAWKY; SADIK, 2012). A resposta a exercícios físicos também é diferente de acordo com o componente genético, sendo que determinados genótipos podem modular o efeito de exercícios físicos sobre parâmetros antropométricos e metabólicos (LEOŃSKA-DUNIEC; AHMETOV; ZMIJEWSKI, 2016).

Nos dias atuais, os GWAS possibilitaram a descoberta de uma grande quantidade de polimorfismos envolvidos em doenças complexas. Entretanto, ainda são necessários estudos de replicação, que possam elucidar o efeito de cada um desses polimorfismos em diferentes contextos e diferentes populações, bem como sua interação com fatores ambientais de favorecimento e/ou proteção a essas doenças (LOOS; BOUCHARD, 2008). Portanto, esse estudo pretende analisar a influência de polimorfismos nos genes *FTO, ABCA1, ABCA7* e *ABCG1* na alteração de variáveis antropométricas e bioquímicas de crianças e adolescentes em resposta a exercícios físicos. A análise do efeito de polimorfismos no *FTO* na resposta a exercícios físicos é especialmente interessante, visto que os resultados sobre a influência do *FTO* no gasto energético são contraditórios. Com relação aos genes

ABCA7 e *ABCG1*, esse estudo foi um dos primeiros a analisar o efeito dos SNPs rs2279796 (*ABCA7*) e rs692383 (*ABCG1*) e rs3827225 (*ABCG1*) em variáveis metabólicas.

5 METODOLOGIA

Esse estudo provém de uma parceria entre o Departamento de Genética da UFPR (Laboratório de Polimorfismos e Ligação) e o Departamento de Educação Física da UFPR (Núcleo de Qualidade de Vida – NQV).

5.1 PARTICIPANTES DO ESTUDO

A amostra foi constituída por 557 crianças e adolescentes (254 obesos, 98 com excesso de peso e 205 eutróficos), estudantes da rede de ensino pública do estado do Paraná. A média de idade foi de 13,46 ± 1,91, sendo que 63,91% da amostra foi composta por meninos e 36,09% por meninas.

Uma parte desses indivíduos (179 obesos, 42 com excesso de peso e 10 eutróficos, totalizando 231 indivíduos) foi submetida à realização de exercícios físicos, sendo que foram realizados diferentes tipos de treinamento (FIGURA 8).

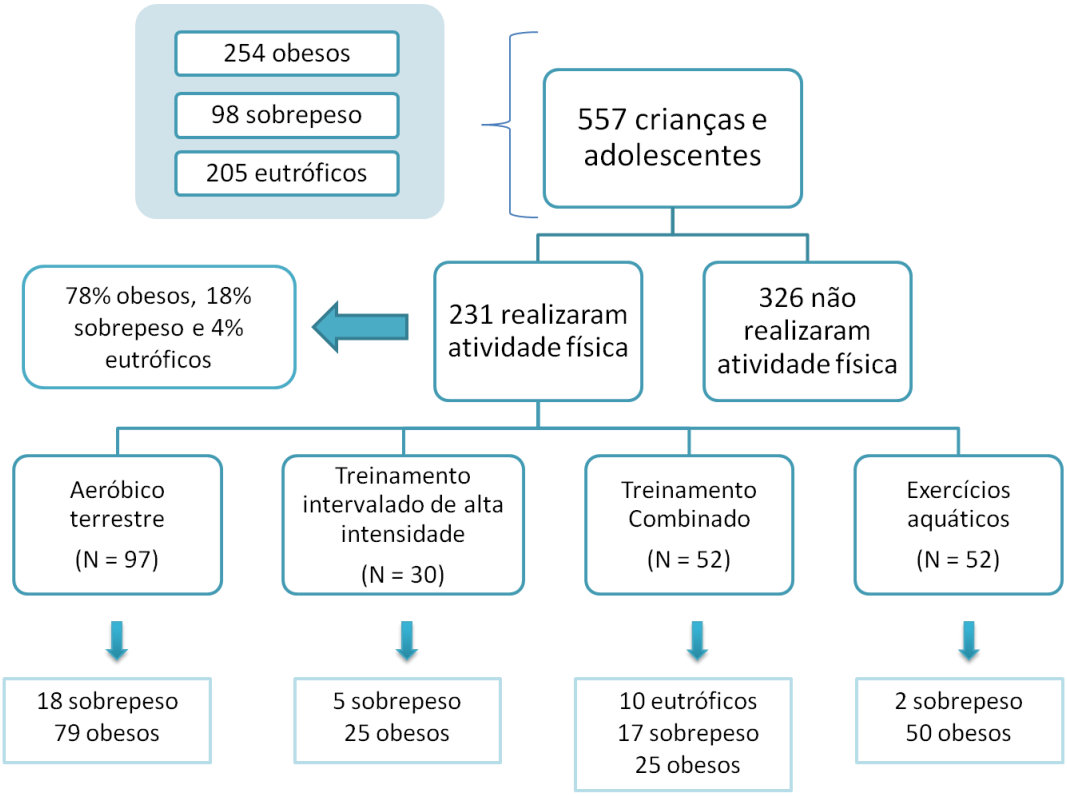

FIGURA 8 – ESQUEMA DA METODOLOGIA DO ESTUDO

Das crianças e adolescentes que concluíram os programas de exercícios físicos, 96% eram obesos ou tinham sobrepeso, assim, somente estes foram considerados nas análises de efetividade dos treinamentos, sendo que o grupo eutrófico que não realizou nenhum dos programas de exercícios físicos foi tido como grupo de referência nessas análises.

Os critérios de inclusão foram: a) idade entre sete e 18 anos; b) liberação médica para prática de exercícios físicos; c) não utilizar medicamentos que possam interferir no controle do peso e/ou na hiperinsulinemia; d) apresentar o Termo de Consentimento Livre e Esclarecido assinado pelos pais ou responsáveis.

Esse trabalho reuniu dados e amostras de vários projetos independentes também resultantes da parceria entre o laboratório de Polimorfismos e Ligação e o Núcleo de Qualidade de Vida. Por esse motivo, o número de indivíduos com dados coletados é variado, como mostra a TABELA 2.

TABELA 2 - DISTRIBUIÇÃO DAS AMOSTRAS SEGUNDO OS DADOS OBTIDOS PARA CADA UM DOS POLIMORFISMOS

Dados – rs9939609 (*FTO*)	Geral	Eutróficos	Sobrepeso e obesos
	N	N	N
Presença de ao menos um dado antropométrico ou bioquímico	557	205	352
Dados genotípicos (rs9939609)	444	172	267
Dados antropométricos			
IMC escore-Z	435	171	264
CA	284	129	155
CC	181	70	111
GC	133	16	117
%GC	140	16	124
MM	133	16	117
%MM	104	15	89
Dados bioquímicos			
LT	94	27	67
CT	322	90	232
VLDL-C	139	63	76
HDL-C	423	161	262
LDL-C	322	90	232
TG	423	161	262
Glicose	432	169	263
Glicose 120	90	15	75
Insulina	383	161	222
Insulina 120	71	16	55
HOMA-IR	232	127	105
QUICKI	182	86	96

Dados – rs1800977 (*ABCA1)*	Geral	Eutróficos	Sobrepeso e obesos
	N	N	N
Presença de ao menos um dado antropométrico ou bioquímico	557	205	352
Dados genotípicos (rs1800977)	434	174	260
Dados antropométricos			
IMC escore-Z	425	174	251
CA	288	135	153
CC	137	57	80
GC	129	16	113
%GC	136	16	120
MM	129	16	113
%MM	103	15	88
Dados bioquímicos			
LT	91	26	65
CT	317	89	228
VLDL-C	138	64	74
HDL-C	418	164	254
LDL-C	316	89	227
TG	418	163	255
Glicose	427	172	255
Glicose 120	84	15	69
Insulina	377	164	213
Insulina 120	67	16	51
HOMA-IR	231	131	100
QUICKI	177	90	87

Dados – rs2230806 (*ABCA1)*	Geral	Eutróficos	Sobrepeso e obesos
	N	N	N
Presença de ao menos um dado antropométrico ou bioquímico	557	205	352
Dados genotípicos (rs2230806)	456	172	284
Dados antropométricos			
IMC escore-Z	447	172	275
CA	322	137	185
CC	126	53	73
GC	156	22	134
%GC	164	22	142
MM	156	22	134
%MM	109	21	88
Dados bioquímicos			
LT	88	25	63
CT	346	91	255
VLDL-C	134	62	72
HDL-C	438	160	278
LDL-C	345	91	254
TG	437	159	278
Glicose	446	168	278
Glicose 120	125	23	102
Insulina	396	160	232
Insulina 120	107	24	83
HOMA-IR	258	130	128
QUICKI	209	92	117

Dados – rs2279796 (*ABCA7)*	Geral	Eutróficos	Sobrepeso e obesos
	N	N	N
Presença de ao menos um dado antropométrico ou bioquímico	557	205	352
Dados genotípicos (rs2279796)	414	166	248
Dados antropométricos			
IMC escore-Z	406	166	240
CA	272	128	144
CC	135	56	79
GC	105	11	94
%GC	113	11	102
MM	105	11	94
%MM	79	11	68
Dados bioquímicos			
LT	90	26	64
CT	297	82	215
VLDL-C	130	58	72
HDL-C	398	156	242
LDL-C	296	82	214
TG	398	155	243
Glicose	405	164	241
Glicose 120	87	11	76
Insulina	378	156	222
Insulina 120	70	11	59
HOMA-IR	233	125	108
QUICKI	180	85	95

Dados – rs692383 (*ABCG1)*	Geral	Eutróficos	Sobrepeso e obesos
	N	N	N
Presença de ao menos um dado antropométrico ou bioquímico	557	205	352
Dados genotípicos (rs692383)	377	160	217
Dados antropométricos			
IMC escore-Z	370	160	210
CA	234	121	113
CC	137	58	79
GC	75	2	73
%GC	82	2	80
MM	75	2	73
%MM	58	2	56
Dados bioquímicos			
LT	90	26	64
CT	257	75	182
VLDL-C	128	56	72
HDL-C	360	149	211
LDL-C	256	75	181
TG	360	148	212
Glicose	369	157	212
Glicose 120	61	2	59
Insulina	351	149	202
Insulina 120	43	2	41
HOMA-IR	211	118	93
QUICKI	156	76	80

Dados – rs3827225 (*ABCG1)*	Geral	Eutróficos	Sobrepeso e obesos
	N	N	N
Presença de ao menos um dado antropométrico ou bioquímico	557	205	352
Dados genotípicos (rs3827225)	378	155	223
Dados antropométricos			
IMC escore-Z	370	155	215
CA	233	116	117
CC	136	57	79
GC	84	3	81
%GC	91	3	88
MM	84	3	81
%MM	63	3	60
Dados bioquímicos			
LT	90	26	64
CT	268	76	192
VLDL-C	127	56	71
HDL-C	361	144	217
LDL-C	267	76	191
TG	361	143	218
Glicose	370	152	218
Glicose 120	67	3	64
Insulina	349	144	205
Insulina 120	50	3	47
HOMA-IR	205	112	93
QUICKI	151	71	80

Os participantes do estudo receberam explicações sobre a pesquisa e seus pais ou responsáveis assinaram o Termo de Consentimento Livre e Esclarecido, conforme documento aprovado no Comitê de Ética do Setor de Saúde da Universidade Federal do Paraná (UFPR), sob o número CEP – 05/09, atendendo a resolução 196/96 do Conselho Nacional de Saúde.

5.1.1 Tipos de treinamento aplicados

Foram realizados quatro diferentes programas de treinamento, conforme mostra a FIGURA 9.

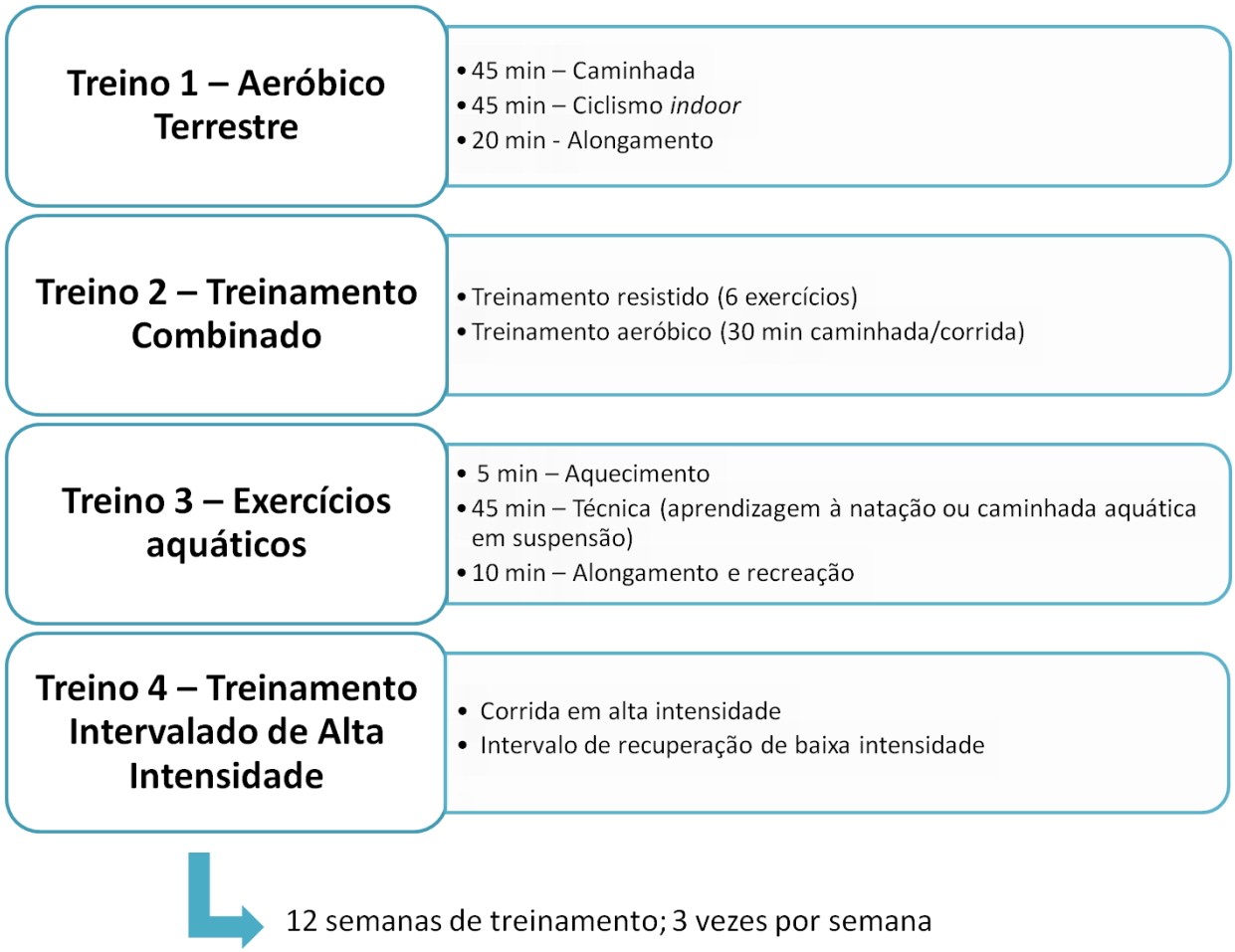

FIGURA 9 – DIFERENTES TIPOS DE PROGRAMAS DE TREINAMENTO APLICADOS ÀS CRIANÇAS E ADOLESCENTES

No treinamento aeróbico terrestre (programa 1), composto por 12 semanas, foram realizados 45 minutos de caminhada, 45 minutos de ciclismo *indoor* e 20 minutos de alongamento, em uma frequência de três vezes por semana. O ciclismo

indoor e a caminhada foram iniciados na intensidade entre 35 a 55% da frequência cardíaca de reserva (FCR), aumentando para 45 a 65% na 5ª a 8ª semana, e atingindo entre 55 e 75% da FCR na 9ª a 12ª semana (MILANO, 2013).

No treinamento combinado (programa 2), os participantes também realizaram treinos três vezes por semana, totalizando 12 semanas de treinamento. O treino era composto por treinamento resistido e aeróbio, realizados na mesma sessão, em um total de 60 minutos. O treinamento resistido era constituído por seis exercícios (*leg press, leg extension, leg curl, bench press, lateral pulldown* e *arm Curl*), sendo três séries de 6-10 repetições a 60-70% 1 RM (1 repetição máxima do participante, que é a carga máxima que pode ser levantada de uma vez só para um dado exercício (MANN; BEEDIE; JIMENEZ, 2014)), e o aeróbio por 30 minutos de caminhada/corrida em uma pista de atletismo. A intensidade alcançada no treino aeróbio era de 50-80% do VO_{2pico}, e a carga do treinamento resistido era ajustada semanalmente (LOPES *et al*., 2016).

No programa de exercícios aquáticos (programa 3), as crianças e adolescentes realizaram o programa três vezes por semana, totalizando 12 semanas de treinamento. Cada sessão consistia de cinco minutos de aquecimento, 45 minutos de técnica (exercícios de aprendizagem de técnicas de natação ou caminhada aquática em suspensão) e dez minutos de alongamento e recreação (LEITE *et al*., 2010). Na caminhada aquática em suspensão, o indivíduo permanece em posição vertical e seu corpo fica submerso até a altura dos ombros, com o auxílio de um colete flutuador preso à cintura. Não há contato dos pés com o fundo da piscina, e são realizados movimentos semelhantes à caminhada em terra (LEITE *et al*., 2010).

O programa de treinamento intervalado de alta intensidade (HIIT - programa 4) também foi realizado três vezes por semana, em um total de 12 semanas. Os exercícios consistiam de períodos de corrida em alta intensidade, em que o indivíduo deveria correr na maior velocidade possível por 30 segundos, seguidos de intervalo de recuperação de baixa intensidade, em que era feito caminhada em velocidade moderada/rápida. Na primeira e segunda semanas, foram realizadas duas séries de quatro repetições de corrida de alta intensidade de 30 segundos, seguidos de um minuto de recuperação ativa (caminhada), com intervalo passivo de quatro minutos entre as séries. Da terceira semana até a sexta semana foi aumentado uma repetição de corrida por série, totalizando oito tiros de corrida por série. O número de

séries, tempo de recuperação ativa e tempo de intervalo passivo permaneceram os mesmos das semanas anteriores. Da sétima semana até a nona semana foram realizadas duas séries de oito repetições de corrida de alta intensidade, com 45 segundos de recuperação ativa entre os tiros e quatro minutos de intervalo passivo entre as séries. A partir da décima semana até o final do programa de treinamento foram realizadas duas séries de oito repetições de corrida de alta intensidade com recuperação ativa de 30 segundos entre os tiros e quatro minutos de intervalo passivo entre as séries (PIZZI, 2014).

5.2 VARIÁVEIS ANTROPOMÉTRICAS E BIOQUÍMICAS ANALISADAS

Foram obtidas as variáveis antropométricas e bioquímicas das crianças e adolescentes que compuseram o presente estudo no espaço físico das escolas onde estas estudavam, pelos estudantes de pós-graduação do Departamento de Educação Física da UFPR, participantes do projeto.

Dentre as medidas antropométricas, encontram-se a massa corporal (kg), estatura (m), IMC (kg/m²), CA (cm), CC (cm), % GC, GC (kg), % MM e MM (kg). Os dados antropométricos foram coletados de acordo com o *Anthropometric Standardization Reference Manual* (BRUCE, 2003), sendo que foram obtidas três medidas e o valor mediano entre elas foi considerado. Peso e altura foram medidos com uma precisão de 0,1 kg e 0,1 cm, respectivamente. O IMC foi calculado como o peso (kg) dividido pela altura (m) ao quadrado, e então convertido a IMC escore-Z de acordo com as especificações da OMS (WHO, 2007). A verificação da composição corporal foi realizada por absorciometria por dupla emissão de raios X Lunar Prodigy Primo (General Electric Healthcare; Madison, WI).

As crianças e adolescentes que compuseram a amostra tiveram amostras de sangue coletadas, e variáveis bioquímicas foram analisadas por procedimentos padronizados em laboratórios particulares parceiros e no laboratório de análises clínicas da UFPR. A coleta das amostras sanguíneas foi realizada no período da manhã, com jejum de 8 a 12 horas. Foram medidos glicemia em jejum (mg/dL), glicemia 120 (a medição é realizada 120 minutos após ingestão de glicose) (mg/dL), insulina em jejum (microUI/mL), insulina 120 (mg/dL) e perfil lipídico (lipídeos totais (LT), CT, colesterol da VLDL (VLDL-C), HDL-C e TG) (mg/dL). Os níveis de LDL-C foram calculados usando a equação de Friedwald (FRIEDEWALD; LEVY;

FREDRICKSON, 1972), HOMA-IR foi calculado como (glicose em jejum [µU/ml] x insulina [mMol/l]/22.5) (MATTHEWS *et al*., 1985) e QUICKI foi calculado como 1/[log (insulina em jejum)(mU/ml) x log (glicose em jejum) (mMol/l)] (KATZ *et al*., 2000).

5.3 GENOTIPAGEM DOS POLIMORFISMOS INVESTIGADOS

Aproximadamente cinco ml do sangue coletado foram encaminhados ao Laboratório de Polimorfismos e Ligação do Departamento de Genética da UFPR, onde o sangue foi processado e submetido à técnica de *salting out*, de acordo com o método de Lahiri e Nurnberger (1991) com modificações, para a extração do DNA.

A concentração do DNA foi estimada através de espectrofotometria (*Nanodrop*). De acordo com essa concentração, foi feita a diluição das amostras com água Mili-Q para que fosse atingida a concentração desejada de DNA (20 ng/µl).

A genotipagem de todos os SNPs investigados foi realizada por ensaio de discriminação alélica TaqMan (*Applied Biosystems*). Para cada 3µl de DNA, foram utilizados 3µl de MasterMix, 0,3µl de primer e 1,7µl de água mili-Q. O aparelho utilizado foi o *Applied Biosystems Viia 7 Real-Time PCR System*, sendo que foram feitas as seguintes etapas: 1) 50ºC por 2 minutos, 2) 95º por 10 minutos, 3) 50 ciclos de 95ºC por 15 segundos, intercalados por 62ºC por 1 minuto, 4) 60ºC por 2 minutos.

5.4 ANÁLISE ESTATÍSTICA

As frequências dos genótipos e alelos foram obtidas por contagem direta e comparadas entre o grupo de sobrepesos/obesos e eutróficos por teste do qui-quadrado. Foi verificado o equilíbrio de Hardy e Weinberg, referente às frequências genotípicas observadas e as esperadas dos SNPs investigados, também por meio do teste de qui-quadrado. As variáveis foram testadas quanto à normalidade por meio do teste de Kolmogorov-Smirnov com correção de Lilliefors. Os indivíduos foram estratificados por genótipos para cada SNP (segundo os modelos recessivo, dominante, ou ausência de dominância), e suas médias, quanto às variáveis analisadas, foram comparadas por testes paramétricos (teste T – pareado ou não pareado, segundo a natureza da comparação), ou não paramétricos (teste Mann

Whitney para amostras independentes, ou Wilcoxon para dependentes). Análises de regressão múltipla foram utilizadas para testar a significância de modelos estatísticos construídos a fim de se verificar a causalidade entre variáveis (dependentes e independentes). Também foi realizada uma análise de predição de risco, que determina o risco que cada fator gera para determinada variável, através de valores de *odds ratio* (OR) e AUC (*Area Under the Curve*, determinado através de curvas ROC - *Receiver Operating Characteristic*). A significância estatística adotada para os testes foi de 0,05 (5%).

CAPÍTULO I

FTO SNP influences the response to dietary intervention but not to physical exercise program

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ABSTRACT

Background and Aims

The fat mass and obesity-associated (*FTO*) gene is involved in energy homeostasis. The A-allele of the rs9939609 single nucleotide polymorphism (SNP; T>A) is associated with obesity and higher food intake, while its effect in energy expenditure is unclear. The aim of this study is to evaluate the interaction of the rs9939609 with the anthropometric responses to a physical exercise program and to a dietary intervention.

Methods and Results

We studied two independent samples. The first was composed by children and adolescents in which overweight and obese individuals were submitted to a physical

exercise program (N = 136) and normal weight served as a control group (N = 172).The second sample was composed by obese women submitted to a hypocaloric dietary intervention (N = 126). Physical exercise and dietary intervention were effective, independently of genotype. We found no association of *FTO* rs9939609 with obesity in children and adolescents (p = 0.67). The rs9939609 affected the response to dietary intervention in obese women: A-allele carriers reduced 2.7cm less of abdominal circumference (AC) than homozygous TT (p= 0.04), while no effect was observed in response to physical exercise in overweight and obese children and adolescents.

Conclusion

Obese women exhibited resistance to abdominal circumference reduction in function of the A-allele presence. The same allele did not show interaction with the exercise program applied, which suggests that the *FTO* rs9939609 influence on energy expenditure may be small, or dependent of other factors such as sex and age.

Keywords: *FTO*, rs9939609 SNP, obesity, dietary intervention, physical exercise, obese women, childhood obesity.

INTRODUCTION

The common obesity, whose prevalence has been increasing worldwide, has a complex etiology, that result of interactions from the endogenous (genetic) and exogenous (lifestyle) factors [1]. It is well established the role of healthy feeding and lifestyle in the prevention and treatment of common obesity, but the impact of genetic factors in this context is still not well understood. In this sense, many research studies are conducted seeking to identify genetic variants in genome that contribute to phenotypes associated with obesity, such as variants that contribute to the BMI increase [2-4].

In addition to this identification, it is necessary to analyze the effect of variants in specific contexts in order to identify the interaction factors (genotype-environment) and the direction of these interactions, which may contribute to the predisposition and the response to obesity treatments [5-6].

The fat mass and obesity-associated (*FTO*) gene seems to be an excellent candidate gene, since it has been related to weight gain [2]. *FTO* gene product is a 2-oxoglutarate dependent nucleic acid demethylase [7] and has more affinity for single strand DNA/RNA [7,8]. *FTO* is expressed in the whole body, especially in the hypothalamus, which is involved in regulation of energy balance [2,9]. Stratigopoulos *et al*. [10] found that fasted mice had a reduced *FTO* expression in hypothalamus compared to fed mice. This result suggests that the variation in *FTO* levels in hypothalamus can be a signal to promote feeding [9].

FTO rs9939609 single nucleotide polymorphism (SNP) (T>A) is localized in the first intron of the gene, and the risk allele (A-allele) is associated with a higher body mass index (BMI) and increased food intake [2,11,12].

Thus, with the objective of adding efforts in the elucidation of the genotype x environment interactions that predispose and/or interfere in therapeutic approaches of obesity, this study verified the *FTO* rs9939609 SNP interactions with two interventions: physical exercise in overweight and obese children and adolescents and hypocaloric dietary intervention in obese women.

METHODS

Study Design

This study presents the analysis of interaction of the same anthropometric and genetic variables in two independent sample groups, which were structured and submitted to interventions at different times. The experimental design in each sample group was longitudinal.

In total, 434 individuals were analyzed, 308 of which constituted one sample (children and adolescents), and 126 constituted another independent sample (obese women). Thus, the analyzes were concentrated in each group, and not between them, due to the differences between the applied interventions, and the participants profile. However, both samples were composed of individuals from Curitiba and neighboring cities, Brazil, with predominantly Euro-Brazilian ancestry.

The studies were approved by the ethics committee of the Federal University of Paraná (UFPR) (Protocol number 2460.067/2011) and Pontifical Catholic

University of Parana's Institutional Ethics Board (IEB approval number: 0005306/11). Informed Consent was obtained from every participant.

Sample Groups and Interventions

Children and adolescents group – Physical exercise program

This group was composed of 308 children and adolescents of both sexes (204 boys and 104 girls), of which 172 had normal weight and 136 were overweight or obese (31overweight and 105 obese; according to parameters defined by WHO).The mean overall age was 13.55 ± 2 years old (aged 8-17 y).

They were recruited in public schools of the state of Paraná, Brazil. The inclusion criteria in this group were: medical liberation for practicing physical exercise and do not use drugs that could interfere on weight control and/or lipid levels. Those who were in agreement with the established criteria were invited to participate in this research, and those who accepted, with the legal responsible consent, had the free and informed consent term signed by them. The blood samples were collected and BMI Z-score, waist circumference (WC) and abdominal circumference (AC) measured.

The 136 overweight or obese children and adolescents were subjected to physical exercises composed of four different types of training. The 172 children and adolescents with normal weight were included in some analyzes as a reference group.

The physical exercises were conducted by Physical Education professionals, and applied three times a week during 12 weeks on students in their home schools.

Four kinds of physical exercise were conducted: land-based aerobic exercise, high intensity interval training (HIIT), combined training and aquatic exercise. However, for the statistical analyzes the physical exercise groups were analyzed together, since there was no significant impact of the different trainings in the analyzed variables. Details of the applied exercises are in the supplemental material.

After the conclusion of the exercise program, the anthropometric data were collected again. It was not possible to obtain AC and WC data from all individuals who completed the program (n = 136), therefore the analyzes of these variables count with a smaller number of individuals (n = 94 for AC and n = 58 for WC).

Obese women – Dietetic intervention

This group was initially constituted by 199 obese women (BMI ≥ 30, according to parameters defined by WHO). At the end of the study 126 women completed the hypocaloric dietary intervention. Only this group was statistically analyzed.

The women that participated of this study were invited to participate by local radio and television, aiming to reduce weight. The inclusion criteria in this group were: to be obese, woman, have 20 years or more, being in reproductive period (not in menopause), not pregnant and not breastfeeding. Women in drug treatment for weight control, with hypothyroidism, type I diabetes, kidney disease, hypertension or who have undergone stomach reduction surgery were excluded from the study.

Those who were in agreement with the established criteria were invited to participate in this research, and those who accepted signed the free and informed consent term.

The nutritional intervention design, and the application of the same, counted with a multidisciplinary team of professionals and postgraduates of the Nutrition Department of the Pontifical Catholic University of Paraná. Psychologists, nutritionists, nurses and genetic postgraduate collected preliminary information from women who fit in the study. The blood samples were collected and BMI, AC and WC were measured. A questionnaire containing eating habits was also applied to provide the basis for the elaboration of a personalized diet.

Then, the dietetic intervention was started, which had two components: (1) a group nutritional intervention with two sessions, one consisted in readings about choosing healthy foods and one workshop about food labels in the third and fifth week, and (2) an individual dietetic intervention with three sessions. The five sessions occurred during seven weeks.

The individual dietetic intervention was performed by a nutritionist and consisted in a hypocaloric diet based on estimates of their daily energy needs (total energy expenditure) with a deficit of 600 Kcal/per day. Because of this, the diets ranged between 1000 and 2200 Kcal/per day, and had two options for dinner (salad, bread and cheese or salad, rice, beans and chicken). The dinner options were based on previously reported dietary habits. The sessions of individual dietetic intervention occurred in the second, fourth and sixth weeks, changing foods of the diet to avoid food monotony [13].

After the seven weeks of intervention, the anthropometric data were collected again. It was not possible to obtain AC and WC data from all individuals who completed the program (n = 126), hence the analyzes of these variables count with a smaller number of individuals (n = 125 for AC and n = 124 for WC).

The experimental procedure applied in all the sample groups is demonstrated in Figure 1.

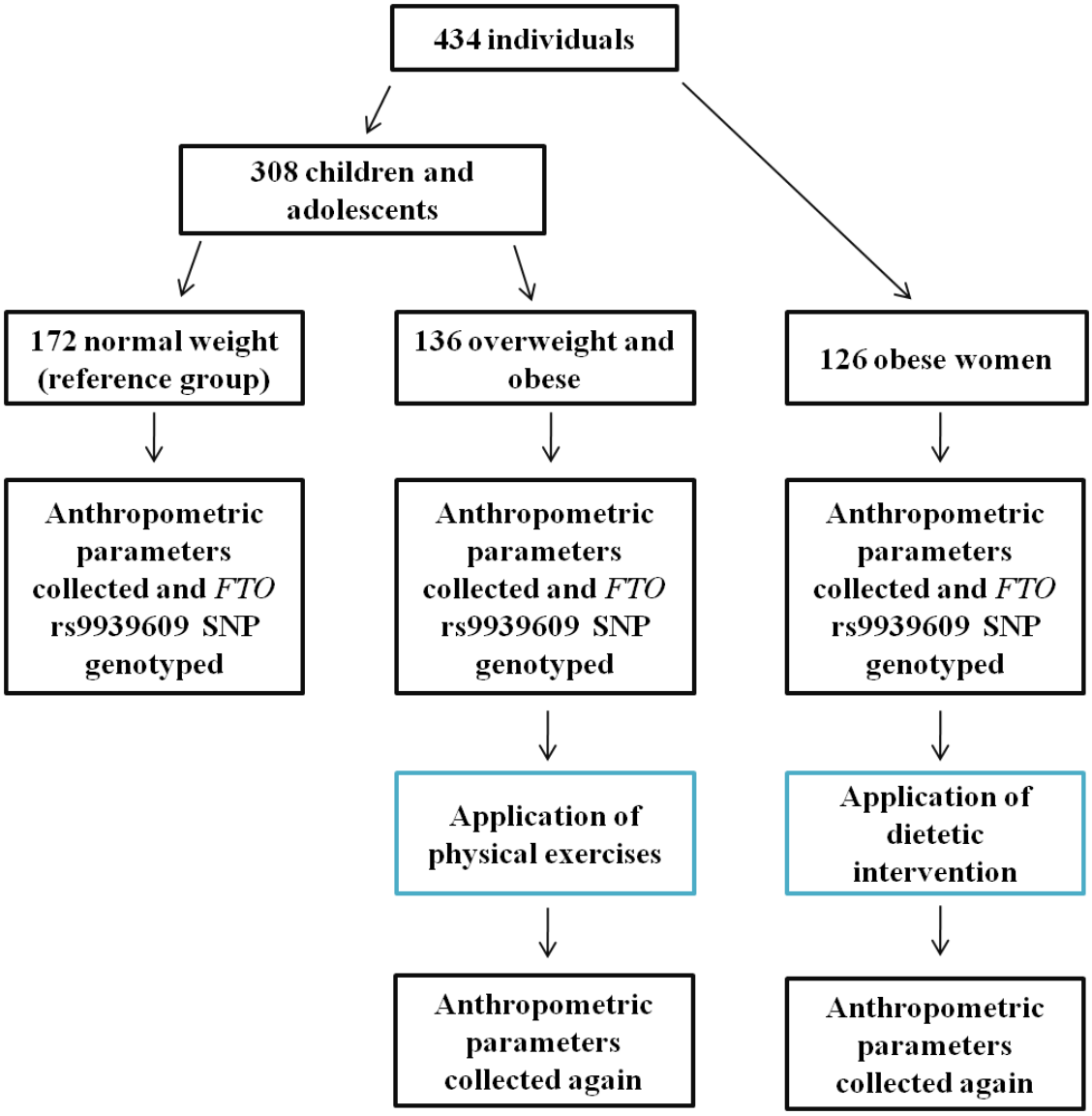

Figure 1. Study design.

Anthropometric variables

The anthropometric variables were collected according to the Anthropometric Indicators Measurement Guide [14], with the individuals wearing light clothes and without shoes.

Three measurements were obtained and the median between them was considered. The children and adolescents were considered overweight when their BMI Z-score was between +1 and +2, and obese when their BMI Z-score was more than +3. Women were classified as obese when BMI ≥ 30 [15].

DNA extraction and genotyping

The DNA was extracted from peripheral blood according to the salting-out technique Lahiri and Nurnberger [16], and then diluted to 20ng/µl. The *FTO* rs9939609 SNP was genotyped with a TaqMan SNP genotyping assay (Applied Biosystems). The reactions were done using the following conditions: 60°C for 30s, 95°C for 10min, 50 cycles of 95°C for 15s and 60°C for 1 min, and 60ºC for 30s. Three previously sequenced control samples, representative of each of the possible genotypes, were included in each reaction.

Statistical analysis

The frequencies of genotypes and alleles were obtained by direct counting and, regarding children and adolescents, compared between the group of overweight/obese and normal weight by chi-square test. The Hardy-Weinberg equilibrium was verified, also using the chi-square test.

The continuous variables were tested for normality using the Kolmogorov-Smirnov test with Lilliefors correction. The initial and final mean of the variables (before and after the interventions) were compared by paired parametric or no parametric tests (t test paired or Wilcoxon test, respectively).

The recessive, dominant and co-dominant models of allelic interaction were tested. The dominant model fitted our results, and henceforth adopted for analyzes that involved the sample stratification by rs9939609 SNP genotype. Independent comparison tests of mean were used to evaluate the mean differences (initial – final)

in the anthropometric parameters between genotypes (Parametric – t test or nonparametric – Mann Whitney). Multiple regression analyzes were also applied. Statistical significance adopted for the tests was 0.05 (5%).

RESULTS

The physical exercise and dietary intervention promoted changes in anthropometric variables of overweight/obese children and adolescents and obese women, respectively (Table 1A and 1B).

The physical exercise contributed to reduction of 0.23kg/m² in BMI Z-score ($p = 10^{-4}$) in overweight/obese children and adolescents (Table 1A). The means of the variables analyzed in the normal weight group served as reference in order to check whether variables that initially were different between overweight/obese and normal weight groups had become similar due to the physical exercise program. However, all anthropometric measures that initially were different between these groups remained higher in overweight/obese (Table 1A).

Similar to the exercise effect, the diet was also effective: reduction of 0.9kg/m² in BMI ($p = 10^{-4}$), 7.04cm in AC ($p = 10^{-4}$) and 3.28cm in WC ($p = 10^{-4}$) was found in obese women (Table 1B).

Table 1A. Comparisons of initial and final means of anthropometric variables (before and after physical exercise) in overweight and obese children and adolescents, and their comparisons with means of anthropometric variables of normal weight children and adolescents.

Children and adolescents								
Variables	Overweight and obese				Normal weight			
	N	Initial mean ± SD	Mean after 12 weeks ± SD	p	N	Mean ± SD	p*	p**
BMI Z-score (kg/m²)	136	2.88 ± 1.09	2.80 ± 1.08	**0.0008**	172	-0.21 ± 0.83	$\mathbf{10^{-4}}$	$\mathbf{10^{-4}}$
AC (cm)	83	96.84 ± 12.19	96.05 ± 12.62	0.29	129	67.63 ± 6.35	$\mathbf{10^{-4}}$	$\mathbf{10^{-4}}$
WC (cm)	55	93.31 ± 10.99	92.84 ± 11.38	0.22	58	67.30 ± 5.74	$\mathbf{10^{-4}}$	$\mathbf{10^{-4}}$

BMI: Body mass index; AC: Abdominal circumference; WC: Waist circumference; SD: Standard deviation; p: comparison between the initial and after 12 weeks means of physical exercise in overweight and obese children and adolescents; p*: comparison between the initial mean in the overweight and obese individuals and the mean in normal weight individuals; p**: comparison between the mean after 12 weeks in the overweight and obese individuals and the mean in the normal weight individuals.

Table 1B. Comparison of initial and final means of anthropometric variables (before and after dietetic intervention) in obese women.

Obese women				
Variables	N	Initial mean ± SD	Mean after 7 weeks ± SD	P
BMI (kg/m²)	126	35.11 ± 5.15	34.19 ± 5.09	$\mathbf{10^{-4}}$
AC (cm)	125	109.44 ± 11.56	101.88 ± 10.49	$\mathbf{10^{-4}}$
WC (cm)	124	95.91 ± 9.77	92.08 ± 10.93	$\mathbf{10^{-4}}$

BMI: Body mass index; AC: Abdominal circumference; WC: Waist circumference; SD: Standard deviation; p: comparison between the initial and after 7 weeks means of nutritional intervention in obese woman.

The allele and genotype frequencies of rs9939609 SNP in children and adolescents (overweight/obese and normal weight groups) and in obese women are shown in table 2. The rs9939609SNP genotypes distribution are in Hardy-Weinberg equilibrium in all sample groups (p>0.05).

Table 2. Genotype and allele frequencies of *FTO* rs9939609 SNP in overweight and obese children and adolescents, in normal weight children and adolescents, and in obese women.

Children and adolescents - Overweight and obese				
Genotype	N	%	Allele	% ± SE
TT	53	38.97	T	62.13 ± 0.01
AT	63	46.32		
AA	20	14.71	A	37.87 ± 0.01
Total	136	100		
Children and adolescents - Normal weight				
Genotype	N	%	Allele	% ± SE
TT	65	37.79	T	63.95 ± 0.01
AT	90	52.33		
AA	17	9.88	A	36.05 ± 0.01
Total	172	100		
Obese women				
Genotype	N	%	Allele	% ± SE
TT	35	27.78	T	50.4 ± 0.01
AT	55	43.65		
AA	36	28.57	A	49.6 ± 0.01
Total	126	100		

SE: Standard error.

The risk allele (A-allele), frequently associated with obesity, was found at similar frequency among overweight/obese group, compared to children and adolescents with normal weight (p = 0.67).

The rs9939609 A-allele effect on anthropometric variables was found only in interaction with dietary intervention. The A-allele carriers reduced on average 2.7cm less of AC than homozygous TT (p= 0.04) (figure 2B). No rs9939609 A-allele influence was observed in response to exercise in obese/overweight children and adolescents (figure 2A).

In transversal analyzes (at baseline and at the final moment), in both sample groups, no rs9939609 A-allele effect was found (analyses in supplemental material).

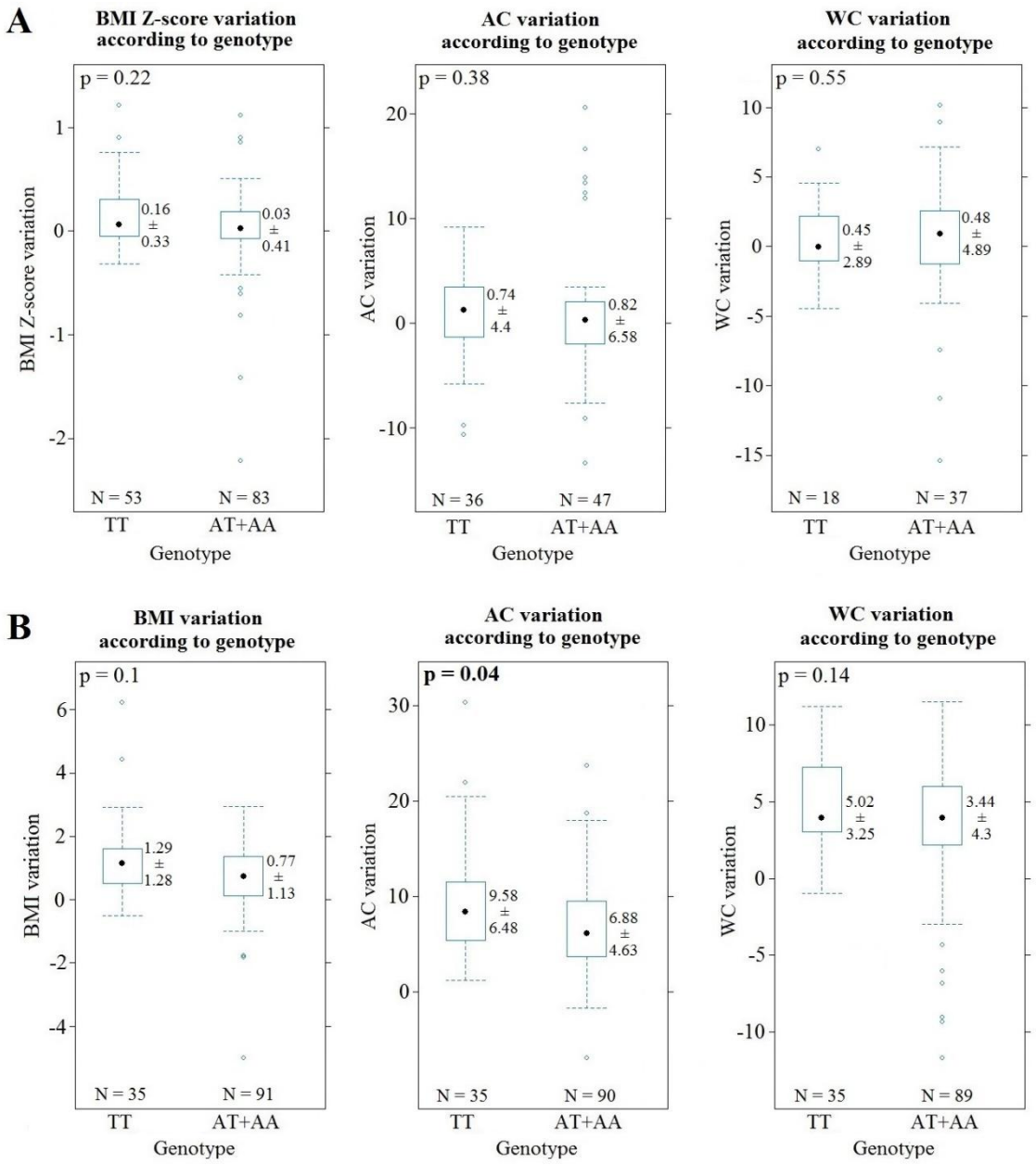

Figure 2. Comparisons of mean variation (± SD) of anthropometric variables between carriers and non-carriers of rs9939609 A-allele. (A) Overweight and obese children and adolescents subjected to physical exercises, mean variation in body mass index (BMI) Z-score, abdominal circumference (AC) and waist circumference (WC) according to genotype. (B) Obese woman subjected to dietary intervention, mean variation in BMI, AC and WC according to genotype.

Multiple regression analyzes were applied in models in which the dependence of variation of anthropometric measurements was evaluated as a function of possible independent variables in both sample groups. These analyzes confirmed the interaction between rs9939609 SNP and dietary intervention on AC change in obese women (p = 0.047) in a dominant model of A-allele (Table 3). The AC change was

also dependent on the BMI variation (p = 0.001), which was expected because of the correlation between these variables. We found no relation between rs9939609 SNP and WC variation (p = 0.16). The change in this variable was only dependent of the BMI change (p = 0.003) in obese women.

The lack of rs9939609 SNP effect on physical exercise response in children and adolescents was also confirmed in obese/overweight children and adolescents, in whom the AC variation was dependent of the BMI variation only (p = 0.001) (Table 3). The analysis was corrected for type of training.

Table 3. Models of multiple regression analysis in overweight and obese children and adolescents and in obese women.

Overweight and obese children and adolescents			
Dependent variable	Independent variables considered	β ± SD	p
BMI Z-score variation	Genotype	0.06 ± 0.08	0.46
	Age	0.05 ± 0.08	0.52
	Sex	0.02 ± 0.08	0.78
AC variation	Genotype	0.05 ± 0.10	0.6
	BMI Z-score variation	0.35 ± 0.10	**0.001**
	Age	0.17 ± 0.10	0.11
	Sex	0.12 ± 0.10	0.25
WC variation	Genotype	0.05 ± 0.13	0.68
	BMI Z-score variation	0.20 ± 0.13	0.14
	Age	0.03 ± 0.13	0.8
	Sex	0.04 ± 0.13	0.78
Obese women			
Dependent variable	Independent variables considered	β ± SD	p
AC variation	Genotype	2.03 ± 1.02	**0.047**
	BMI variation	1.27 ± 0.38	**0.001**
WC variation	Genotype	0.12 ± 0.09	0.160
	BMI variation	0.26 ± 0.09	**0.003**

BMI: Body mass index; AC: Abdominal circumference; WC: Waist circumference; β: Regression coefficient; SD: Standard deviation. Genotypes: AT+AA and TT (dominant model).

DISCUSSION

In the present study, it was possible to evaluate the interaction between *FTO* rs9939609 SNP and metabolic changes induced by dietary intervention or physical

exercise, which were reflected in the anthropometric measures variation, in two independent samples.

It is known that in obesity, environmental factors such as diet and physical exercise play an important role in its prevention and are widely used as treatment. The presence of specific genetic variants leads to individual variation in response to these approaches, which, in general, indicates that more individualized approaches could be more efficient.

In our study, although both interventions demonstrated beneficial effect on the anthropometric variables evaluated, obese women carriers of the A-allele appeared to benefit less from the applied diet compared to non-carriers obese women; while the same allele did not influence the variables change in children and adolescents submitted to physical exercise. This finding suggests that the A-allele, besides contributing negatively to the baseline anthropometric and metabolic profile [17-19], may also influence the results of obesity therapeutic approaches.

In our study, the A-allele carriers obese women decreased in mean 2.7cm less of abdomen circumference compared to non-carriers, submitted to the same calorie restriction orientation. This finding is interesting, since the *FTO* genotype did not influence the BMI reduction in response to diet, but specifically modified the fat central deposit response to it. The harmful effect of increased central fat deposition for whole metabolic health is well known. It has unique characteristics of development and function that differentiate it from the adipose tissue distributed in the rest of the body [20], and its accumulation is correlated with increased susceptibility to various metabolic complications [21-23]. In this context, the A-allele effect may be of particular importance in women, since postmenopausal women show an increase in visceral fat, compared to premenopausal women because of the decline in the estrogen protective effect [24,25], which may be aggravated by the presence of the rs9939609 risk allele.

Several studies demonstrate the association of the *FTO* rs9939609 SNP with obesity and metabolic disorder traits [26-28]. Because it is intronic, its functional role is not fully understood, but studies suggest that the risk allele is functional, and leads to increased *FTO* expression [29]. Berulava and Horsthemke [29] found higher levels of primary *FTO* transcript from the risk allele, compared to levels obtained from the non risk allele in blood cells and skin fibroblasts. The association between the risk allele and increased *FTO* expression is consistent with the observed in *FTO*

knockout mice, which presented less weight and less fat mass compared to wild-type [30].

It is not well established how the *FTO* overexpression affects the demethylase function of the encoded protein, and consequently, its physiological contribution to adiposity and associated metabolic disorders. However, Merkestein *et al*. [31] demonstrated that mice that overexpressed *FTO* exhibited altered expression of many genes previously associated with obesity. Among these genes, the adiponectin, leptin and adrenergic receptor beta 3 and beta 2, related to food intake control, inflammatory profile and energy expenditure, suggesting that the physiological effect of *FTO* overexpression may involve all these pathways.

In addition to the fact that the *FTO* mRNA is found at high levels in the hypothalamus, a region responsible for energy balance regulation [32], studies have associated the presence of the A-allele with the increase in food and fat intake [11,12,33].

Considering all the above mentioned studies, it is possible that the differential regulation of caloric restriction-responsive pathways have resulted in greater resistance to fat loss in the central area of the body in A-allele carriers. However, it is not possible to rule out the possibility that, in our study, the obese women carriers of the A-allele may have ingested a greater amount and more energetic foods, compared to A-allele non carriers, even with the same caloric restriction orientation, which reflected in lower abdominal circumference losses. To elucidate this issue, more studies involving dietary intervention are needed, as well as functional studies considering the energy pathways preferentially activated in function of *FTO* overexpression.

Despite the lack of interaction between A-allele and the metabolic changes stimulated by physical exercise in our study, it could be involved in energy expenditure front during physical activity due to its participation in the energy homeostasis regulation via hypothalamus. According to Merkestein *et al*. [31] this interaction may involve the exacerbated activation of anabolic pathways in white adipose tissue and skeletal muscles due to the *FTO* overexpression, which could contribute to weight gain, and potentially negatively influence the response to physical exercise, since this route could be preferentially used in detriment of the catabolic pathway.

However, a pathway that clearly explains the effect of *FTO* gene variants on energy expenditure stimulated by physical exercise is unknown, which explains in part the controversial results of studies evaluating this relationship [34].

Our results agree with other studies that found no association of rs9939609 A-allele with energy expenditure [11,12,35]. However, these comparisons should be interpreted with caution, considering that such studies had different methodologies, some measuring basal energy expenditure, using calorimetric approaches [35], others assessed the physical activity level by questionnaires that allowed to classify the individuals of the sample in physically active or inactive [36]. Our study is one of the few that evaluates the interaction of the rs9939609 SNP with the practice of controlled physical exercise in terms of anthropometric profile changes in obese and overweight children and adolescents.

Other factors also contribute to the lack of consensus in the studies that evaluate the physical activity and rs9939609 SNP interaction, such as the ethnicity, gender and age of participants. Kilpeläinen *et al*. [37], in a meta-analysis, found that physical activity attenuates the odds ratio for obesity in 27% in adults with the A allele, but in children and adolescents this interaction was not observed.

Despite promising results, our work has some restrictions. The largest of these refers to the samples size, which generally affect the identification of minor effects. This restriction also influenced the analyzes performed in the obese children and adolescents group, which could not be stratified according to sex neither to specific age groups, which could be important for the identification of sex and age dependent *FTO* interactions.

Knowing the magnitude of contributing factors for obesity and associated co morbidities is extremely important, given the particularities of treatment and prevention that may arise from this knowledge. In this sense, we found that the obese women A-allele carriers, who composed our sample, were less benefited by applied dietary intervention, compared to non-carriers, being this difference represented by the smaller decrease in abdominal circumference, a characteristic that is of great importance in terms of metabolic health.

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REFERENCES

[1] Heni M, Kullmann S, Veit R, Ketterer C, Frank S, Machicao F, et al. Variation in the obesity risk gene FTO determines the postprandial cerebral processing of food stimuli in the prefrontal cortex. Mol Metab 2014;3:109–13. doi:10.1016/j.molmet.2013.11.009.

[2] Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, et al. A Common Variant in the FTO Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. Science 2007;316:889–93.

[3] Speliotes EK, Willer CJ, Berndt SI, Monda KL, Thorleifsson G, Jackson AU, et al. Association analyses of 249,796 individuals reveal eighteen new loci associated with body mass index. Nat Genet 2011;42:937–48. doi:10.1038/ng.686.Association.

[4] Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 2015;518:197–206. doi:10.1038/nature14177.

[5] O'Rahilly S, Sadaf Farooqi I, Yeo GSH, Challis BG. Minireview: Human obesity - Lessons from monogenic disorders. Endocrinology 2003;144:3757–64. doi:10.1210/en.2003-0373.

[6] Leońska-duniec A, Jastrzębski Z, Zarębska A, Maciejewska A, Ficek K, Cięszczyk P. Assessing effect of interaction between the FTO A/T polymorphism (rs9939609) and Physical Activity on Obesity-related traits. J Sport Heal Sci 2016;352. doi:10.1016/j.jshs.2016.08.013.

[7] Gerken T, Girard CA, Tung Y-CL, Webby CJ, Saudek V, Hewitson KS, et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 2007;318:1469–72. doi:10.1126/science.1151710.

[8] Jia G, Yang CG, Yang S, Jian X, Yi C, Zhou Z, et al. Oxidative demethylation of 3-methylthymine and 3-methyluracil in single-stranded DNA and RNA by

mouse and human FTO. FEBS Lett 2008;582:3313–9. doi:10.1016/j.febslet.2008.08.019.

[9] Fawcett KA, Barroso I. The genetics of obesity: FTO leads the way. Trends Genet 2010;26:266–74. doi:10.1016/j.tig.2010.02.006.

[10] Stratigopoulos G, Padilla S, LeDuc CA, Watson E, Hattersley AT, McCarthy MI, et al. Regulation of Fto/Ftm gene expression in mice and humans. Am J Physiol 2010;294:R1185–96. doi:10.1152/ajpregu.00839.2007.Regulation.

[11] Cecil JE, Tavendale R, Watt P, et al. An Obesity-Associated FTO Gene Variant and Increased Energy Intake in Children. N Engl J Med 2008;359:2558–66.

[12] Speakman JR, Rance KA, Johnstone AM. Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. Obesity (Silver Spring) 2008;16:1961–5. doi:10.1038/oby.2008.318.

[13] Saliba LF, Reis RS, Brownson RC, Hino AA, Tureck LV, Valko C, et al. Obesity-related gene ADRB2, ADRB3 and GHRL polymorphisms and the response to a weight loss diet intervention in adult women. Genet Mol Biol 2014;37:15–22. doi:10.1590/S1415-47572014000100005.

[14] Cogill B. Anthropometric indicators measurement guide. Food Nutr Tech Assist Proj 2003; 8-92.

[15] World Health Organization. Obesity and overweight. World Health Organization, 2016.http://www.who.int/topics/obesity/en/. 23/09/2017.

[16] Lahiri DK, Numberger JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 1991;19:5444. doi:10.1093/nar/19.19.5444.

[17] Shahid A, Rana S, Saeed S, Imran M, Afzal N, Mahmood S. Common Variant of FTO Gene, rs9939609, and Obesity in Pakistani Females. BioMed Research International 2013;2013:1-7.

[18] Muñoz-Yáñez C, Pérez-Morales R, Moreno-Macías H, Calleros-Rincón E, Ballesteros G, González RA, et al. Polymorphisms FTO rs9939609, PPARG rs1801282 and ADIPOQ rs4632532 and rs182052 but not lifestyle are associated with obesity related-traits in Mexican children. Genet Mol Biol 2016;553:547–53. doi:10.1590/1678-4685-GMB-2015-0267.

[19] Prakash J, Mittal B, Srivastava A, Awasthi S, Srivastava N. Association of FTO rs9939609 SNP with Obesity and Obesity- Associated Phenotypes in a North Indian Population. Oman Med J 2016;31:99–106. doi:10.5001/omj.2016.20.

[20] White UA, Tchoukalova YD. Sex dimorphism and depot differences in adipose tissue function. BBA - Mol Basis Dis 2014;1842:377–92. doi:10.1016/j.bbadis.2013.05.006.

[21] Smith SR, Lovejoy JC, Greenway F, Ryan D, Bretonne J De, Volafova J, et al. Contributions of Total Body Fat, Abdominal Subcutaneous Adipose Tissue Compartments, and Visceral Adipose Tissue to the Metabolic Complications of Obesity. Metabolism 2001;50. doi:10.1053/meta.2001.21693.

[22] Amati F, Pennant M, Azuma K, Dubé JJ, Toledo FGS, Rossi AP, et al. Lower Thigh Subcutaneous and Higher Visceral Abdominal Adipose Tissue Content Both Contribute to Insulin Resistance. Obesity 2012;20:1115–7. doi:10.1038/oby.2011.401.

[23] Tordjman J, Divoux A, Prifti E, Poitou C, Pelloux V, Hugol D, et al. Structural and inflammatory heterogeneity in subcutaneous adipose tissue : Relation with liver histopathology in morbid obesity. J Hepatol 2012;56:1152–8. doi:10.1016/j.jhep.2011.12.015.

[24] Awad NS, El-tarras AE. Analysis of the APO B R3500Q Mutation and APOE Polymorphism in Taif Saudi Population using Polymerase Chain Reaction-Reveres Hybridization Technique. J Mol Biomark Diagn 2011;2:2–5. doi:10.4172/2155-9929.1000109.

[25] Kanaley JA, Sames C, Swisher L, Swick AG, Ploutz-Snyder LL, Steppan CM, et al. Abdominal Fat Distribution in Pre- and Postmenopausal Women: The Impact of Physical Activity, Age, and Menopausal Status. Metabolism 2001;50:976–82. doi:10.1053/meta.2001.24931.

[26] Al-Attar SA, Pollex RL, Ban MR, Young TK, Bjerregaard P, Anand SS, et al. Association between the FTO rs9939609 polymorphism and the metabolic syndrome in a non-Caucasian multi-ethnic sample. Cardiovasc Diabetol 2008;7:5. doi:10.1186/1475-2840-7-5.

[27] Kring SII, Holst C, Zimmermann E, Jess T, Berentzen T, Toubro S, et al. FTO gene associated fatness in relation to body fat distribution and metabolic traits throughout a broad range of fatness. PLoS One 2008;3:1–7. doi:10.1371/journal.pone.0002958.

[28] Liguori R, Labruna G, Alfieri A, Martone D, Farinaro E, Contaldo F, et al. The FTO gene polymorphism (rs9939609) is associated with metabolic syndrome in morbidly obese subjects from southern Italy. Mol Cell Probes 2014;28:195–9.

doi:10.1016/j.mcp.2014.03.004.

[29] Berulava T, Horsthemke B. The obesity-associated SNPs in intron 1 of the FTO gene affect primary transcript levels. Eur J Hum Genet 2010;18:1054–6. doi:10.1038/ejhg.2010.71.

[30] Fischer J, Koch L, Emmerling C, Vierkotten J, Peters T, Brüning JC, et al. Inactivation of the Fto gene protects from obesity. Nature 2009;458:894–8. doi:10.1038/nature07848.

[31] Merkestein M, McTaggart JS, Lee S, Kramer HB, McMurray F, Lafond M, et al. Changes in gene expression associated with FTO overexpression in mice. PLoS One 2014;9:1–11. doi:10.1371/journal.pone.0097162.

[32] Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature 2006;443:289–95. doi:10.1038/nature05026.

[33] Church C, Moir L, McMurray F, Girard C, Banks GT, Teboul L, et al. Overexpression of Fto leads to increased food intake and results in obesity. Nat Genet 2010;42:1086–92. doi:10.1038/ng.713.

[34] Petkeviciene J, Smalinskiene A, Klumbiene J, Petkevicius V, Kriaucioniene V, Lesauskaite V. Physical activity, but not dietary intake, attenuates the effect of the FTO rs9939609 polymorphism on obesity and metabolic syndrome in Lithuanian adult population. Public Health 2015;135:23–9. doi:10.1016/j.puhe.2016.02.009.

[35] Berentzen T, Kring SII, Holst C, Zimmermann E, Jess T, Hansen T, et al. Lack of association of fatness-related FTO gene variants with energy expenditure or physical activity. J Clin Endocrinol Metab 2008;93:2904–8. doi:10.1210/jc.2008-0007.

[36] Kim JY, DeMenna JT, Puppala S, Chittoor G, Schneider J, Duggirala R, et al. Physical activity and FTO genotype by physical activity interactive influences on obesity. BMC Genet 2016;17:47. doi:10.1186/s12863-016-0357-6.

[37] Kilpeläinen TO, Qi L, Brage S, Sharp SJ, Sonestedt E, Demerath E, et al. Physical Activity Attenuates the Influence of FTO Variants on Obesity Risk : A Meta-Analysis of 218 , 166. PLoS Med 2011;8:e1001116.

[38] Milano GE, Leite N, Chaves TJ, Eisfeld G, Lehtonen R, Souza R De, et al. Atividade da butirilcolinesterase e fatores de risco cardiovascular em adolescentes obesos submetidos a um programa de exercícios físicos. Arq

Bras Endocrinol Metab 2013;57:533–7.

[39] Lopes WA, Leite N, Silva LR, Brunelli DT, Gáspari AF, Radominski RB, et al. Effects of 12 weeks of combined training without caloric restriction on inflammatory markers in overweight girls. Nutrition and Biochemistry 2016;34:1902-12.

[40] Leite N, Lazarotto L, Cavazza JF, Lopes MDFA, Bento PCB, Torres R, et al. Efeitos de exercícios aquáticos e orientação nutricional na composição corporal de crianças e adolescentes obesos. Rev Bras Cineantropometria E Desempenho Hum 2010;12:232–8. doi:10.5007/1980-0037.2010v12n4p232.

SUPPLEMENTARY MATERIAL

Details of the applied exercises

Land-based aerobic exercise: It was performed 45 minutes of walking, 45 minutes of indoor cycling and 20 minutes of stretching [38].

HIIT: Consisted of running periods at high intensity, in which the individual should run at maximal speed for 30 seconds, followed by low intensity recovery interval, which was walking in moderate/fast speed. The training intensity increased as the weeks pass.

Combined training: It was composed of resistance and aerobic training performed in a 60 minutes session. Resistance training was composed by six exercises (leg press, leg extension, leg curl, bench press, lateral pull down and arm curl) and aerobic consisted of walking/running in an athletic track [39].

Aquatic exercise: Each session consisted of five minutes of warming-up, 45 minutes of technique (swimming techniques learning exercises or deep water running) and 10 minutes of stretching and recreation. The deep water running consisted of the individual remains in a vertical position and his body is submerged to shoulder height with the support of a float vest attached to the waist. There is no contact of the feet with the bottom of the pool, and similar movements to walk on land are made [40].

Table 1A. Comparison of variables means between carriers and non-carriers of rs9939609 A-allele before and after the physical exercise in overweight and obese children and adolescents, and comparison of variables means between carriers and non-carriers of rs9939609 A-allele in normal weight children and adolescents.

VARIABLES	Children and adolescents														
	Overweight and obese										Normal weight				
	Before					After									
	AT+AA		TT			AT+AA		TT			AT+AA		TT		
	N	Mean ± SD	N	Mean ± SD	p	N	Mean ± SD	N	Mean ± SD	p	N	Mean ± SD	N	Mean ± SD	p
BMI Z-score (kg/m²)	83	2.78 ± 0.90	53	3.03 ± 1.33	0.73	83	2.75 ± 0.99	53	2.87 ± 1.20	0.95	107	-0.26 ± 0.87	65	-0.12 ± 0.74	0.43
AC (cm)	47	95.14 ± 12.22	36	99.70 ± 12.64	0.10	47	93.82 ± 12.02	36	98.96 ± 12.96	0.08	80	67.38 ± 5.88	49	68.03 ± 7.09	0.79
WC (cm)	37	94.11 ± 11.30	18	91.67 ± 10.43	0.63	37	93.63 ± 11.82	18	91.22 ± 10.56	0.81	37	67.58 ± 5.65	21	66.81 ± 6.02	0.47

BMI: Body mass index; AC: Abdominal circumference; WC: Waist circumference; SD: Standard deviation; p: comparison between carriers and non-carriers of rs9939609 A-allele.

Table 1B. Comparison of variables means between carriers and non-carriers of rs9939609 A-allele before and after the dietetic intervention in obese women.

VARIABLES	Obese women									
	Before					After				
	AT+AA		TT		p	AT+AA		TT		p
	N	Mean ± SD	N	Mean ± SD		N	Mean ± SD	N	Mean ± SD	
BMI (kg/m²)	91	35.06 ± 4.57	35	35.26 ± 6.48	0.54	91	34.28 ± 4.81	35	33.96 ± 5.82	0.43
AC (cm)	90	109.3 ± 11.3	35	110.08 ± 12.4	0.96	90	102.42 ± 10.97	35	100.49 ± 9.16	0.47
WC (cm)	89	95.66 ± 9.67	35	96.74 ± 10.25	0.65	89	92.22 ± 11.15	35	91.71 ± 10.53	0.91

BMI: Body mass index; AC: Abdominal circumference; WC: Waist circumference; SD: Standard deviation; p: comparison between carriers and non-carriers of rs9939609 A-allele.

CAPÍTULO II

FTO SNP effect on insulin sensitivity markers and their lack of interaction with the physical exercise

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Abstract

Introduction and Aims

The rs9939609 single nucleotide polymorphism (SNP) in *FTO* gene is associated with obesity and type 2 diabetes. The aim of this study is to verify the rs9939609 A-allele effect on biochemical variables in 432 children and adolescents (obese, overweight and normal weight), as well as evaluate this

SNP effect on biochemical variables in response to a physical exercise program realized in 136 overweight/obese children and adolescents.

Methods and Results

432 children and adolescents were genotyped. The AA genotype carriers have higher levels of insulin (p = 0.05), HOMA (p = 0.01) and lower levels of QUICKI (p = 0.04). The rs9939609 A-allele did not influence the response to physical exercise.

Conclusion

The rs9939609 A-allele influenced parameters related to glucose metabolism, and did not interact with physical exercise.

Keywords

FTO, rs9939609 SNP, obesity, insulin, HOMA-IR, QUICKI, physical exercise, childhood obesity.

Introduction

The fat mass and obesity associated (*FTO)* gene product is a 2-oxoglutarate dependent nucleic acid demethylase (GERKEN et al., 2007). It can have several target genes, and it seems that among them are adiponectin and ghrelin genes (MERKESTEIN et al., 2014). These two hormones have many functions in the body, including a relation with glucose and insulin metabolism (MIHALACHE et al., 2016; SHEHZAD et al., 2012).

Single nucleotide polymorphism (SNPs) in *FTO* gene are associated with obesity (FRAYLING et al., 2007), type 2 diabetes (HERTEL et al., 2011) and other metabolic complications (LIGUORI et al., 2014). One of the most studied SNPs is rs9939609 SNP (T>A), which, beyond association with higher body mass index (BMI) (FRAYLING et al., 2007), was also associated with levels of cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein

cholesterol (HDL-C), triglycerides (TG), glucose and insulin (DE LUIS et al., 2016; FREATHY et al., 2008; MUÑOZ-YÁÑEZ et al., 2016; PRAKASH et al., 2016).

Considering the potential effect of this *FTO* SNP on biochemical variables that predict essential metabolic functions, the present study investigated this relationship in 432 children and adolescents (divided into an overweight/obese group and a normal weight group) of Brazil, and their possible interaction with metabolic changes induced in these individuals by physical exercise.

Methods

Subjects

The study was composed by 432 children and adolescents of both sexes (290 boys and 142 girls), of which 169 had normal weight and 263 were overweight or obese (80 overweight and 183 obese) (according to parameters defined by WHO). The mean overall age was 13.51 ± 0.09 years old (aged 8-17 y).

They were recruited in public schools of the state of Paraná, Brazil. The inclusion criteria were: medical liberation for physical exercise and do not use drugs that could interfere on weight control and/or lipid levels. Those who were in agreement with the established criteria were invited to participate in this research, and those who accepted, with the legal responsible consent, had the free and informed consent term signed by them. The study was approved by the ethics committee of the Federal University of Paraná (UFPR) (Protocol number 2460.067/2011) (NASCIMENTO *et al*., 2017).

Weight and height were measured with an accuracy of 0.1 kg and 0.1 cm, respectively. BMI was calculated as weight in kilograms divided by the square of height in meters, and then converted into BMI Z-score according to WHO (2016). The children and adolescents were considered overweight when their BMI Z-score was between +1 and +2, and obese when their BMI Z-score was more than +3 (WHO, 2016).

Biochemical variables

The blood samples were collected and total cholesterol (TC), HDL-C and TG were measured by standard procedures in private partner laboratories and in the clinical analyzes laboratory of UFPR. Blood glucose levels were determined by the enzymatic method and insulin was measured by the chemiluminescence immunoassay technique, by automated equipment. LDL-C levels were calculated using the Friedewald equation (FRIEDEWALD; LEVY; FREDRICKSON, 1972), homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as (fasting blood glucose [µU/ml] x insulin [mMol/l]/22.5) (MATTHEWS et al., 1985) and the quantitative insulin sensitivity check index (QUICKI) was calculated as 1/[log (fasting insulin)(mU/ml) x log (fasting blood glucose) (mMol/l)] (KATZ et al., 2000).

Physical exercise

Of the 263 overweight or obese children and adolescents that participate in the study, 136 were submitted to a physical exercise program. The 169 children and adolescents with normal weight were included in some analyzes as a reference group (NASCIMENTO *et al*., 2017).

The physical exercises were composed of four different types of training. The physical exercises were conducted by Physical Education professionals, and applied three times a week during 12 weeks on students in their home schools (NASCIMENTO *et al*., 2017).

Four types of physical exercise were realized: land-based aerobic exercise, high intensity interval training (HIIT), combined training and aquatic exercise. However, for the statistical analyzes the physical exercise groups were analyzed together, since there was no significant impact of the different trainings in the analyzed variables. Details of the applied exercises are in the supplemental material (NASCIMENTO *et al*., 2017).

After the conclusion of the exercise program, the biochemical data were collected again. It was not possible to obtain data on all variables from all individuals who completed the program (n = 136), therefore the analyzes of some variables count with a smaller number of individuals.

The experimental procedure applied is demonstrated in Figure 1.

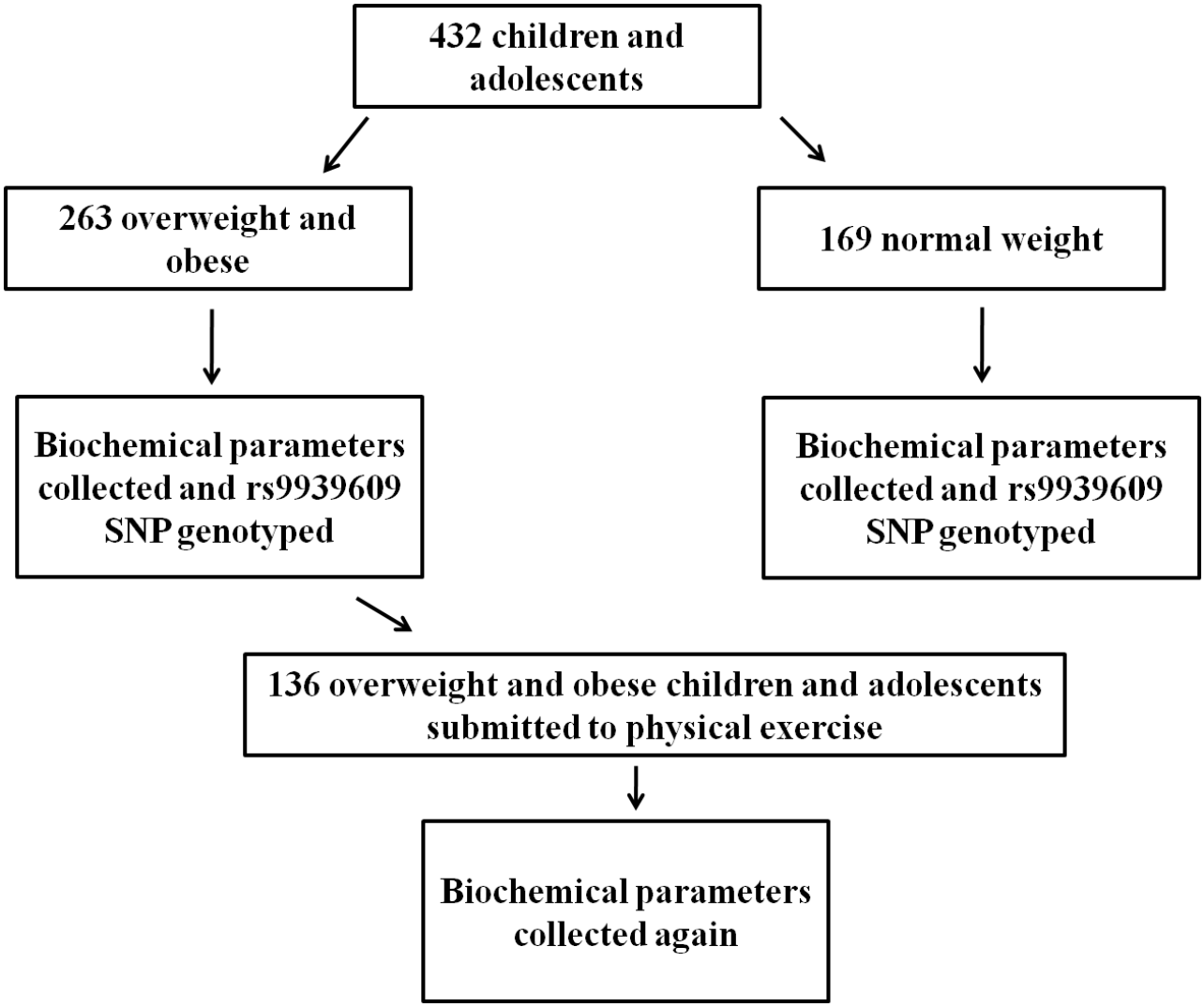

Figure 1. Study design.

DNA extraction and genotyping

DNA was extracted from peripheral blood according to the salting-out technique Lahiri and Nurnberger (LAHIRI; NUMBERGER, 1991), and then diluted to 20ng/µl. *FTO* rs9939609 SNP was genotyped with a TaqMan SNP genotyping assay (Applied Biosystems). The reactions were done using the following conditions: 60°C for 30s, 95°C for 10min, 50 cycles of 95°C for 15s and 60°C for 1 min, and 60ºC for 30s. Three previously sequenced control

samples, representative of each of the possible genotypes, were included in each reaction (NASCIMENTO *et al*., 2017).

Statistical analysis

The frequencies of genotypes and alleles were obtained by direct counting and compared between the group of overweight/obese and normal weight by chi-square test, which was also used to check the Hardy-Weinberg equilibrium.

The continuous variables were tested for normality using the Kolmogorov-Smirnov test with Lilliefors correction. The initial and final mean of the variables (before and after the interventions) were compared by paired parametric or no parametric tests (t test paired or Wilcoxon test, respectively).

The recessive, dominant and lack of dominance models of allelic interaction were tested. The recessive model was more adequate to our results, being therefore adopted for analyzes that involved the sample stratification by rs9939609 SNP genotype. The variables means were compared between genotypes by parametric or no parametric tests (t test or Mann Whitney, respectively). Independent comparison tests of means were used to evaluate the means differences (initial – final) in the biochemical parameters between genotypes (Parametric – t test or nonparametric – Mann Whitney). Multiple regression analyzes were also applied. Statistical significance adopted for the tests was 0.05 (5%).

Results

In the overweight and obese group, TT genotype frequency was 38.02%, AT genotype frequency was 48.67% and AA genotype frequency was 13.31%. In the normal weight group, TT genotype frequency was 37.28%, AT genotype frequency was 52.66% and AA genotype frequency was 10.06%. The rs9939609 SNP genotypic distributions were in Hardy-Weinberg equilibrium in all sample groups ($p > 0.05$).

The risk allele (A), frequently associated with obesity, was not found at a higher frequency in overweight/obese individuals than in the normal weight group ($p = 0.84$).

We analyzed the SNP effect on the investigated variables at baseline (in all overweight or obese individuals, including the subgroup that subsequently participated of the physical intervention, and in normal weight group) and at the final moment (in the overweight or obese subgroup that participated of the physical intervention only). We found that overweight or obese individuals with AA genotype presented less favorable insulin sensitivity profile compared with AT and TT individuals (table 1).

These individuals showed higher values of HOMA-IR ($p = 0.006$), lower values of QUICKI ($p = 0.04$) and a trend toward higher levels of insulin ($p = 0.08$) compared to TT+AT carriers. The AA genotype effect remained in the subgroup that participated of the intervention, since HOMA-IR values remained higher in AA genotype carriers ($p = 0.02$), QUICKI values remained lower ($p = 0.04$), the trend toward higher levels of insulin remained ($p = 0.05$) and also appeared an effect in glucose levels ($p = 0.002$), that is lower in individuals with AA genotype. Regarding the normal weight group, no AA genotype effect was observed (table 1).

Multiple regression analyzes corrected for type of training were applied in the overweight or obese group. The genotype effect on HOMA-IR values was confirmed before the physical exercise ($p = 0.009$), but on QUICKI was lost. Regarding the variables values after physical exercise, the genotype effect was confirmed also on HOMA-IR ($p = 0.006$) and insulin ($p = 0.04$). The results are shown in table 2.

Now, evaluating the physical exercise program effect on overweight or obese individuals subgroup, regardless of genotype, the training was effective (since an improvement was observed in TC ($p = 0.002$), LDL-C ($p = 0.03$), glucose ($p = 0.02$), insulin ($p = 10^{-4}$), HOMA-IR ($p = 0.0004$) and QUICKI ($p = 10^{-4}$) parameters - Table 1 in supplementary material).

The *FTO* SNP showed no interaction with the metabolic effects of physical exercise, since the genotype did not determine differences in the changes induced by it, as shown in table 3. The lack of AA genotype effect on biochemical variables in response to exercise was confirmed through multiple

regression analyzes (corrected for type of training), where we considered the means variations (initial – final) as dependent variable.

Table 1. Comparison of variables means between individuals stratified according to a recessive model before and after the physical exercise in overweight and obese children and adolescents, and comparison of variables means between individuals stratified according to a recessive model in normal weight children and adolescents.

	Overweight and obese										Normal weight				
	Before					After									
	TT+AT		AA			TT+AT		AA			TT+AT		AA		
Variables	N	Mean ± SD	N	Mean ± SD	p	N	Mean ± SD	N	Mean ± SD	p	N	Mean ± SD	N	Mean ± SD	p
TC (mg/dl)	202	161.83 ± 37.07	30	165.35 ± 33.11	0.62	119	156.13 ± 34.40	17	148.3 ± 29.26	0.22	85	157.46 ± 29.54	5	153.56 ± 4.75	0.77
HDL-C (mg/dl)	227	47.67 ± 12.16	35	50.20 ± 11.14	0.11	118	47.06 ± 14.29	17	45.62 ± 9.91	0.88	144	46.76 ± 11.24	17	43.24 ± 9.43	0.22
LDL-C (mg/dl)	202	92.22 ± 29.54	30	90.17 ± 23.26	0.72	119	90.10 ± 27.64	17	82.62 ± 30.94	0.31	85	91.36 ± 26.83	5	92.17 ± 13.90	0.95
TG (mg/dl)	227	108.57 ± 57.90	35	104.87 ± 52.37	0.83	118	102.51 ± 50.33	17	98.06 ± 68.54	0.44	144	73.73 ± 31.37	17	78.74 ± 25.69	0.26
Glucose (mg/dl)	228	87.09 ± 10.12	35	85.37 ± 10.30	0.22	117	85.76 ± 8.06	18	79.57 ± 5.76	**0.002**	152	91.50 ± 11.96	17	94.35 ± 9.23	0.32
Insulin (uUI/ml)	192	14.14 ± 11.68	30	18.74 ± 16.25	0.08	95	12.89 ± 8.05	15	17.58 ± 9.80	0.05	144	5.92 ± 4.82	17	5.55 ± 3.63	0.94
HOMA-IR	88	1.64 ± 1.22	17	2.56 ± 1.59	**0.006**	48	1.40 ± 0.88	8	2.53 ± 1.43	**0.01**	110	1.12 ± 0.77	17	1.35 ± 1.09	0.64
QUICKI	81	0.36 ± 0.06	15	0.34 ± 0.08	**0.04**	43	0.36 ± 0.04	7	0.32 ± 0.03	**0.04**	73	0.41 ± 0.08	13	0.40 ± 0.09	0.29

TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: triglycerides; HOMA-IR: Homeostatic model assessment for insulin resistance; QUICKI: Quantitative insulin sensitivity check index; SD: Standard deviation; p: comparison between individuals stratified according to a recessive model.

Table 2. Models of multiple regression analysis before and after the physical exercise in overweight and obese children and adolescents.

Before the physical exercise			
Dependent variable	Independent variables considered	β ± SD	p
Insulin	Genotype	-0.13 ± 0.07	0.05
	Age	0.07 ± 0.07	0.25
	Sex	0.12 ± 0.07	0.07
HOMA-IR	Genotype	-0.25 ± 0.09	**0.009**
	Age	0.07 ± 0.09	0.46
	Sex	0.14 ± 0.09	0.14
QUICKI	Genotype	0.13 ± 0.10	0.19
	Age	0.15 ± 0.10	0.14
	Sex	-0.24 ± 0.10	**0.02**
After the physical exercise			
Dependent variable	Independent variables considered	β ± SD	p
Insulin	Genotype	-0.20 ± 0.10	**0.04**
	Age	0.09 ± 0.10	0.32
	Sex	0.06 ± 0.10	0.53
HOMA-IR	Genotype	-0.36 ± 0.13	**0.006**
	Age	0.17 ± 0.13	0.18
	Sex	0.04 ± 0.13	0.77
QUICKI	Genotype	0.27 ± 0.14	0.05
	Age	-0.19 ± 0.14	0.17
	Sex	-0.11 ± 0.14	0.41

HOMA-IR: Homeostatic model assessment for insulin resistance; QUICKI: Quantitative insulin sensitivity check index; β: Regression coefficient; SD: Standard deviation; Genotype: TT+AT and AA (recessive model).

Table 3. Comparisons of means variations (initial – final) of biochemical variables between overweight/obese children and adolescents stratified according to a recessive model.

Variables	N	TT+AT Mean ± SD	N	AA Mean ± SD	p
TC variation	119	5.37 ± 21.90	17	13.14 ± 26.34	0.12
HDL-C variation	118	1.23 ± 10.68	17	6.73 ± 11.73	0.05
LDL-C variation	119	3.72 ± 21.29	17	8.97 ± 20.69	0.18
TG variation	118	1.61 ± 45.32	17	-12.21 ± 62.68	0.70
GLU variation	117	1.67 ± 8.29	18	2.29 ± 9.15	0.79
INS variation	95	3.27 ± 8.82	15	2.41 ± 8.44	0.54
HOMA-IR variation	48	0.46 ± 1.03	8	0.33 ± 1.24	0.77
QUICKI variation	43	-0.02 ± 0.03	7	-0.007 ± 0.02	0.44

TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: triglycerides; HOMA-IR: Homeostatic model assessment for insulin resistance; QUICKI: Quantitative insulin sensitivity check index; SD: Standard deviation; p: comparison between individuals stratified according to a recessive model.

Discussion

The children and adolescents carriers of the AA genotype presented a less favorable metabolic profile regarding the parameters that predict glucose metabolism, demonstrating lower insulin sensitivity. There are other studies that also found an association between *FTO* rs9939609 SNP and parameters related to glucose metabolism, like higher levels of insulin and/or decreased insulin sensitivity (DE LUIS et al., 2016; KRING et al., 2008; PASCOE et al., 2007; TAN et al., 2010).

FTO is a demethylase (GERKEN et al., 2007) and can have several target genes, including some related to glucose metabolism like adiponectin and ghrelin genes (MERKESTEIN et al., 2014).

Merkestein and colleagues (2014) found that *FTO* overexpression - which appears to be a rs9939609 A-allele effect (BERULAVA; HORSTHEMKE, 2010) - promotes a decrease in adiponectin expression in male rats after 20 weeks, what agrees with the reduction in adiponectin levels found in obese humans (ARITA et al., 1999). Adiponectin is a hormone produced mainly in white adipose tissue and has a negative correlation with obesity. It is not known exactly what are the adiponectin functions, but it is probably involved in glucose, TG and fatty acids decrease, and low levels of this hormone increases the

susceptibility to insulin resistance and type 2 diabetes (SHEHZAD et al., 2012). Adiponectin is regulated by several genes and molecules, and *FTO* may have a contribution in its regulation. This could be an explanation for the altered values of insulin, HOMA-IR and QUICKI observed in AA genotype carriers of this study: rs9939609 A-allele would promote an increase in *FTO* expression, which could contribute to a decrease in adiponectin expression, leading to a decrease in insulin sensitivity, which was expressed through increased insulin, HOMA-IR and reduced QUICKI.

Another possible target of FTO is ghrelin gene. Karra and colleagues (2013) found that cells with *FTO* overexpression have increased expression of ghrelin mRNA (KARRA et al., 2013). Ghrelin is a hormone synthesized by the stomach that promotes increase of appetite and food intake and decrease in insulin sensitivity, among other functions (MIHALACHE et al., 2016). Therefore, the effects in glucose metabolism observed in this study can also be explained by the increased ghrelin expression. The increased food intake promoted by ghrelin also contributes to this hypothesis, since this is a characteristic observed in individuals with the rs9939609 risk allele.

It is not known exactly which are the *FTO* target genes, so there is a wide variety of genes related to metabolism that may have had their expression altered, resulting in differences in metabolic variables in function of *FTO* rs9939609 genotype.

Besides, rs9939609 SNP by itself promotes increased food intake (CECIL et al., 2008; SPEAKMAN; RANCE; JOHNSTONE, 2008; WARDLE et al., 2009). In this way, there may have been an increase in carbohydrate intake, which would increase glucose levels and, consequently, insulin levels. Glucose lower levels in AA carriers may have been observed because the high insulin was effective in the intracellular uptake of glucose, decreasing its plasma levels. As well, the faster metabolism of children and adolescents may have compensated the possible energy overload due to this increment in dietary intake, so this increase did not represent weight gain associated with the presence of the rs9939609 risk allele.

Tschritter and colleagues (2007) found an association between a variant in *FTO* gene and cerebrocortical insulin resistance in humans (TSCHRITTER et al., 2007). In normal conditions, insulin promotes a signal of adiposity and

satiety to the brain, but, in obese individuals, this hormone is not capable of increase the spontaneous cortical activity. The risk allele reduces the insulin effect in cortical activity, which decreases the cerebrocortical response to insulin (TSCHRITTER et al., 2007). Without the insulin effect in the brain, the body could try to increase insulin production, what would influence the values of insulin, HOMA-IR and QUICKI, as we observed in our study.

Grunnet and colleagues (2009) observed an association between rs9939609 and higher levels of glucose and insulin, hepatic insulin resistance and shorter recovery half-times of phosphocreatine and inorganic phosphate after exercise in a primarily type I muscle (GRUNNET et al., 2009). This last parameter means there might be an increased coupling of oxidative phosphorylation in the homozygous carriers of the rs9939609 risk allele. This characteristic could be related to an enhanced susceptibility to obesity and type 2 diabetes, because the decreased coupling of oxidation phosphorylation in animals reduces fat tissue accumulation, gluconeogenesis, glucose and insulin levels, and reverts peripheral insulin resistance (COSTFORD; GOWING; HARPER, 2007; GRUNNET et al., 2009; ISHIGAKI et al., 2005). Therefore, the increased coupling of oxidative phosphorylation could be another explanation for the influence of rs9939609 SNP in parameters of glucose metabolism observed in our study.

Regarding the physical exercise, the training was effective, promoting improvements in the levels of CT, LDL-C, glucose, insulin, HOMA-IR and QUICKI. However, we did not found an effect of rs9939609 A-allele on physical exercise response. The studies about *FTO* and physical exercise interaction have some controversial results, since some works found an association (MUC; PADEZ; MANCO, 2015; PETKEVICIENE et al., 2015) and some did not found (BERENTZEN et al., 2008; CECIL et al., 2008; JONSSON et al., 2009; LEOŃSKA-DUNIEC et al., 2016; LIEM et al., 2010; SPEAKMAN; RANCE; JOHNSTONE, 2008). Those discrepant results could be because of the different samples and different ways to analyze the physical activity. Regarding the samples, some studies analyzed children and adolescents (CECIL et al., 2008; LIEM et al., 2010) while others analyzed adults (BERENTZEN et al., 2008; JONSSON et al., 2009; MUC; PADEZ; MANCO, 2015; PETKEVICIENE et al., 2015). The study realized by Kilpeläinen and colleagues (2011) showed

that this difference in age is important, since they observed and interaction of rs9939609 SNP and physical activity in adults but not in children and adolescents (KILPELÄINEN et al., 2011). The variation in gender and ethnicity between the sample studies may also have contributed to the different outcomes. The method to measure physical activity also can differ from one work to another, since some authors used questionnaires (JONSSON et al., 2009; LIEM et al., 2010; PETKEVICIENE et al., 2015), that were different from each other, and other studies analyzed leisure time physical activity, maximal oxygen uptake (VO_2max), resting energy expenditure and basal metabolic rate (BERENTZEN et al., 2008; CECIL et al., 2008; SPEAKMAN; RANCE; JOHNSTONE, 2008). Our study is one of the few that evaluated rs9939609 SNP effect on variations of biochemical variables in response to a physical exercise program.

Our work has some limitations, as the sample size, which did not make possible to separate children and adolescents by gender or age groups.

In conclusion, we found that rs9939609 SNP A-allele influenced parameters related to glucose metabolism and did not interact with physical exercise. Studies that seek to identify SNPs effects in determined variables are important because can help in the prevention of obesity and other metabolic diseases, besides try to make the treatment of these diseases more individualized, according to the genetic background of each patient.

References

ARITA, Y. et al. Paradoxical decrease of an adipose-specific protein, Adiponectin, in obesity. **Biochemical and Biophysical Research Communications**, v. 257, p. 79–83, 1999.

BERENTZEN, T. et al. Lack of association of fatness-related FTO gene variants with energy expenditure or physical activity. **Journal of Clinical Endocrinology and Metabolism**, v. 93, n. 7, p. 2904–2908, 2008.

BERULAVA, T.; HORSTHEMKE, B. The obesity-associated SNPs in intron 1 of the FTO gene affect primary transcript levels. **European Journal of Human Genetics**, v. 18, n. 9, p. 1054–1056, 2010.

CECIL, J. E. et al. An Obesity-Associated FTO Gene Variant and Increased

Energy Intake in Children. **N Engl J Med**, v. 359, p. 2558–2566, 2008.
COSTFORD, S.; GOWING, A.; HARPER, M.-E. Mitochondrial uncoupling as a target for drug development for the treatment of obesity. **Current Opinion in Clinical Nutrition and Metabolic Care**, v. 10, p. 671–678, 2007.
DE LUIS, D. A. et al. Association of the rs9939609 gene variant in FTO with insulin resistance, cardiovascular risk factor and serum adipokine levels in obese patients. **Nutrición Hospitalaria**, v. 33, n. 5, p. 1108–1115, 2016.
FRAYLING, T. M. et al. A Common Variant in the FTO Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. **Science**, v. 316, n. May, p. 889–893, 2007.
FREATHY, R. M. et al. Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on bmi. **Diabetes**, v. 57, n. 5, p. 1419–1426, 2008.
FRIEDEWALD, W. T.; LEVY, R. I.; FREDRICKSON, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. **Clinical Chemistry**, v. 18, n. 6, p. 499–502, 1972.
GERKEN, T. et al. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. **Science (New York, N.Y.)**, v. 318, n. 5855, p. 1469–72, 2007.
GRUNNET, L. G. et al. Increased recovery rates of phosphocreatine and inorganic phosphate after isometric contraction in oxidative muscle fibers and elevated hepatic insulin resistance in homozygous carriers of the A-allele of FTO rs9939609. **Journal of Clinical Endocrinology and Metabolism**, v. 94, n. 2, p. 596–602, 2009.
HERTEL, J. K. et al. FTO, type 2 diabetes, and weight gain throughout adult life: A meta-analysis of 41,504 subjects from the scandinavian HUNT, MDC, and MPP studies. **Diabetes**, v. 60, n. 5, p. 1637–1644, 2011.
ISHIGAKI, Y. et al. Dissipating excess energy stored in the liver is a potential treatment strategy for diabetes associated with obesity. **Diabetes**, v. 54, n. 2, p. 322–332, 2005.
JONSSON, A. et al. Assessing the effect of interaction between an FTO variant (rs9939609) and physical activity on obesity in 15,925 Swedish and 2,511 Finnish adults. **Diabetologia**, v. 52, n. 7, p. 1334–1338, 2009.
KARRA, E. et al. A link between FTO, ghrelin, and impaired brain food-cue

responsivity Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases. **The Journal of Clinical Investigation**, v. 123, n. 8, p. 3539–3551, 2013.

KATZ, A. et al. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. **Journal of Clinical Endocrinology and Metabolism**, v. 85, n. 7, p. 2402–2410, 2000.

KILPELÄINEN, T. O. et al. Physical activity attenuates the influence of FTO variants on obesity risk: A meta-analysis of 218,166 adults and 19,268 children. **PLoS Medicine**, v. 8, n. 11, 2011.

KRING, S. I. I. et al. FTO gene associated fatness in relation to body fat distribution and metabolic traits throughout a broad range of fatness. **PLoS ONE**, v. 3, n. 8, p. 1–7, 2008.

LAHIRI, D. K.; NUMBERGER, J. I. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. **Nucleic Acids Research**, v. 19, n. 19, p. 5444, 1991.

LEITE, N. et al. Efeitos de exercícios aquáticos e orientação nutricional na composição corporal de crianças e adolescentes obesos. **Revista Brasileira de Cineantropometria e Desempenho Humano**, v. 12, n. 4, p. 232–238, 2010.

LEOŃSKA-DUNIEC, A. et al. Assessing effect of interaction between the FTO A/T polymorphism (rs9939609) and Physical Activity on Obesity-related traits. **Journal of Sport and Health Science**, v. 352, 2016.

LIEM, E. T. et al. Influence of common variants near INSIG2, in FTO, and near MC4R genes on overweight and the metabolic profile in adolescence: The TRAILS (TRacking Adolescents' Individual Lives Survey) Study. **American Journal of Clinical Nutrition**, v. 91, n. 2, p. 321–328, 2010.

LIGUORI, R. et al. The FTO gene polymorphism (rs9939609) is associated with metabolic syndrome in morbidly obese subjects from southern Italy. **Molecular and Cellular Probes**, v. 28, n. 4, p. 195–199, 2014.

LOPES, W. A. et al. Effects of 12 weeks of combined training without caloric restriction on inflammatory markers in overweight girls. **Journal of Sports Sciences**, v. 34, n. 0, p. 1902–12, 2016.

MATTHEWS, D. R. et al. Homeostasis model assessment: IR and beta-cell function from fasting plasma glucose and insulin concentration in man.

Diabetologia, v. 28, p. 412–9, 1985.
MERKESTEIN, M. et al. Changes in gene expression associated with FTO overexpression in mice. **PLoS ONE**, v. 9, n. 5, p. 1–11, 2014.
MIHALACHE, L. et al. Effects of ghrelin in energy balance and body weight homeostasis. **Hormones**, v. 15, n. 2, p. 186–196, 2016.
MILANO, G. E. et al. Atividade da butirilcolinesterase e fatores de risco cardiovascular em adolescentes obesos submetidos a um programa de exercícios físicos. **Arq Bras Endocrinol Metab**, v. 57, p. 533–537, 2013.
MUC, M.; PADEZ, C.; MANCO, L. Influence of physical activity on the association between the FTO variant rs9939609 and adiposity in young adults. **American journal of human biology : the official journal of the Human Biology Council**, v. 27, n. 5, p. 734–738, 2015.
MUÑOZ-YÁÑEZ, C. et al. Polymorphisms FTO rs9939609, PPARG rs1801282 and ADIPOQ rs4632532 and rs182052 but not lifestyle are associated with obesity related-traits in Mexican children. **Genetics and Molecular Biology**, v. 553, p. 547–553, 2016.
NASCIMENTO, G. A. et al., 2017. ***FTO* SNP influences the response to dietary intervention but not to physical exercise program**. Curitiba, 2017. Submetido a publicação.
PASCOE, L. et al. Common variants of the novel type 2 diabetes genes. **Diabetes**, v. 56, n. 12, p. 3101–3104, 2007.
PETKEVICIENE, J. et al. Physical activity, but not dietary intake, attenuates the effect of the FTO rs9939609 polymorphism on obesity and metabolic syndrome in Lithuanian adult population. **Public Health**, v. 135, p. 23–29, 2015.
PRAKASH, J. et al. Association of FTO rs9939609 SNP with Obesity and Obesity- Associated Phenotypes in a North Indian Population. **Oman medical journal**, v. 31, n. 2, p. 99–106, 2016.
SHEHZAD, A. et al. Adiponectin: Regulation of its production and its role in human diseases. **Hormones-International Journal of Endocrinology and Metabolism**, v. 11, n. 1, p. 8–20, 2012.
SPEAKMAN, J. R.; RANCE, K. A.; JOHNSTONE, A. M. Polymorphisms of the FTO gene are associated with variation in energy intake, but not energy expenditure. **Obesity (Silver Spring, Md.)**, v. 16, n. 8, p. 1961–5, 2008.
TAN, S. et al. Large effects on body mass index and insulin resistance of fat

mass and obesity associated gene (FTO) variants in patients with polycystic ovary syndrome (PCOS). **BMC medical genetics**, v. 11, p. 12, 2010.
TSCHRITTER, O. et al. Variation in the FTO gene locus is associated with cerebrocortical insulin resistance in humans [2]. **Diabetologia**, v. 50, n. 12, p. 2602–2603, 2007.
WARDLE, J. et al. The FTO gene and measured food intake in children. **International journal of obesity (2005)**, v. 33, n. 1, p. 42–45, 2009.
World Health Organization (WHO). Obesity and overweight. World Health Organization, 2016.http://www.who.int/topics/obesity/en/. 23/09/2017.

Supplementary material

Details of the applied exercises

Land-based aerobic exercise: 45 minutes of walking, 45 minutes of indoor cycling and 20 minutes of stretching was performed (MILANO et al., 2013).

HIIT: Consisted of running periods at high intensity, in which the individual should run at maximal speed for 30 seconds, followed by low intensity recovery interval, which was walking in moderate/fast speed. The training intensity increased as the weeks pass.

Combined training: It was composed of resistance and aerobic training performed in a 60 minutes session. Resistance training was composed by six exercises (leg press, leg extension, leg curl, bench press, lateral pulldown and arm curl) and aerobic consisted of walking/running in an athletic track (LOPES et al., 2016).

Aquatic exercise: Each session consisted of five minutes of warming-up, 45 minutes of technique (swimming techniques learning exercises or deep water running) and 10 minutes of stretching and recreation. The deep water running consisted of the individual remains in a vertical position and his body is submerged to shoulder height with the support of a float vest attached to the waist. There is no contact of the feet with the bottom of the pool, and similar movements to walk on land are made (LEITE et al., 2010).

Table 1. Comparison of initial and final means of biochemical variables (before and after physical exercise) in overweight and obese children and adolescents.

	Obese and overweight			
Variables	N	Initial mean ± SD	Mean after 3 months ± SD	p
TC (mg/dl)	136	161.49 ± 37.07	155.15 ± 33.80	**0.002**
HDL-C (mg/dl)	135	48.79 ± 13.35	46.87 ± 13.80	**0.03**
LDL-C (mg/dl)	136	93.54 ± 28.97	89.16 ± 28.06	**0.02**
TG (mg/dl)	135	101.82 ± 51.77	101.95 ± 52.68	0.46
Glucose (mg/dl)	135	86.69 ± 9.57	84.93 ± 8.05	**0.02**
Insulin (uUI/ml)	110	16.69 ± 12.69	13.53 ± 8.41	$\mathbf{10^{-4}}$
HOMA-IR	56	2.01 ± 1.30	1.56 ± 1.04	**0.0004**
QUICKI	50	0.34 ± 0.04	0.35 ± 0.04	$\mathbf{10^{-4}}$

TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: triglycerides; HOMA-IR: Homeostatic model assessment for insulin resistance; QUICKI: Quantitative insulin sensitivity check index; SD: Standard deviation; p: comparison between the initial and after 12 weeks means of physical exercise in overweight and obese children and adolescents.

Note: It was not possible to obtain HDL-C, TG, glucose, insulin, HOMA-IR and QUICKI data from all individuals who completed the program (n = 136), so, the analyzes of these variables count with a smaller number of individuals (n = 135 for HDL-C, TG and glucose, n = 110 for insulin, n = 56 for HOMA-IR and n = 50 for QUICKI).

Table 2. Models of multiple regression analysis before and after the physical exercise in overweight and obese children and adolescents.

Before the physical exercise			
Dependent variable	Independent variables considered	β ± SD	p
Insulin	Genotype	-0.13 ± 0.07	0.05
	Age	0.07 ± 0.07	0.25
	Sex	0.12 ± 0.07	0.07
HOMA-IR	Genotype	-0.25 ± 0.09	**0.009**
	Age	0.07 ± 0.09	0.46
	Sex	0.14 ± 0.09	0.14
QUICKI	Genotype	0.13 ± 0.10	0.19
	Age	0.15 ± 0.10	0.14
	Sex	-0.24 ± 0.10	**0.02**
After the physical exercise			
Dependent variable	Independent variables considered	β ± SD	p
Insulin	Genotype	-0.20 ± 0.10	**0.04**
	Age	0.09 ± 0.10	0.32
	Sex	0.06 ± 0.10	0.53
HOMA-IR	Genotype	-0.36 ± 0.13	**0.006**
	Age	0.17 ± 0.13	0.18
	Sex	0.04 ± 0.13	0.77
QUICKI	Genotype	0.27 ± 0.14	0.05
	Age	-0.19 ± 0.14	0.17
	Sex	-0.11 ± 0.14	0.41

HOMA-IR: Homeostatic model assessment for insulin resistance; QUICKI: Quantitative insulin sensitivity check index; β: Regression coefficient; SD: Standard deviation; Genotype: TT+AT and AA (recessive model).

CAPÍTULO III

Effect of *ABC* transporters genes polymorphisms on adiposity, glucose and lipid metabolism and interaction with physical exercise

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Abstract

Introduction and Aims

ATP Binding-Cassette (ABC) transporters mediate lipid efflux and single nucleotide polymorphisms (SNPs) in these genes could affect metabolism. The objective of this study is to analyze the rs1800977 (*ABCA1*), rs2230806

(*ABCA1*), rs2279796 (*ABCA7*), rs692382 (*ABCG1*) and rs3827225 (*ABCG1*) SNPs effects on anthropometric and biochemical variables in 451 children and adolescents (obese, overweight and normal weight), and their effect on anthropometric and biochemical variables in 184 overweight/obese children and adolescents in response to a physical exercise program.

Methods and Results

451 children and adolescents were genotyped. The rs1800977 SNP (*ABCA1*) C-allele was associated to higher BMI Z-score, AC, FM and insulin 120 and lower QUICKI. The rs2230806 SNP (*ABCA1*) A-allele was associated to higher BMI Z-score and AC and reduced %LBM. The rs2279796 SNP (*ABCA7*) C-allele was associated to higher BMI Z-score. The rs692383 SNP (*ABCG1)* was associated to higher BMI Z-score, AC, HDL-C, glucose, insulin and HOMA-IR. The rs3827225 SNP (*ABCG1)* G-allele was associated to higher VLDL-C and glucose. The response to physical exercise was affected by rs1800977 SNP (higher BMI Z-score reduction and better response to QUICKI) and rs2230806 SNP (higher LBM gain).

Conclusion

SNPs in ABC transporters genes could influence adiposity, glucose and lipid metabolism, besides interaction with physical exercise.

Keywords
ABC, *ABCA1, ABCA7, ABCG1*, lipid, glucose, adiposity.

Introduction

ATP-Binding Cassette (ABC) transporters are a family of transmembrane proteins that use energy from ATP hydrolysis to transport substances through cell membranes (TARLING; DE AGUIAR VALLIM; EDWARDS, 2013). The ABC genes are classified into subfamilies based on similarity in gene structure, order of the domains (nucleotide binding folds – NBFs – and transmembrane domains

– TMDs) and sequence homology in NBF and TMD (SINGARAJA et al., 2003). In this study, we will focus on ABCA1, ABCA7 and ABCG1, which are all involved in lipid transport.

ABCA1 is expressed in the whole body, and participates of high-density lipoprotein (HDL) formation by the transference of cholesterol to apoA1 (the principal apolipoprotein of HDL) (QUAZI; MOLDAY, 2011). The *ABCA1* gene is located at 9q31.1 (KAMINSKI; PIEHLER; WENZEL, 2006) and several single nucleotide polymorphisms (SNPs) have already been described in this gene. In this study, we analyzed the rs1800977 SNP (T>C) and rs2230806 SNP (G>A).

ABCA7 is high homology to ABCA1 and also participates of HDL formation. However, unlike ABCA1, it generates small and cholesterol-poor HDL particles (KAMINSKI et al., 2000; QUAZI; MOLDAY, 2011). *ABCA7* gene is located at 19p13.3 (KAMINSKI; PIEHLER; WENZEL, 2006) and the rs2279796 SNP (C>T) was analyzed in this study.

ABCG1 mediates lipid efflux to HDL and low-density lipoprotein (LDL) (WANG et al., 2004; CAVELIER et al., 2006; KOBAYASHI et al., 2006) and it is expressed in the whole body, with higher levels in macrophages (CAVELIER et al., 2006; QUAZI; MOLDAY, 2011). The *ABCG1* gene is located at 21q22.3 (CAVELIER et al., 2006), and rs692383 SNP (G>A) and rs3827225 SNP (G>A) were analyzed in this study.

Besides the involvement with lipid metabolism, it was proposed that polymorphisms in ABC transporters genes could also influence the adiposity and glucose metabolism (DE HAAN et al., 2014; FRISDAL; GOFF, 2015). Therefore, the aim of this study is to verify the *ABCA1*, *ABCA7* and *ABCG1* above mentioned SNPs effects on anthropometric and biochemical variables in 451 children and adolescents (divided into an overweight/obese group and a normal weight group) of Brazil, and their possible interaction with metabolic changes induced by physical exercise.

Methods

Subjects

The study was composed by 451 children and adolescents of both sexes (297 boys and 154 girls), 172 of which were within the normal weight range and 279 were overweight or obese (80 overweight and 199 obese) (according to parameters defined by WHO). The children and adolescents with normal weight were included in some analyzes as a reference group. The mean overall age was 13.47 ± 1.90 years old (aged 8-17 y).

They were recruited in public schools of the state of Paraná, Brazil. The inclusion criteria were: medical liberation for physical exercise and do not use drugs that could interfere on weight control and/or lipid levels. Those who were in agreement with the established criteria were invited to participate in this research, and those who accepted, with the legal responsible consent, had the free and informed consent term signed by them. The study was approved by the ethics committee of the Federal University of Paraná (UFPR) (Protocol number 2460.067/2011) (NASCIMENTO *et al*., 2017).

Weight and height were measured with an accuracy of 0.1 kg and 0.1 cm, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters, and then converted into BMI Z-score according to WHO (2016). The children and adolescents were considered overweight when their BMI Z-score was between +1 and +2, and obese when their BMI Z-score was more than +3 (WHO, 2016). Body composition assessment was performed by dual X-ray absorptiometry Lunar Prodigy Primo (General Electric Healthcare; Madison, WI).

Biochemical variables

Blood samples were collected and total lipids (TL), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) were measured by standard procedures in private partner laboratories and in the clinical analyzes laboratory of UFPR. Blood glucose levels were determined by the enzymatic method and

insulin was measured by the chemiluminescence immunoassay technique, by automated equipment. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald equation (FRIEDEWALD; LEVY; FREDRICKSON, 1972), homeostatic model assessment for insulin resistance (HOMA-IR) was calculated as (fasting blood glucose [µU/ml] x insulin [mMol/l]/22.5) (MATTHEWS et al., 1985) and the quantitative insulin sensitivity check index (QUICKI) was calculated as 1/[log (fastinginsulin)(mU/ml) x log (fasting blood glucose) (mMol/l)] (KATZ et al., 2000).

Physical exercise

Of the 279 overweight or obese children and adolescents that participate in the study, 184 were submitted to a physical exercise program.

The physical exercises were composed of four different types of training. The physical exercises were conducted by Physical Education professionals, and applied three times a week during 12 weeks on students in their home schools (NASCIMENTO *et al*., 2017).

Four types of physical exercise were performed: land-based aerobic exercise, high intensity interval training (HIIT), combined training and aquatic exercise. However, for the statistical analyzes the physical exercise groups were analyzed together, since there was no significant impact of the different trainings in the analyzed variables. Details of the applied exercises are in the supplemental material (NASCIMENTO *et al*., 2017).

After the conclusion of the exercise program, the anthropometric and biochemical data were collected again. It was not possible to obtain data on all variables from all individuals who completed the program (n= 184), therefore the analyzes of some variables count with a smaller number of individuals.

The experimental procedure applied is demonstrated in Figure 1.

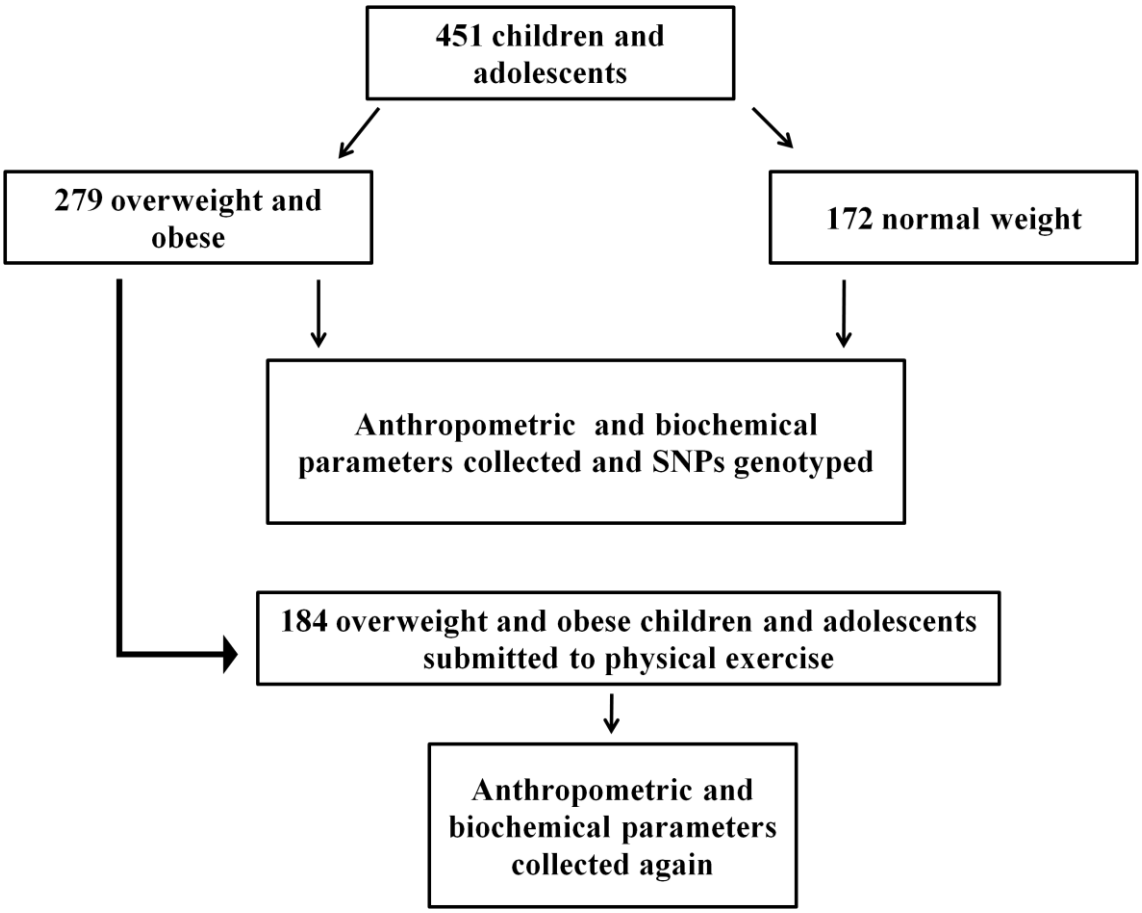

Figure 1. Study design.

Choice of SNPs

These SNPs were chosen based on interesting results found in literature (WANG et al., 2004; VASQUEZ; FARDO; ESTUS, 2013; HAGHVIRDIZADEH et al., 2015; CYRUS et al., 2016), which allowed us to think about some questions for our sample. As *ABCA1, ABCA7* and *ABCG1* act on cholesterol metabolism and are present in several tissues (TARLING; DE AGUIAR VALLIM; EDWARDS, 2013), it would be interesting to verify the effect of SNPs in these genes on anthropometric and biochemical variables of obese, overweight or normal weight children and adolescents.

These SNPs are not in linkage disequilibrium and are representative of linkage disequilibrium blocks (tag SNPs; D' between rs1800977 and rs2230806 (*ABCA1*) = 0.1525; D' between rs692383 and rs3827225 (*ABCG1*) = 0.067).

The SNPs frequencies were also considered: rs1800977 minor allele frequency (MAF) is 0.36, rs2230806 MAF is 0.33, rs2279796 MAF is 0.43, rs692383 MAF is 0,5 and rs3827225 MAF is 0.17 (NCBI, 2017).

DNA extraction and genotyping

DNA was extracted from peripheral blood according to the salting-out technique Lahiri and Nurnberger (LAHIRI; NUMBERGER, 1991), and then diluted to 20ng/µl. *ABCA1* rs1800977 SNP and rs2230806 SNP, *ABCA7* rs2279796 SNP and *ABCG1* rs692383 SNP and rs3827225 SNP were genotyped with a TaqMan SNP genotyping assay (Applied Biosystems). The reactions were done using the following conditions: 60°C for 30s, 95°C for 10min, 50 cycles of 95°C for 15s and 60°C for 1 min, and 60ºC for 30s. Three previously sequenced control samples, representative of each of the possible genotypes, were included in each reaction. Samples that failed the reaction, or genotypes identified doubtfully were excluded from statistical analyzes (NASCIMENTO *et al*., 2017).

Statistical analysis

The frequencies of genotypes and alleles were obtained by direct counting and compared between the group of overweight/obese and normal weight by chi-square tests, which were also used to check the Hardy-Weinberg equilibrium.

The continuous variables were tested for normality using the Kolmogorov-Smirnov test with Lilliefors correction. The initial and final means of the variables (before and after the interventions) were compared by paired parametric or no parametric tests (t test paired or Wilcoxon test, respectively).

The recessive, dominant and co-dominance models of allelic interaction were tested. The co-dominance model was more adequate to our results, being therefore adopted for analyzes that involved the sample stratification by

genotype. The variables means were compared between genotypes by parametric or no parametric independent tests (t test or Mann Whitney, respectively). Independent comparison tests of means were used to evaluate the means differences (initial – final) in the anthropometric and biochemical parameters between genotypes (Parametric – t test or nonparametric – Mann Whitney). Multiple regression analyzes were applied. A risk prediction analysis was also realized (using the R software, package PredictABEL), and for this analysis the quantitative variables had their values transformed in classificatory binary code from the median values, with each observation classified as below or above the median. Statistical significance adopted for the tests was 0.05 (5%).

Results

The allele and genotype frequencies of the overweight/obese and normal weight groups for the five SNPs are shown in table 1. The genotype distributions for all SNPs were in Hardy-Weinberg equilibrium in both sample groups ($p>0.05$).

The allele frequencies for each SNP were compared between the overweight/obese and normal weight group, but no association with obesity was observed ($p > 0.05$).

Table 1.Genotype and allele frequencies of rs1800977 (*ABCA1*), rs2230806 (*ABCA1*), rs2279796 (*ABCA7*), rs692383 (*ABCG1*) and rs3827225 (*ABCG1*) in overweight and obese children and adolescents and in normal weight children and adolescents.

	Overweight and obese					Normal weight				
SNP	Genotype	N	%	Allele	% ± SE	Genotype	N	%	Allele	% ± SE
rs1800977	TT	36	14.12	T	35.10 ± 0.01	TT	26	14.94	T	35.63 ± 0.01
	CT	107	41.96			CT	72	41.38		
	CC	112	43.92	C	64.9 ± 0.01	CC	76	43.68	C	64.37 ± 0.01
	Total	255	100			Total	174	100		
rs2230806	GG	117	41.94	G	63.44 ± 0.01	GG	71	41.28	G	64.54 ± 0.01
	AG	120	43.01			AG	80	46.51		
	AA	42	15.05	A	36.56 ± 0.01	AA	21	12.21	A	35.47 ± 0.01
	Total	279	100			Total	172	100		
rs2279796	CC	72	29.63	C	52.88 ± 0.01	CC	42	25.3	C	52.41 ± 0.01
	TC	113	46.5			TC	90	54.22		
	TT	58	23.87	T	47.12 ± 0.01	TT	34	20.48	T	47.59 ± 0.01
	Total	243	100			Total	166	100		
rs692383	GG	26	12.27	G	38.92 ± 0.01	GG	23	14.37	G	37.5 ± 0.01
	AG	113	53.3			AG	74	46.25		
	AA	73	34.43	A	61.09 ± 0.01	AA	63	39.38	A	62.5 ± 0.01
	Total	212	100			Total	160	100		
rs3827225	GG	127	58.25	G	76.61 ± 0.009	GG	87	56.13	G	76.45 ± 0.01
	AG	80	36.7			AG	63	40.64		
	AA	11	5.05	A	23.39 ± 0.009	AA	5	3.23	A	23.55 ± 0.01
	Total	218	100			Total	155	100		

SE: Standard error.

We analyzed the effects of the five SNPs on anthropometric and biochemical variables means in the overweight/obese group (before and after the physical exercise) and in the normal weight group. The results are shown in supplementary material.

Multiple regression analyzes corrected for age, gender, BMI Z-score and type of training were also perform to confirm the SNPs effects. Some of these variables also presented different means depending of specific genotypes, in others the genotype effect appeared only in the multiple regression analysis. Only the variables with significant results in multiple regression analysis ($p < 0.05$) are shown in table 2.

The rs1800977 SNP (*ABCA1*) influenced BMI Z-score before and after the exercise, respectively, ($p = 0.002$; $p = 0.001$), just like AC ($p = 0.005$; $p = 0.03$), insulin 120 ($p = 0.03$; $p = 0.03$) and QUICKI ($p = 0.02$; $p = 0.002$) in overweight/obese children and adolescents; and influenced FM levels ($p = 0.003$) in these group only before the exercise. The SNP effect on QUICKI levels was also seen in the means comparison test and individuals with CC genotype had lower levels of QUICKI ($p = 0.03$ for TT vs CC).

The rs2230806 SNP (*ABCA1*) influenced the levels of BMI Z-score ($p = 0.007$), AC ($p = 0.02$) and %LBM ($p = 0.008$) in the overweight/obese children and adolescents before the exercise. The SNP effect on %LBM was also seen in the means comparison test and individuals with the AG genotype had higher %LBM ($p = 0.04$ for AG vs AA).

The rs2279796 SNP (*ABCA7*) influenced BMI Z-score levels in overweight/obese children and adolescents after the exercise ($p = 0.007$). This SNP effect what was also seen in the means comparison test, but in the normal weight individuals carriers of CT genotype that had higher BMI Z-score compared with CC carriers ($p = 0.008$ for CC vs CT).

The rs692383 SNP (*ABCG1)* influenced BMI Z-score in overweight/obese individuals before ($p = 10^{-4}$) and after the exercise ($p = 0.02$), influenced AC ($p = 0.04$) and glucose ($p = 0.02$) in overweight/obese individuals before the exercise, and influenced HDL-C ($p = 0.03$), insulin ($p = 0.02$) and HOMA-IR ($p = 0.009$) in normal weight individuals. The SNP effect on BMI Z-score was also seen in the means comparison test, but only after the exercise.

Overweight/obese individuals with the AG genotype had higher BMI Z-score compared with AA ($p = 0.04$ for AG vs AA).

The rs3827225 SNP (*ABCG1)* influenced levels of VLDL-C in normal weight children and adolescents ($p = 0.02$) and glucose levels in overweight/obese individuals after the exercise ($p = 0.02$). The SNP effect on VLDL-C was also seen in the means comparison test, and individuals with the GG genotype had higher levels of VLDL-C ($p = 0.04$ for GG vs AG).

Table 2. Models of multiple regression analysis in overweight and obese children (before and after the exercise program) and in normal weight children and adolescents.

Independent	BMI Z-score						AC					
	Overweight and obese				Normal weight		Overweight and obese				Normal weight	
	Before		After				Before		After			
variables	β ± SE	p	β ± SE	p	β ± SE	P	β ± SE	P	β ± SE	p	β ± SE	p
rs1800977	0.19 ± 0.06	**0.002**	0.28 ± 0.08	**0.001**	-0.06 ± 0.08	0.45	0.22 ± 0.08	**0.005**	0.25 ± 0.11	**0.03**	0.06 ± 0.08	0.49
rs2230806	0.15 ± 0.05	**0.007**	0.09 ± 0.07	0.2	-0.11 ± 0.07	0.14	0.16 ± 0.07	**0.02**	0.13 ± 0.1	0.19	-0.07 ± 0.07	0.36
rs2279796	-0.02 ± 0.06	0.8	-0.23 ± 0.08	**0.007**	0.09 ± 0.08	0.3	-0.006 ± 0.08	0.93	-0.13 ± 0.11	0.23	0.03 ± 0.08	0.68
rs692383	-0.35 ± 0.08	**10^{-4}**	-0.24 ± 0.1	**0.02**	-0.0009 ± 0.1	0.99	-0.19 ± 0.09	**0.04**	-0.07 ± 0.13	0.58	-0.07 ± 0.1	0.51
rs3827225	-0.12 ± 0.07	0.09	-0.12 ± 0.09	0.19	-0.03 ± 0.09	0.7	-0.15 ± 0.09	0.1	-0.11 ± 0.13	0.41	-0.08 ± 0.09	0.41

Independent	FM						%LBM					
	Overweight and obese				Normal weight		Overweight and obese				Normal weight	
	Before		After				Before		After			
variables	β ± SE	p	β ± SE	p	β ± SE	p	β ± SE	P	β ± SE	p	β ± SE	p
rs1800977	0.24 ± 0.08	**0.003**	0.2 ± 0.11	0.09	0.04 ± 0.17	0.8	-0.11 ± 0.1	0.26	0.22 ± 0.49	0.67	-0.06 ± 0.17	0.73
rs2230806	0.14 ± 0.07	0.06	0.12 ± 0.1	0.23	-0.14 ± 0.17	0.4	-0.26 ± 0.1	**0.008**	-0.39 ± 0.55	0.51	0.15 ± 0.16	0.37
rs2279796	-0.11 ± 0.09	0.2	-0.13 ± 0.1	0.23	-0.01 ± 0.15	0.94	0.04 ± 0.11	0.7	0.07 ± 0.48	0.89	0.04 ± 0.15	0.78
rs692383	-0.2 ± 0.1	0.05	-0.07 ± 0.13	0.6	0.15 ± 0.27	0.58	0.21 ± 0.13	0.1	-0.03 ± 0.54	0.95	-0.14 ± 0.26	0.6
rs3827225	0.01 ± 0.1	0.9	-0.05 ± 0.12	0.71	0.03 ± 0.23	0.9	-0.04 ± 0.12	0.77	0.09 ± 0.51	0.87	-0.03 ± 0.22	0.89

Independent variables	VLDL-C						HDL-C					
	Overweight and obese				Normal weight		Overweight and obese				Normal weight	
	Before		After				Before		After			
	β ± SE	p	β ± SE	p	β ± SE	P	β ± SE	P	β ± SE	p	β ± SE	p
rs1800977	-0.17 ± 0.12	0.15	0.03 ± 0.22	0.89	-0.13 ± 0.13	0.32	0.07 ± 0.07	0.33	0.14 ± 0.1	0.14	0.02 ± 0.08	0.76
rs2230806	0.01 ± 0.12	0.92	-0.27 ± 0.22	0.22	-0.17 ± 0.12	0.14	-0.01 ± 0.06	0.82	0.05 ± 0.08	0.53	0.11 ± 0.07	0.12
rs2279796	-0.2 ± 0.13	0.15	-0.1 ± 0.24	0.68	0.18 ± 0.13	0.19	0.04 ± 0.07	0.56	-0.03 ± 0.09	0.75	-0.05 ± 0.08	0.54
rs692383	-0.2 ± 0.15	0.17	0.07 ± 0.23	0.77	0.3 ± 0.16	0.06	0.09 ± 0.08	0.28	0.14 ± 0.11	0.2	-0.19 ± 0.09	**0.03**
rs3827225	-0.04 ± 0.12	0.71	0.1 ± 0.23	0.68	-0.35 ± 0.14	**0.02**	0.04 ± 0.07	0.62	-0.14 ± 0.11	0.19	-0.13 ± 0.08	0.14

Independent variables	Glucose						Insulin					
	Overweight and obese				Normal weight		Overweight and obese				Normal weight	
	Before		After				Before		After			
	β ± SE	p	β ± SE	p	β ± SE	P	β ± SE	p	β ± SE	p	β ± SE	p
rs1800977	-0.003 ± 0.07	0.96	-0.18 ± 0.09	0.06	-0.02 ± 0.08	0.79	-0.05 ± 0.08	0.55	-0.07 ± 0.12	0.56	-0.11 ± 0.08	0.15
rs2230806	-0.04 ± 0.06	0.49	-0.03 ± 0.08	0.71	0.09 ± 0.07	0.25	0.008 ± 0.06	0.9	-0.07 ± 0.09	0.45	-0.12 ± 0.07	0.09
rs2279796	0.006 ± 0.07	0.93	0.08 ± 0.09	0.35	0.04 ± 0.08	0.63	-0.03 ± 0.07	0.64	0.01 ± 0.1	0.91	-0.02 ± 0.08	0.78
rs692383	-0.2 ± 0.08	**0.02**	-0.11 ± 0.11	0.34	0.04 ± 0.1	0.65	0.09 ± 0.09	0.31	0.07 ± 0.13	0.57	0.22 ± 0.09	**0.02**
rs3827225	-0.02 ± 0.07	0.76	-0.25 ± 0.1	**0.02**	0.009 ± 0.009	0.92	0.05 ± 0.08	0.47	0.13 ± 0.12	0.25	-0.01 ± 0.09	0.88

Independent variables	Insulin 120						HOMA-IR					
	Overweight and obese				Normal weight		Overweight and obese				Normal weight	
	Before		After				Before		After			
	β ± SE	p	β ± SE	p	β ± SE	P	β ± SE	p	β ± SE	p	β ± SE	p
rs1800977	-0.42 ± 0.19	**0.03**	-0.49 ± 0.22	**0.03**	-0.01 ± 021	0.95	-0.12 ± 0.12	0.34	-0.22 ± 0.19	0.24	-0.06 ± 0.09	0.51
rs2230806	0.15 ± 0.11	0.2	0.11 ± 0.16	0.5	-0.18 ± 0.21	0.39	0.14 ± 0.09	0.15	0.006 ± 0.14	0.96	-0.15 ± 0.08	0.06
rs2279796	-0.09 ± 0.12	0.45	0.08 ± 0.15	0.61	0.22 ± 0.19	0.28	0.08 ± 0.1	0.45	0.16 ± 0.14	0.25	0.009 ± 0.09	0.93
rs692383	-0.04 ± 0.19	0.84	-0.22 ± 0.22	0.33	0.29 ± 0.32	0.38	0.05 ± 0.13	0.69	0.2 ± 0.18	0.28	0.28 ± 0.11	**0.009**
rs3827225	0.19 ± 0.18	0.29	0.26 ± 0.21	0.23	-0.27 ± 0.29	0.35	0.07 ± 0.12	0.59	0.11 ± 0.18	0.56	0.05 ± 0.1	0.65

Independent variables	QUICKI					
	Overweight and obese				Normal weight	
	Before		After			
	β ± SE	p	β ± SE	P	β ± SE	P
rs1800977	0.27 ± 0.12	**0.02**	0.63 ± 0.19	**0.002**	0.13 ± 0.11	0.24
rs2230806	-0.08 ± 0.09	0.4	-0.15 ± 0.15	0.31	0.17 ± 0.1	0.09
rs2279796	-0.03 ± 0.1	0.73	-0.15 ± 0.14	0.3	0.003 ± 0.11	0.98
rs692383	-0.003 ± 0.13	0.98	-0.12 ± 0.18	0.5	-0.23 ± 0.14	0.1
rs3827225	-0.04 ± 0.12	0.72	-0.15 ± 0.19	0.43	0.14 ± 0.13	0.3

BMI: Body Mass Index; AC: Abdominal Circumference; FM: Fat Mass; % LBM: Lean Body Mass Percentage; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; β: Regression coefficient; SD: Standard deviation. *ABCA1*: rs1800977 and rs2230806; *ABCA7*: rs2279796; *ABCG1*: rs692383 and rs3827225.

Regardless the genotype, physical exercise promoted positive changes in values of BMI Z-score ($p = 10^{-4}$), AC ($p = 0.003$), %BF ($p = 10^{-4}$), FM ($p = 10^{-4}$), LBM ($p = 10^{-4}$), TC (10^{-4}), LDL-C ($p = 0.0007$), glucose ($p = 0.03$), glucose 120 ($p = 0.004$), insulin ($p = 10^{-4}$), insulin 120 ($p = 10^{-4}$), HOMA-IR ($p = 0.0004$) and QUICKI ($p = 10^{-4}$).

In order to check the genotype interaction with physical exercise, the variables mean differences resulting from exercise (initial – final) were compared between the genotypes for each SNP and the results are shown in supplementary material. Multiple regression analyzes corrected for age, gender, BMI Z-score and type of training were applied considering the variables that had significant results in mean differences comparison test as dependent variables, as shown in table 3. We found two SNPs that influenced the response to physical exercise: rs1800977 and rs2230806 SNPs.

The rs1800977 SNP (*ABCA1*) influenced BMI Z-score ($p = 0.04$) and QUICKI ($p = 0.02$) response to exercise. For BMI Z-score variation, individuals with CT genotype had higher BMI Z-score reduction than TT individuals ($p = 0.04$), and individuals with CC genotype had higher BMI Z-score reduction than TT individuals ($p = 0.04$). For QUICKI variation, individuals with CT genotype had a better response to exercise than TT individuals ($p = 0.02$), and individuals with CC genotype had a better response to exercise than TT individuals ($p = 0.02$). The rs2230806 SNP (*ABCA1*) influenced the response to exercise for LBM ($p = 0.03$), and individuals with AA genotype had higher LBM gain than AG individuals.

Table 3. Models of multiple regression analysis in overweight and obese children submitted to physical exercise.

	BMI Z-score variation		WC variation		%BF variation		FM variation		LBM variation	
Independent Variables	β ± SE	p	β ± SE	p	β ± SE	p	β ± SE	p	β ± SE	p
rs1800977	-0.19 ± 0.09	**0.04**	0.07 ± 0.15	0.65	0.14 ± 0.11	0.21	0.22 ± 0.13	0.08	-0.02 ± 0.13	0.86
rs2230806	0.03 ± 0.08	0.74	-0.01 ± 0.14	0.94	0.07 ± 0.09	0.45	0.14 ± 0.11	0.2	-0.24 ± 0.11	**0.03**
rs2279796	0.03 ± 0.09	0.76	-0.12 ± 0.15	0.43	-0.008 ± 0.11	0.94	-0.06 ± 0.12	0.61	-0.02 ± 0.12	0.87
rs692383	-0.09 ± 0.11	0.43	0.07 ± 0.15	0.62	0.14 ± 0.13	0.28	-0.16 ± 0.14	0.28	-0.1 ± 0.14	0.49
rs3827225	-0.05 ± 0.1	0.63	-0.25 ± 0.15	0.1	-0.1 ± 0.13	0.44	0.03 ± 0.14	0.8	-0.13 ± 0.14	0.35

	TC variation		VLDL-C variation		HDL-C variation		TG variation		QUICKI variation	
Independent Variables	β ± SE	p	β ± SE	p	β ± SE	p	β ± SE	p	β ± SE	p
rs1800977	0.06 ± 0.01	0.56	0.16 ± 0.18	0.38	0.03 ± 0.1	0.78	-0.07 ± 0.1	0.48	-0.46 ± 0.19	**0.02**
rs2230806	-0.04 ± 0.08	0.66	0.1 ± 0.18	0.57	-0.05 ± 0.09	0.6	-0.02 ± 0.08	0.78	-0.15 ± 0.15	0.32
rs2279796	-0.05 ± 0.09	0.58	-0.2 ± 0.21	0.34	0.1 ± 0.1	0.32	0.08 ± 0.09	0.41	0.11 ± 0.15	0.44
rs692383	-0.22 ± 0.11	0.06	-0.15 ± 0.21	0.5	0.001 ± 0.12	0.99	-0.19 ± 0.12	0.1	-0.07 ± 0.19	0.71
rs3827225	0.13 ± 0.11	0.24	0.2 ± 0.19	0.3	0.06 ± 0.11	0.61	0.01 ± 0.11	0.92	0.13 ± 0.19	0.48
BMI Z-score variation	0.13 ± 0.09	0.13	-0.006 ± 0.21	0.98	0.04 ± 0.09	0.69	-0.05 ± 0.09	0.6	-0.27 ± 0.16	0.1

BMI: Body Mass Index; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; LBM: Lean Body Mass; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; TG: Triglycerides; QUICKI: Quantitative Insulin Sensitivity Check Index; β: Regression coefficient; SD: Standard deviation. *ABCA1*: rs1800977 and rs2230806; *ABCA7*: rs2279796; *ABCG1*: rs692383 and rs3827225.

We also performed a risk prediction analysis, considering individual allele effects. The risk prediction values were used to generate ROC curves (Receiver Operating Characteristic) and values of AUC (Area Under the Curve). The five SNPs were included as independent factors in the logistic regressions, and the individual binary classification (above and below the median of the anthropometric and biochemical variables) as dependent variables.

Considering the overweight and obese group, for FM, we found that rs2279796 (*ABCA7*) SNP was a risk factor, with AUC (with the respective CI) = 0.652 [0.515 – 0.788], OR = 0.31 [0.10 – 0.90] and p = 0.03), and, for insulin, rs692383 (*ABCG1*) was a risk factor, with AUC = 0.632 [0.544 – 0.721], OR = 1.73 [1.04 – 2.88] and p = 0.04. Considering the normal weight group, for VLDL-C, rs3827225 (*ABCG1*) was a risk factor, with AUC = 0.697 [0.557 – 0.837], OR = 0.25 [0.07 – 0.92] and p = 0.04. The ROC curves for these variables are shown in figure 1.

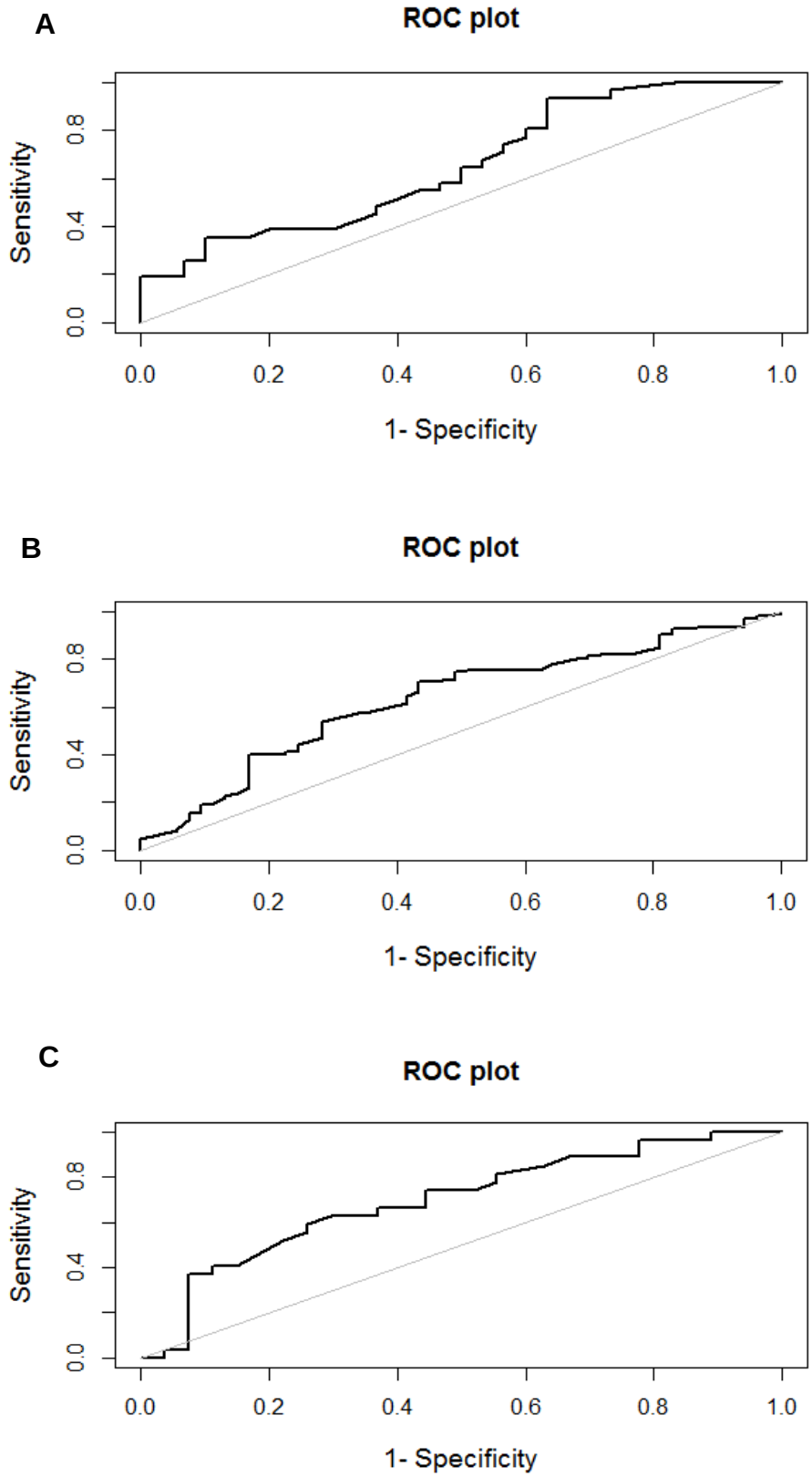

Figure 1. ROC curves in risk prediction analysis. (A) Roc curve for FM, with rs2279796 SNP (*ABCA7*) as risk factor. (B) Roc curve for insulin, with rs692383 SNP (*ABCG1*) as risk factor. (C) Roc curve for VLDL-C, with rs3827225 SNP (*ABCG1*) as risk factor.

Discussion

We found that the SNPs analyzed in this study had effect on anthropometric and biochemical variables of children and adolescents.

Regarding the *ABCA1*, both SNPs investigated (rs1800977 and rs2230806) were associated to the variables analyzed.

The rs1800977 SNP influenced BMI Z-score, AC, FM, insulin 120 and QUICKI in overweight/obese children and adolescents. Studies show that this SNP is located in the 5'UTR region, and the T-allele increases transcriptional activity and it is associated with increased HDL-C levels (PORCHAY et al., 2006). Therefore, while the T-allele would promote increased expression of *ABCA1*, the C-allele could be associated to *ABCA1* reduced expression (HODOĞLUGIL et al., 2005). In our sample, the results suggest that C-allele could be involved in obesity and insulin resistance. Since ABCA1 mediates the cholesterol efflux, the C-allele could promote an alteration of lipid transport performed by ABCA1, which can be involved in TG metabolism in adipose tissue (LAY et al., 2001; DE HAAN et al., 2014). A disturbance in lipid metabolism in adipose tissue could lead to a dysfunction characteristic of obesity and a risk factor for insulin resistance (GUILHERME et al., 2008; DE HAAN et al., 2014). An adipose tissue dysfunction could change the AG and adipokines liberation, which could reduce insulin sensitivity (GUILHERME et al., 2008; DE HAAN et al., 2014). Moreover, mice lacking ABCA1 in adipose tissue have more fat mass and reduction in insulin sensitivity (DE HAAN et al., 2014). Besides the reduced *ABCA1* expression promoted by rs1800977 SNP C-allele, another possible explanation for the effects observed is the linkage disequilibrium with other functional SNPs (PORCHAY et al., 2006).

The rs2230806 SNP influenced BMI Z-score, AC and %LBM. The rs2230806 SNP A-allele promotes changes in the first extracellular loop of ABCA1, which is important for interaction with apoA1 (PORCHAY et al., 2006). Ma, Liu and Song (2011) found an association of A-allele with increased HDL-C levels (MA; LIU; SONG, 2011), however, this effect appears to be weight-dependent, since there is an increase in HDL-C levels in lean individuals, and a decrease in overweight individuals (PORCHAY et al., 2006). Our results suggest involvement of rs2230806 SNP A-allele with weight gain, what is in

accordance with the assumption that the A-allele would be a risk allele for obese individuals (according to results found by Porchay et al (2006)). One possible explanation for this enhance in adiposity is the alteration of lipid transport, which could lead to a dysfunction of adipose tissue (GUILHERME et al., 2008; DE HAAN et al., 2014).

The rs2279796 SNP (*ABCA7*) affected BMI Z-score and FM, proposing that the C-allele could be related to weight gain. ABCA7 has homology of 54% to ABCA1 (KAMINSKI et al., 2000), and it is also expressed in the adipose tissue (KIM et al., 2005; ABE-DOHMAE; UEDA; YOKOYAMA, 2006). Besides, female mice without ABCA7 had lower white adipose tissue (KIM et al., 2005). In this sense, the C-allele could interfere in the lipid metabolism in adipose tissue, what could promote weight gain. The functional effect of the risk allele is not known, and this study is one of the few to evaluate the SNP rs2279796 effect on anthropometric and biochemical variables.

In relation to *ABCG1*, rs692383 SNP influenced BMI Z-score, AC and glucose in overweight/obese individuals and affected HDL-C, insulin and HOMA-IR in normal weight individuals, what suggests that this SNP may be related to adiposity, lipid and glucose metabolism. ABCG1 mediates the lipid efflux to HDL and LDL (WANG et al., 2004; CAVELIER et al., 2006). The rs692383 SNP could modificate the interaction of ABCG1 with HDL, thus, altering the HDL-C levels. Although ABCG1 mediates the cholesterol efflux in a cholesterol-rich environment, it also acts in lipid storage, especially TG, in a glycerolipid-rich environment (FRISDAL; GOFF, 2015). In this sense, if the *ABCG1* expression was increased, TG storage would be enhanced, thus increasing adiposity. Buchmann and colleagues (2007) found that mice without *ABCG1* have reduced fat mass and body weight gain (BUCHMANN et al., 2007), the opposite of our results. The effects on the variables related to glucose metabolism suggest that rs692383 SNP is associated to insulin resistance. Sturek and colleagues found an association of *ABCG1* and glucose metabolism, since loss of *ABCG1* expression leads to impaired insulin secretion (STUREK et al., 2010). Therefore, considering our results about adiposity, lipid and glucose metabolism, it is possible to propose that the effects promoted by rs692383 SNP are related to *ABCG1* increased expression, or to linkage disequilibrium with other causal variant.

The G-allele of rs3827225 SNP (*ABCG1*) was associated to higher levels of VLDL-C in normal weight children and adolescents and higher glucose levels in overweight/obese children and adolescents, what suggests that the G-allele could be related to lipid and glucose metabolism. Since ABGC1 participates of the TG storage (FRISDAL; GOFF, 2015) and VLDL is composed mostly by TG (LEE; OLSON; EVANS, 2003), an increase in ABCG1 expression could enhance TG storage, and consequently increase VLDL-C levels. To the best of our knowledge, the functional effect of rs692383 and rs3827225 SNPs risk alleles is not known.

Physical exercise promotes several benefits to the body, such as lipid profile improvement (GORDON; CHEN; DURSTINE, 2014). Since ABC transporters are involved in lipid efflux (TARLING; DE AGUIAR VALLIM; EDWARDS, 2013) and adiposity (as seen in this study), we verified whether there was interaction between genotype and physical exercise in the variables analyzed, in order to determine if any of the analyzed genotypes could favor or impair the response to physical exercise.

We found that individuals with the C-allele of rs1800977 had higher reduction of BMI Z-score and better response in QUICKI, and individuals with the A-allele of rs2230806 had higher gain in LBM in response to exercise. In the transversal analyzes, these SNPs had promoted negative effects (which we suggest are due to reduced expression of *ABCA1*). However, these negative results seem to be outweigh by physical exercise. Considering that *ABCA1* overexpression would have positive effects, our results are in line with studies that found an increase in *ABCA1* expression after practice of physical exercise (BUTCHER et al., 2008; HOANG et al., 2008; GHANBARI-NIAKI; SAGHEBJOO; HEDAYATI, 2011; TOFIGHI et al., 2015).

Butcher and colleagues (2008) found an increased *ABCA1* expression in leukocyte after low-intensity exercise (walking 10.000 steps three times per week for 8 weeks) (BUTCHER et al., 2008). Hoang and colleagues found an increased *ABCA1* expression in leukocytes in individuals with higher physical activity (assessed by a questionnaire) (HOANG et al., 2008). Ghanbari-Niaki, Saghebjoo and Hedayatis (2011) observed an increased *ABCA1* expression in lymphocytes after a single session of circuit-resistance exercise applied at three intensities (40%, 60% and 80% of the individual one-repetition maximum –

1RM). The exercise program consisted of nine exercises (25 s for each exercise, 8 repeats, 3 non-stop circuits with 1 min rest period between circuits), and the *ABCA1* expression increased in all the intensities, but it was more pronounced in 60% 1RM. The mechanism by which the resistance exercise increased *ABCA1* expression is not known, but it could be related to the lymphocyte increased expression promoted by physical exercise. Since *ABCA1* is highly expressed at this type of cell, an increased in lymphocyte expression could enhance *ABCA1* expression (GHANBARI-NIAKI; SAGHEBJOO; HEDAYATI, 2011). Tofighi and colleagues (2015) observed and increased expression of ABCA1 and apoA1 genes in blood after aerobic exercise (five-minute stretching program, 10 to 15-minute dynamic warm-up program, 20 to 30 minutes of core exercises, ten-minute cooling program and recovery; three sessions a week for 12 weeks) (TOFIGHI et al., 2015). Therefore, the negative results generated by the SNPs observed in the transversal analyzes (enhanced adiposity markers and reduced insulin sensitivity) would have been surpassed by physical exercise through the increased *ABCA1* expression.

One of the restrictions of our work is the sample size, which did not allow us to separate the children and adolescents by age, sex and type of physical exercise to which they were submitted.

One of the strengths of this work is that it is one of the first to evaluate *ABCA1* (rs1800977 and rs2230806), *ABCA7* (rs2279796) and *ABCG1* (rs692383 and rs3827225) SNPs interaction with physical exercise, opening the possibility to other functional and interaction studies in other contexts. In this study, we found that polymorphisms in *ABCA1, ABCA7* and *ABCG1* determined variations in basal levels of variables related to adiposity, lipid metabolism and glucose metabolism, as well as variations in response to a physical exercise program. These polymorphisms presence could affect the individual's response to treatments or physical exercise, and the knowledge of these SNPs effects could help to determine more individualized treatments according to the genetic background of each patient.

References

ABE-DOHMAE, S.; UEDA, K.; YOKOYAMA, S. ABCA7, a molecule with unknown function. **FEBS Letters**, v. 580, n. 4, p. 1178–1182, 2006.

BUCHMANN, J. et al. Ablation of the cholesterol transporter adenosine triphosphate-binding cassette transporter G1 reduces adipose cell size and protects against diet-induced obesity. **Endocrinology**, v. 148, n. 4, p. 1561–1573, 2007.

BUTCHER, L. R. et al. Low-Intensity Exercise Exerts Beneficial Effects on Plasma Lipids via PPAR. **Medicine and science in sports and exercise**, v. 40, n. 7, p. 1263–70, 2008.

CAVELIER, C. et al. Lipid efflux by the ATP-binding cassette transporters ABCA1 and ABCG1. **Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids**, v. 1761, n. 7, p. 655–666, 2006.

DE HAAN, W. et al. ABCA1 in adipocytes regulates adipose tissue lipid content, glucose tolerance, and insulin sensitivity. **Journal of lipid research**, v. 55, n. 3, p. 516–23, 2014.

FRIEDEWALD, W. T.; LEVY, R. I.; FREDRICKSON, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. **Clinical Chemistry**, v. 18, n. 6, p. 499–502, 1972.

FRISDAL, E.; GOFF, W. LE. Adipose ABCG1: A potential therapeutic target in obesity? **Adipocyte**, v. 4, n. 4, p. 315–318, 2015.

GHANBARI-NIAKI, A.; SAGHEBJOO, M.; HEDAYATI, M. A single session of circuit-resistance exercise effects on human peripheral blood lymphocyte ABCA1 expression and plasma HDL-C level. **Regulatory Peptides**, v. 166, n. 1–3, p. 42–47, 2011.

GORDON, B.; CHEN, S.; DURSTINE, J. L. The effects of exercise training on the traditional lipid profile and beyond. **Current sports medicine reports**, v. 13, n. 4, p. 253–9, 2014.

GUILHERME, A. et al. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. **Nature Reviews Molecular …**, v. 9, n. 5, p. 367–377, 2008.

HOANG, A. et al. ABCA1 expression in humans is associated with physical activity and alcohol consumption. **Atherosclerosis**, v. 197, p. 197–203, 2008.

HODOĞLUGIL, U. et al. Common polymorphisms of ATP binding cassette transporter A1, including a functional promoter polymorphism, associated with plasma high density lipoprotein cholesterol levels in Turks. **Atherosclerosis**, v. 183, n. 2, p. 199–212, 2005. KAMINSKI, W. E. et al. Identification of a novel human sterol-sensitive ATP-binding cassette transporter (ABCA7). **Biochemical and biophysical research communications**, v. 273, n. 2, p. 532–538, 2000.

KAMINSKI, W. E.; PIEHLER, A.; WENZEL, J. J. ABC A-subfamily transporters: Structure, function and disease. **Biochimica et Biophysica Acta - Molecular Basis of Disease**, v. 1762, n. 5, p. 510–524, 2006.

KATZ, A. et al. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. **Journal of Clinical Endocrinology and Metabolism**, v. 85, n. 7, p. 2402–2410, 2000.

KIM, W. S. et al. Abca7 null mice retain normal macrophage phosphatidylcholine and cholesterol efflux activity despite alterations in adipose mass and serum cholesterol levels. **Journal of Biological Chemistry**, v. 280, n. 5, p. 3989–3995, 2005.

KOBAYASHI, A. et al. Efflux of sphingomyelin, cholesterol, and phosphatidylcholine by ABCG1. **J. Lipid Res.**, v. 47, p. 1791-1802, 2006.

LAHIRI, D. K.; NUMBERGER, J. I. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. **Nucleic Acids Research**, v. 19, n. 19, p. 5444, 1991.

LAY, S. LE et al. Cholesterol, a Cell Size-dependent Signal That Regulates Glucose Metabolism and Gene Expression in Adipocytes. **Journal of Biological Chemistry**, v. 276, n. 20, p. 16904–16910, 2001.

LEE, C.-H.; OLSON, P.; EVANS, R. M. Minireview: Lipid Metabolism, Metabolic Diseases, and Peroxisome Proliferator-Activated Receptors. **Endocrinology**, v. 144, n. 6, p. 2201–2207, 2003. 4 LEITE, N. et al. Efeitos de exercícios aquáticos e orientação nutricional na composição corporal de crianças e adolescentes obesos. **Revista Brasileira de Cineantropometria e Desempenho Humano**, v. 12, n. 4, p. 232–238, 2010.

LOPES, W. A. et al. Effects of 12 weeks of combined training without caloric restriction on inflammatory markers in overweight girls. **Journal of Sports Sciences**, v. 34, n. 0, p. 1902–12, 2016.

MA, X. Y.; LIU, J. P.; SONG, Z. Y. Associations of the ATP-binding cassette transporter A1 R219K polymorphism with HDL-C level and coronary artery disease risk: A meta-analysis. **Atherosclerosis**, v. 215, n. 2, p. 428–434, 2011.

MATTHEWS, D. R. et al. Homeostasis model assessment: IR and beta-cell function from fasting plasma glucose and insulin concentration in man. **Diabetologia**, v. 28, p. 412–9, 1985.

MILANO, G. E. et al. Atividade da butirilcolinesterase e fatores de risco cardiovascular em adolescentes obesos submetidos a um programa de exercícios físicos. **Arq Bras Endocrinol Metab**, v. 57, p. 533–537, 2013.

NASCIMENTO, G. A. et al., 2017. ***FTO* SNP influences the response to dietary intervention but not to physical exercise program**. Curitiba, 2017. Submetido a publicação.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION (NCBI). 2017. https://www.ncbi.nlm.nih.gov/snp. 03/02/2017.

PORCHAY, I. et al. ABCA1 single nucleotide polymorphisms on high-density lipoprotein-cholesterol and overweight: the D.E.S.I.R. study. **Obesity (Silver Spring, Md.)**, v. 14, n. 11, p. 1874–1879, 2006.

QUAZI, F.; MOLDAY, R. S. Lipid transport by mammalian ABC proteins. **Essays in Biochemistry**, v. 50, n. 1, p. 265–90, 2011.

SINGARAJA, R. R. et al. Efflux and atherosclerosis: The clinical and biochemical impact of variations in the ABCA1 gene. **Arteriosclerosis, Thrombosis, and Vascular Biology**, v. 23, n. 8, p. 1322–1332, 2003.

STUREK, J. M. et al. An intracellular role for ABCG1-mediated cholesterol transport in the regulated secretory pathway of mouse pancreatic β cells. **The Journal of Clinical Investigation**, v. 120, n. 7, p. 2575–2589, 2010.

TARLING, E. J.; DE AGUIAR VALLIM, T. Q.; EDWARDS, P. A. Role of ABC transporters in lipid transport and human disease. **Trends Endocrinol Metab**, v. 24, n. 7, p. 342–50, 2013.

TOFIGHI, A. et al. The Effect of Regular Aerobic Exercise on Reverse Cholesterol Transport A1 and Apo Lipoprotein A-I Gene Expression in Inactive Women. **Iran Red Crescent Med**, v. 17, n. 4, p. 1–5, 2015.

WANG, N. et al. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. **PNAS**, v. 2004, 2004.

World Health Organization (WHO). Obesity and overweight. World Health Organization, 2016. http://www.who.int/topics/obesity/en/. 23/09/2016.

Supplementary material

Details of the applied exercises

Land-based aerobic exercise: It was performed 45 minutes of walking, 45 minutes of indoor cycling and 20 minutes of stretching (MILANO et al., 2013).

HIIT: Consisted of running periods at high intensity, in which the individual should run at maximal speed for 30 seconds, followed by low intensity recovery interval, which was walking in moderate/fast speed. The training intensity increased as the weeks pass.

Combined training: It was composed of resistance and aerobic training performed in a 60 minutes session. Resistance training was composed by six exercises (leg press, leg extension, leg curl, bench press, lateral pull down and arm curl) and aerobic consisted of walking/running in an athletic track (LOPES et al., 2016).

Aquatic exercise: Each session consisted of five minutes of warming-up, 45 minutes of technique (swimming techniques learning exercises or deep water running) and 10 minutes of stretching and recreation. The deep water running consisted of the individual remains in a vertical position and his body is submerged to shoulder height with the support of a float vest attached to the waist. There is no contact of the feet with the bottom of the pool, and similar movements to walk on land are made (LEITE et al., 2010).

Table 1. Comparison of variables means between genotypes of rs1800977 (*ABCA1*) SNP before and after the physical intervention in overweight and obese children and adolescents, and comparison of variables means between genotypes of rs1800977 (*ABCA1*) SNP in normal weight children and adolescents.

VARIABLES	Overweight and obese								
	Before								
	TT		CT		CC		P	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	36	2.48 ± 0.95	105	2.73 ± 1.8	110	2.8 ± 1.11	0.68	0.15	0.21
AC (cm)	17	93.05 ± 17.73	71	93.5 ± 15.61	65	97 ± 14.93	0.77	0.26	0.15
WC (cm)	16	91.02 ± 8.71	31	92.68 ± 12.4	33	91.33 ± 10.01	0.74	0.9	
% BF	14	42.51 ± 7.32	57	38.37 ± 6.90	49	39.48 ± 8.20	0.09	0.18	0.63
FM (kg)	14	31.22 ± 15.14	53	28.27 ± 13.54	46	31.13 ± 11.36	0.32	0.67	0.14
% LBM	13	57.6 ± 7.52	42	61.56 ± 6.24	33	58.53 ± 6.67	**0.04**	0.55	**0.01**
LBM (kg)	14	41.86 ± 12.13	53	45.73 ± 11.45	46	46.36 ± 11.51	0.29	0.18	0.5
TL (mg/dl)	11	518.21 ± 104.63	24	547.27 ± 122.15	30	537.16 ± 94.22	0.47	0.53	0.76
TC (mg/dl)	31	163.88 ± 46.54	93	164.06 ± 37.12	99	163.85 ± 35.07	0.76	0.68	0.98
VLDL-C (mg/dl)	11	17.97 ± 7.29	27	21.62 ± 11.12	32	19.74 ± 7.6	0.6	0.41	0.92
HDL-C (mg/dl)	35	49.30 ± 9.81	105	46.91 ± 10.89	110	48.88 ± 14.25	0.16	0.41	0.51
LDL-C (mg/dl)	31	92.01 ± 38.41	93	93.79 ± 30.74	99	93.53 ± 26.5	0.36	0.42	0.78
TG (mg/dl)	35	103.24 ± 66.69	105	112.15 ± 59.88	110	106.76 ± 48.26	0.27	0.27	0.77
Glucose (mg/dl)	35	86.39 ± 10.55	105	86.41 ± 9.68	111	87.14 ± 10.70	0.99	0.71	0.6
Glucose 120 (mg/dl)	5	84.8 ± 13.16	31	95.32 ± 17.84	33	96.15 ± 17.34	0.22	0.17	0.85
Insulin (uUI/ml)	27	11.66 ± 6.9	85	14.31 ± 12.39	97	15.67 ± 13.09	0.58	0.29	0.31
Insulin 120 (uUI/ml)	2	31.5 ± 28.43	23	35.59 ± 35.97	26	40.91 ± 32.34	0.8	0.75	0.33
HOMA-IR	9	1.25 ± 0.92	43	1.82 ± 1.29	44	1.94 ± 1.53	0.2	0.23	0.91
QUICKI	9	0.39 ± 0.07	37	0.36 ± 0.06	41	0.35 ± 0.07	0.32	**0.03**	0.14

VARIABLES	Overweight and obese								
	After								
	TT		CT		CC		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	21	2.65 ± 1.10	57	2.75 ± 1.32	55	3 ± 1.16	0.66	0.15	0.14
AC (cm)	10	97.42 ± 17.47	38	95.41 ± 12.43	35	98.02 ± 13.74	0.99	0.59	0.32
WC (cm)	12	91.82 ± 12.5	21	93.73 ± 12.15	23	92.92 ± 10.1	0.67	0.78	0.81
% BF	12	37.88 ± 7.06	45	35.74 ± 6.85	39	36.31 ± 9.67	0.34	0.6	0.75
FM (kg)	8	33.04 ± 19.41	32	25.14 ± 9.82	31	28.26 ± 11.86	0.5	0.57	0.43
% LBM			6	57.68 ± 12.99	7	59.41 ± 5.54			0.83
LBM (kg)	8	43.73 ± 12.21	32	47.19 ± 12.38	31	47.24 ± 12.96	0.48	0.49	0.99
TL (mg/dl)	7	523.23 ± 131.72	12	564.09 ± 125.36	12	564.09 ± 125.36	0.81	0.53	0.67
TC (mg/dl)	22	148 ± 34.33	58	159.86 ± 32.06	55	158.72 ± 35.25	0.38	0.27	0.64
VLDL-C (mg/dl)	7	19.11 ± 6.32	10	21.18 ± 10.89	12	22.36 ± 9.73	0.61	0.44	0.84
HDL-C (mg/dl)	21	45.38 ± 7.41	58	45.95 ± 12.96	55	48.42 ± 17.1	0.88	0.96	0.62
LDL-C (mg/dl)	22	84.46 ± 30.03	58	90.99 ± 28.18	55	93.39 ± 28.01	0.4	0.25	0.48
TG (mg/dl)	21	95.77 ± 48.64	58	112.59 ± 61.43	55	99.96 ± 46.46	0.16	0.38	0.18
Glucose (mg/dl)	22	82.9 ± 7.79	57	85.49 ± 7.55	54	83.59 ± 6.59	0.18	0.7	0.16
Glucose 120 (mg/dl)	5	88 ± 16.29	15	88.77 ± 13.41	19	93 ± 13.68	0.92	0.49	0.37
Insulin (uUI/ml)	17	12.22 ± 4.01	42	13.93 ± 9.62	47	13.85 ± 9.72	0.89	0.71	0.88
Insulin 120 (uUI/ml)	5	33.92 ± 13.61	15	27.09 ± 17.03	19	29.01 ± 21.59	0.22	0.32	0.84
HOMA-IR	5	1.72 ± 1.09	22	1.74 ± 1.21	27	1.69 ± 1.63	0.85	0.36	0.5
QUICKI	5	0.34 ± 0.01	19	0.36 ± 0.04	23	0.36 ± 0.04	0.41	0.29	0.74

VARIABLES	Normal weight								
	TT		CT		CC		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	26	-0.13 ± 0.86	72	-0.13 ± 0.83	76	-0.31 ± 0.8	0.9	0.35	0.11
AC (cm)	21	68.8 ± 6.49	60	67.48 ± 6.42	54	67.69 ± 7.09	0.32	0.37	0.97
WC (cm)	9	69.02 ± 5.88	25	66.22 ± 6.05	23	67.78 ± 5.48	0.21	0.35	0.33
% BF	3	28.47 ± 2.14	4	24.04 ± 1.86	9	22.44 ± 5.44	0.05	0.1	0.4
FM (kg)	3	14.1 ± 2.2	4	10.34 ± 1.72	9	10.77 ± 2.2	0.11	0.1	0.7
% LBM	2	70.75 ± 2.33	4	75.96 ± 1.86	9	77.56 ± 5.44	0.11	0.13	0.4
LBM (kg)	3	35.53 ± 6.22	4	32.89 ± 6.41	9	37.07 ± 8.91		0.85	0.49
TL (mg/dl)	3	571.75 ± 92.4	8	526.04 ± 63.97	15	489.1 ± 54.53	0.26	0.08	0.23
TC (mg/dl)	14	156.32 ± 25.57	33	160.49 ± 23.95	42	154.38 ± 33.67	0.68	0.67	0.25
VLDL-C (mg/dl)	10	17.05 ± 7.35	25	16.89 ± 5.42	29	16.75 ± 7.51	0.53	0.8	0.48
HDL-C (mg/dl)	25	45.25 ± 12.06	68	44.54 ± 10.65	71	48.6 ± 11.01	0.94	0.09	**0.03**
LDL-C (mg/dl)	14	90.58 ± 20.59	33	96.04 ± 22.68	42	87.63 ± 30.41	0.45	0.5	0.06
TG (mg/dl)	25	69.93 ± 28.99	67	78.1 ± 30.56	71	74.62 ± 33.89	0.18	0.46	0.42
Glucose (mg/dl)	25	92.62 ± 13.89	71	92.82 ± 11.67	76	91.89 ± 11.57	0.92	0.8	0.59
Glucose 120 (mg/dl)	3	81.67 ± 17.62	4	69.5 ± 80	8	80 ± 22.95	0.27	0.91	0.4
Insulin (uUI/ml)	25	6.97 ± 4.28	71	5.5 ± 3.78	71	5.5 ± 3.78	**0.03**	0.14	0.38
Insulin 120 (uUI/ml)	3	22.33 ± 6.97	4	13.23 ± 3.98	9	22.76 ± 11.18	0.11	0.85	0.08
HOMA-IR	20	1.34 ± 0.77	57	1.1 ± 0.78	54	1.08 ± 0.87	0.2	0.12	0.73
QUICKI	13	0.4 ± 0.06	39	0.42 ± 0.09	38	0.41 ± 0.08	0.6	0.69	0.9

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p:TT vs CT; p*: TT vs CC; p**: CT vs CC.

Table 2. Comparison of variables means between genotypes of rs2230806 (*ABCA1*) SNP before and after the physical intervention in overweight and obese children and adolescents, and comparison of variables means between genotypes of rs2230806 (*ABCA1*) SNP in normal weight children and adolescents.

VARIABLES	Overweight and obese								
	Before								
	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	115	2.64 ± 1.11	118	2.69 +± 0.94	42	3.32 ± 2.63	0.2	0.14	0.45
AC (cm)	76	95.35 ± 14.34	78	94.66 ± 13.42	31	98.77 ± 17.78	0.95	0.46	0.42
WC (cm)	34	90.49 ± 10.76	31	90.61 ± 9.34	8	99.51 ± 9.22	0.64	**0.02**	**0.03**
% BF	59	38.73 ± 6.13	56	39.16 ± 6.73	27	40.33 ± 8.91	0.9	0.29	0.18
FM (kg)	53	29.37 ± 9.89	56	27.81 ± 8.99	25	34.4 ± 19.36	0.32	0.67	0.27
% LBM	32	60.17 ± 6.32	39	60.61 ± 6.45	17	55.91 ± 6.36	0.51	0.16	**0.04**
LBM (kg)	53	47.56 ± 10.21	56	43.27 ± 10.08	25	46.37 ± 13.19	**0.04**	0.41	0.45
TL (mg/dl)	28	525.75 ± 108.89	30	547.5 ± 105.99	5	580.42 ± 99.14	0.42	0.24	0.38
TC (mg/dl)	104	163.53 ± 36.26	105	159.47 ± 34.71	41	174.27 ± 41.64	0.61	0.11	0.05
VLDL-C (mg/dl)	30	19.29 ± 9.33	32	20.32 ± 9.36	6	23.46 ± 7.99	0.93	0.18	0.39
HDL-C (mg/dl)	112	49.43 ± 10.76	120	46.7 ± 13.82	42	46.55 ± 9.5	**0.01**	0.15	0.5
LDL-C (mg/dl)	104	91.37 ± 29.45	105	91.02 ± 26.62	41	104.52 ± 34.36	0.99	**0.03**	**0.03**
TG (mg/dl)	111	105.17 ± 54.47	120	112.47 ± 53.46	42	114.44 ± 69.37	0.18	0.64	0.7
Glucose (mg/dl)	115	87.52 ± 10.43	119	87.41 ± 9.77	40	86.26 ± 8.88	0.93	0.49	0.51
Glucose 120 (mg/dl)	44	94.5 ± 18.37	39	94.77 ± 19.19	19	102.16 ± 20.33	0.95	0.15	0.18
Insulin (uUI/ml)	98	13.35 ± 8.39	102	16.57 ± 14	32	13.42 ± 10.08	0.22	0.78	0.2
Insulin 120 (uUI/ml)	41	45.74 ± 34.96	27	38.31 ± 27.11	15	58.19 ± 52.16	0.59	0.56	0.42
HOMA-IR	52	1.68 ± 1.1	52	2.11 ± 1.34	20	2.09 ± 1.53	0.09	0.35	0.34
QUICKI	49	0.36 ± 0.07	48	0.34 ± 0.06	20	0.33 ± 0.04	0.36	0.29	0.87

VARIABLES	Overweight and obese								
	After								
	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	68	2.7 ± 1.04	64	2.76 ± 1	22	3.29 ± 2.06	0.67	0.32	0.45
AC (cm)	45	95.61 ± 13.06	43	95.2 ± 9.52	20	99.46 ± 19.98	0.68	0.66	0.8
WC (cm)	23	91.20 ± 13.05	24	91.54 ± 8.35	5	102.52 ± 9.87	0.91	0.08	0.01
% BF	50	34.75 ± 6.72	46	37.31 ± 7.4	21	35.44 ± 9.01	0.08	0.72	0.37
FM (kg)	36	27.13 ± 10.78	41	26.55 ± 8.64	17	29.36 ± 17.3	0.89	0.85	0.97
% LBM	5	62.16 ± 6.64	6	60.13 ± 4.68	3	51.8 ± 16.98	0.52	0.37	0.9
LBM (kg)	36	48.59 ± 9.61	41	44.36 ± 10.42	17	50.17 ± 15.62	0.07	0.65	0.1
TL (mg/dl)	13	549.51 ± 139.9	15	550.31 ± 120.42			0.89		
TC (mg/dl)	69	158.63 ± 34.58	64	153.58 ± 31.79	23	162.26 ± 33.76	0.86	0.53	0.42
VLDL-C (mg/dl)	13	24.07 ± 10.01	15	18.74 ± 8.42			0.14		
HDL-C (mg/dl)	69	46.78 ± 9.84	63	48.67 ± 17.39	23	44.52 ± 11.46	0.91	0.24	0.4
LDL-C (mg/dl)	69	91.41 ± 29.89	64	88.26 ± 25.91	23	95.89 ± 27.75	0.65	0.35	0.11
TG (mg/dl)	68	101.13 ± 52.32	64	94.85 ± 49.27	23	118.04 ± 66.33	0.58	0.24	0.09
Glucose (mg/dl)	70	84.61 ± 7.66	64	86.27 ± 8.59	21	86.52 ± 8.58	0.24	0.33	0.91
Glucose 120 (mg/dl)	28	89.21 ± 14.84	23	89.93 ± 14.63	11	96.09 ± 20.68	0.86	0.25	0.32
Insulin (uUI/ml)	58	13.12 ± 8.4	52	14.05 ± 7.93	16	12.44 ± 9.02	0.45	0.57	0.23
Insulin 120 (uUI/ml)	28	34.12 ± 25.09	23	31.74 ± 17.1	11	30.01 ± 21.01	0.63	0.78	0.44
HOMA-IR	34	1.72 ± 1.23	29	1.83 ± 1.35	14	1.51 ± 1.05	0.51	0.53	0.35
QUICKI	33	0.35 ± 0.04	24	0.34 ± 0.03	13	0.36 ± 0.05	0.31	0.69	0.19

VARIABLES	Normal weight								
	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	71	−0.20 ± 0.74	80	−0.25 ± 0.95	21	−0.34 ± 0.65	0.93	0.29	0.31
AC (cm)	54	67.79 ± 7.35	67	67.32 ± 6.33	16	68.07 ± 5.82	0.97	0.56	0.59
WC (cm)	22	66.31 ± 5.29	23	68.25 ± 6.14	8	69.08 ± 6.82	0.39	0.43	0.77
% BF	6	25.9 ± 5.99	13	22.62 ± 4.63	3	19.49 ± 2.42	0.36	0.09	0.23
FM (kg)	6	12.94 ± 3.69	13	10.21 ± 3.3	3	10.51 ± 2.46	0.12	0.52	0.79
% LBM	6	74.1 ± 5.99	12	77.74 ± 4.64	3	80.5 ± 2.42	0.28	0.09	0.28
LBM (kg)	6	36.88 ± 5.1	13	34.26 ± 5.92	3	43.45 ± 9.18	0.63	0.37	0.14
TL (mg/dl)	13	502.65 ± 71.09	9	503.09 ± 52.87	3	577.15 ± 62.6		0.14	0.2
TC (mg/dl)	38	152.46 ± 30.07	40	158.38 ± 26.7	13	166.24 ± 23.66	0.42	0.07	0.26
VLDL-C (mg/dl)	28	17.68 ± 8.37	26	15.7 ± 5.69	8	15.62 ± 4.15	0.4	0.62	0.71
HDL-C (mg/dl)	66	46.21 ± 9.56	75	47.78 ± 12.49	19	50.15 ± 13.83	0.74	0.25	0.35
LDL-C (mg/dl)	38	87.5 ± 25.18	40	90.12 ± 26.83	13	97.64 ± 23.82	0.77	0.09	0.26
TG (mg/dl)	66	79.77 ± 37.28	74	71.34 ± 26.6	19	71.1 ± 28.91	0.28	0.47	0.95
Glucose (mg/dl)	68	91.2 ± 11.7	80	93.81 ± 11.58	20	91.48 ± 12.91	0.18	0.93	0.43
Glucose 120 (mg/dl)	7	80.71 ± 17.87	13	82.15 ± 19.76	3	71.33 ± 15.37	0.87	0.45	0.39
Insulin (uUI/ml)	66	5.91 ± 4.05	75	5.15 ± 3.22	19	4.94 ± 3.45	0.45	0.51	0.78
Insulin 120 (uUI/ml)	7	23.96 ± 13.4	14	19.67 ± 8.02	3	11.1 ± 4.86	0.63	0.11	0.09
HOMA-IR	51	1.17 ± 0.83	64	1.04 ± 0.72	15	0.87 ± 0.77	0.44	0.4	0.24
QUICKI	35	0.4 ± 0.07	48	0.41 ± 0.08	9	0.44 ± 0.11		0.48	0.41

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p: GG vs AG; p*: GG vs AA; p**: AG vs AA.

Table 3. Comparison of variables means between genotypes of rs2279796 (*ABCA7*) SNP before and after the physical intervention in overweight and obese children and adolescents, and comparison of variables means between genotypes of rs2279796 (*ABCA7*) SNP in normal weight children and adolescents.

VARIABLES	Overweight and obese								
	Before								
	CC		CT		TT		P	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	71	2.54 ± 1.3	111	2.57 ± 0.96	58	2.71 ± 1.91	0.54	0.74	0.9
AC (cm)	41	93.29 ± 18.49	70	93.89 ± 12.15	33	93.26 ± 14.7	0.31	0.68	0.79
WC (cm)	27	93.58 ± 10.96	33	93.07 ± 10.72	19	86.83 ± 9.38	0.78	**0.04**	**0.02**
% BF	22	39.91 ± 7.19	55	39.42 ± 7.42	25	37.98 ± 8.27	0.67	0.29	0.36
FM (kg)	19	32.9 ± 13.24	53	27.65 ± 9.15	22	25.12 ± 15.56	0.1	**0.01**	0.06
% LBM	14	59.26 ± 5.89	37	59.78 ± 5.97	17	62.65 ± 8.36	0.6	0.08	0.08
LBM (kg)	19	48.99 ± 13.58	53	43.98 ± 11.08	25	42.37 ± 9.82	0.23	0.08	0.44
TL (mg/dl)	19	566.46 ± 140.42	26	540.8 ± 86.53	19	509.7 ± 86.4	0.97	0.31	0.16
TC (mg/dl)	56	165.74 ± 35.6	102	159.96 ± 33.76	52	161.78 ± 38.55	0.58	0.56	0.1
VLDL-C (mg/dl)	21	22.26 ± 11.4	27	19.98 ± 7.89	20	17.74 ± 7.72	0.99	0.33	0.23
HDL-C (mg/dl)	69	47.33 ± 11.51	111	49.34 ± 13.39	58	47.74 ± 12.04	0.29	0.95	0.4
LDL-C (mg/dl)	56	93.22 ± 26.48	102	90.76 ± 25.41	52	90.02 ± 30.54	0.7	0.56	0.7
TG (mg/dl)	69	113.56 ± 55.7	111	105.35 ± 57.52	58	105.39 ± 53.79	0.26	0.42	0.83
Glucose (mg/dl)	71	87.13 ± 10.47	109	85.51 ± 10.15	57	87.88 ± 9.75	0.3	0.68	0.15
Glucose 120 (mg/dl)	18	96.25 ± 20.87	41	94.62 ± 18.12	17	93.06 ± 14.32	0.76	0.6	0.75
Insulin (uUI/ml)	68	13.83 ± 11.29	99	16.18 ± 14.02	51	13.04 ± 7.64	0.23	0.73	0.43
Insulin 120 (uUI/ml)	13	35.73 ± 33.2	33	40.34 ± 35.64	13	36.95 ± 26.79	0.46	0.38	0.85
HOMA-IR	32	1.46 ± 1.06	47	2 ± 1.38	25	1.97 ± 1.43	0.07	0.14	0.82
QUICKI	29	0.37 ± 0.07	44	0.35 ± 0.06	22	0.36 ± 0.07	0.06	0.39	0.37

VARIABLES	Overweight and obese								
	After								
	CC		CT		TT		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	31	2.8 ± 1.64	58	2.66 ± 0.83	32	2.48 ± 1.75	0.74	0.63	0.36
AC (cm)	13	96.98 ± 19.51	39	94.48 ± 10.65	19	92.41 ± 11.53	0.82	0.79	0.47
WC (cm)	21	94.91 ± 12.45	22	93.83 ± 10.83	12	87.67 ± 9.55	0.76	0.09	0.12
% BF	16	34.8 ± 7.84	47	36.22 ± 8.78	23	34.58 ± 6.6	0.57	0.92	0.43
FM (kg)	8	27.92 ± 14.44	35	26.31 ± 10.31	17	23.36 ± 9.13	0.94	0.75	0.51
% LBM	2	61 ± 1.41	8	56.68 ± 11.47	3	62.2 ± 4.18	0.51		0.61
LBM (kg)	8	49.42 ± 20.08	35	46.99 ± 11.82	17	43.35 ± 9.43	0.65	0.31	0.27
TL (mg/dl)	10	585.5 ± 170.72	8	516.38 ± 91.63	10	541.23 ± 102.39	0.45	0.43	0.35
TC (mg/dl)	34	163.5 ± 39.09	58	153.51 ± 33.15	30	160.75 ± 35.61	0.19	0.71	0.43
VLDL-C (mg/dl)	10	22.74 ± 10.65	8	19.63 ± 10.33	10	20.95 ± 8.02	0.54	0.68	0.76
HDL-C (mg/dl)	34	49.11 ± 12.95	57	47.81 ± 16.61	30	45.67 ± 11.38	0.31	0.28	0.98
LDL-C (mg/dl)	34	92.09 ± 30.7	58	88.03 ± 26.58	30	94.78 ± 31.39	0.65	0.76	0.42
TG (mg/dl)	34	113.73 ± 57.71	58	100.1 ± 51.99	30	101.49 ± 58.41	0.23	0.18	0.65
Glucose (mg/dl)	34	83.56 ± 6.27	57	84.15 ± 7.51	30	85.91 ± 10.68	0.7	0.28	0.37
Glucose 120 (mg/dl)	9	99.22 ± 17.83	24	87.15 ± 10.86	8	93.13 ± 11.87	0.02	0.43	0.2
Insulin (uUI/ml)	33	14.04 ± 8.66	50	13.48 ± 8.7	25	13.08 ± 7.85	0.65	0.78	0.93
Insulin 120 (uUI/ml)	9	31.59 ± 17.58	24	29.09 ± 20.61	8	31.31 ± 20.57	0.73	0.81	0.86
HOMA-IR	12	1.51 ± 0.99	30	1.69 ± 1.45	14	1.84 ± 1.29	0.87	0.63	0.55
QUICKI	12	0.35 ± 0.03	25	0.36 ± 0.04	12	0.34 ± 0.05	0.73	0.64	0.42

VARIABLES	Normal weight								
	CC		CT		TT		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	42	-0.47 ± 0.79	90	-0.09 ± 0.74	34	-0.26 ± 1.02	**0.008**	0.11	0.44
AC (cm)	33	66.46 ± 5.75	67	67.6 ± 6.54	28	68.94 ± 7.6	0.39	0.15	0.5
WC (cm)	17	67.01 ± 7.08	29	67.04 ± 5.22	10	68.04 ± 5.61	0.78	0.58	0.53
% BF	2	20.45 ± 3.46	3	22.41 ± 2.6	6	23.36 ± 5.46	0.39	0.62	0.9
FM (kg)	2	11.7 ± 0.14	3	10.86 ± 2.05	6	11.48 ± 3.51	0.77	0.62	0.9
% LBM	2	79.55 ± 3.46	3	77.59 ± 2.6	6	76.64 ± 5.46	0.39	0.62	0.9
LBM (kg)	2	46.4 ± 9.33	3	37.27 ± 1.73	6	37.16 ± 5.63	0.15	0.24	0.7
TL (mg/dl)	7	508.36 ± 46.17	15	514.69 ± 71.63	4	495.3 ± 84.77	0.89		0.96
TC (mg/dl)	22	159.71 ± 19.41	41	152.79 ± 27.16	19	162.41 ± 40.21	0.2	0.91	0.46
VLDL-C (mg/dl)	16	16.30 ± 4	30	17.82 ± 5.55	12	17.46 ± 11.28	0.53	0.32	0.16
HDL-C (mg/dl)	40	46.79 ± 10.81	84	44.92 ± 9.82	32	48.16 ± 13.07	0.29	0.87	0.38
LDL-C (mg/dl)	22	95.54 ± 18.88	41	87.51 ± 25.42	19	93.39 ± 36.43	0.06	0.37	0.71
TG (mg/dl)	40	72.54 ± 26.12	84	76.37 ± 27.45	31	79.28 ± 45.82	0.65	0.82	0.51
Glucose (mg/dl)	42	94.06 ± 11.82	88	91.31 ± 12.28	34	93.94 ± 11.22	0.23	0.96	0.28
Glucose 120 (mg/dl)	2	104.5 ± 21.92	3	84.67 ± 14.19	6	82.67 ± 16.21	0.29	0.17	0.86
Insulin (uUI/ml)	40	5.68 ± 3.79	84	5.72 ± 3.9	32	4.99 ± 3.38	0.78	0.43	0.39
Insulin 120 (uUI/ml)	2	24.85 ± 11.53	3	19.63 ± 6.02	6	26.15 ± 12.34	0.77		0.7
HOMA-IR	31	1.09 ± 0.85	67	1.21 ± 0.84	27	1.02 ± 0.81	0.36	0.83	0.32
QUICKI	20	0.42 ± 0.07	46	0.41 ± 0.09	19	0.41 ± 0.07	0.32	0.62	0.6

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p: CC vs TC; p*: CC vs TT; p**: TC vs TT.

Table 4. Comparison of variables means between genotypes of rs692383 (*ABCG1*) SNP before and after the physical intervention in overweight and obese children and adolescents, and comparison of variables means between genotypes of rs692383 (*ABCG1*) SNP in normal weight children and adolescents.

VARIABLES	Overweight and obese								
	Before								
	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	26	2.36 ± 0.67	111	2.49 ± 0.86	73	2.33 ± 0.76	0.53	0.82	0.16
AC (cm)	15	90.05 ± 15.45	71	91.84 ± 15.29	27	93.91 ± 14.57	0.55	0.37	0.58
WC (cm)	11	89.32 ± 6.59	36	93.48 ± 12.5	32	90.26 ± 9.2	0.33	0.81	0.35
% BF	8	38.99 ± 7.14	46	39.34 ± 6.54	26	39.99 ± 9.04	0.93	0.98	0.78
FM (kg)	7	27.2 ± 5.38	44	28.45 ± 10.36	22	24.91 ± 8.76	0.83	0.43	0.3
% LBM	6	57.18 ± 4.81	33	60.28 ± 5.96	17	62.9 ± 7.3	0.19	0.15	0.26
LBM (kg)	7	38.6 ± 5.69	44	45.36 ± 10.88	22	41.39 ± 11.01	0.15	0.37	0.2
TL (mg/dl)	7	539.23 ± 88.03	32	556.38 ± 124.02	32	525.74 ± 94.76	0.96	0.84	0.61
TC (mg/dl)	20	159.43 ± 29.71	91	166.43 ± 35.39	66	157.23 ± 38.16	0.26	0.93	0.11
VLDL-C (mg/dl)	8	21.85 ± 9.77	27	20.27 ± 11.31	33	19.38 ± 6.89	0.54		0.41
HDL-C (mg/dl)	26	49.6 ± 9.34	110	47.51 ± 11.21	71	50.59 ± 15.14	0.23	0.86	0.19
LDL-C (mg/dl)	20	89.82 ± 25.61	91	94.84 ± 28.05	66	86.52 ± 25.28	0.3	0.56	**0.04**
TG (mg/dl)	26	94.25 ± 52.96	110	104.8 ± 46.88	71	110.89 ± 64.49	0.19	0.2	0.92
Glucose (mg/dl)	26	86.45 ± 10.66	111	87.05 ± 10.67	71	84.37 ± 10.01	0.79	0.38	0.09
Glucose 120 (mg/dl)	8	96.5 ± 19.68	35	94.27 ± 16.97	16	94.28 ± 22.26	0.75	0.81	
Insulin (uUI/ml)	26	12.09 ± 10.14	103	14.69 ± 12.88	69	16.01 ± 12.11	0.28	0.05	0.15
Insulin 120 (uUI/ml)	5	55.24 ± 31.61	26	36.43 ± 30.38	10	36.22 ± 45.97	0.13	0.07	0.68
HOMA-IR	14	1.69 ± 1.49	50	1.72 ± 1.33	25	2.18 ± 1.48	0.59	0.25	0.14
QUICKI	14	0.37 ± 0.07	45	0.36 ± 0.07	21	0.35 ± 0.07	0.61	0.58	0.64

VARIABLES	Overweight and obese								
	After								
	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	13	2.52 ± 0.84	52	2.73 ± 0.74	38	2.42 ± 0.77	0.25	0.71	**0.04**
AC (cm)	7	100.84 ± 17.99	30	93.35 ± 10.82	15	95.89 ± 9.21	0.38	0.81	0.45
WC (cm)	7	90.19 ± 7.08	26	95.2 ± 13.01	22	90.44 ± 9.76	0.34	0.95	0.16
% BF	10	36.3 ± 6.28	37	35.6 ± 7.64	21	36.57 ± 10.09	0.79	0.94	0.68
FM (kg)	5	31.5 ± 11.94	27	24.25 ± 9.37	10	26.62 ± 9.25	0.18	0.5	0.44
% LBM	2	61.2 ± 8.63	9	56.73 ± 10.22	1	62	0.91		
LBM (kg)	5	46.3 ± 9.68	27	46.78 ± 11.68	10	42.03 ± 11.7	0.93	0.5	0.28
TL (mg/dl)	3	494.27 ± 60.09	10	616.55 ± 136.82	15	516.66 ± 117.07	0.11	0.81	0.09
TC (mg/dl)	14	149.99 ± 21.61	53	164.92 ± 35.33	36	151.68 ± 40.59	0.29	0.75	0.2
VLDL-C (mg/dl)	3	14.82 ± 0.64	10	23.57 ± 10.87	15	20.92 ± 9.06	0.2	0.27	0.51
HDL-C (mg/dl)	14	52.45 ± 12.15	53	46.8 ± 11.58	35	49.59 ± 20.04	0.16	0.1	0.74
LDL-C (mg/dl)	14	84 ± 18.87	53	97.04 ± 29.96	36	83.06 ± 32.61	0.15	0.78	0.05
TG (mg/dl)	14	64.16 ± 35.98	53	104.15 ± 48.37	36	119.18 ± 66.86	**0.0005**	**0.0004**	0.62
Glucose (mg/dl)	13	83.25 ± 8.75	53	84.95 ± 6.66	35	82.23 ± 7.98	0.44	0.7	0.09
Glucose 120 (mg/dl)	5	83.8 ± 15.42	16	93.41 ± 13.39	10	84.8 ± 13.05	0.19	0.9	0.12
Insulin (uUI/ml)	13	14.56 ± 11.51	48	13.11 ± 8.13	35	14.44 ± 8.33	0.85	0.58	0.57
Insulin 120 (uUI/ml)	5	31.08 ± 12.91	16	28.45 ± 19.33	10	22.95 ± 10.29	0.39	0.24	0.94
HOMA-IR	7	2.07 ± 1.79	24	1.32 ± 0.75	14	2.26 ± 1.91	0.57	0.91	0.15
QUICKI	7	0.34 ± 0.04	20	0.36 ± 0.04	11	0.35 ± 0.04	0.22	0.72	0.33

VARIABLES	Normal weight								
	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	23	-0.18 ± 0.75	74	-0.17 ± 0.96	63	-0.27 ± 0.76	0.59	0.54	0.14
AC (cm)	21	69.39 ± 6.67	57	67.32 ± 6.47	43	67.89 ± 7.59	0.2	0.39	0.76
WC (cm)	6	68.36 ± 4.85	28	67.46 ± 6.91	24	66.96 ± 4.53	0.67	0.44	0.99
TL (mg/dl)	3	550.9 ± 90.02	11	518.8 ± 68.76	12	491.72 ± 55.62	0.53	0.43	0.56
TC (mg/dl)	10	149.54 ± 31.38	34	164.78 ± 32.24	31	149.96 ± 26.99	0.16	0.88	0.09
VLDL-C (mg/dl)	8	14.68 ± 2.56	27	18.57 ± 8.12	21	16.95 ± 5.49	0.26	0.31	0.67
HDL-C (mg/dl)	22	43.45 ± 10.67	69	45.29 ± 8.77	58	45.55 ± 10.88	0.24	0.39	0.68
LDL-C (mg/dl)	10	87.01 ± 24.41	34	99.22 ± 29.64	31	86.64 ± 25.94	0.23	0.92	0.09
TG (mg/dl)	21	75.79 ± 21.97	69	78.3 ± 35.81	58	75.01 ± 31.95	0.79	0.63	0.71
Glucose (mg/dl)	21	91.69 ± 14.52	73	94.62 ± 11.24	63	91.24 ± 12.4	0.33	0.89	0.1
Insulin (uUI/ml)	22	5.3 ± 2.68	69	5.33 ± 4.07	58	6.01 ± 3.91	0.61	0.71	0.22
HOMA-IR	17	1.1 ± 0.63	55	1.06 ± 0.8	46	1.32 ± 0.94	0.6	0.64	0.17
QUICKI	13	0.42 ± 0.08	35	0.43 ± 0.09	28	0.4 ± 0.07	0.71	0.44	0.1

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p: GG vs AG; p*: GG vs AA; p**: AG vs AA. Variables that did not have enough data for analysis were not presented.

Table 5. Comparison of variables means between genotypes of rs3827225 (*ABCG1*) SNP before and after the physical intervention in overweight and obese children and adolescents, and comparison of variables means between genotypes of rs3827225 (*ABCG1*) SNP in normal weight children and adolescents.

VARIABLES	Overweight and obese								
	Before								
	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	124	2.62 ± 1.64	80	2.43 ± 0.77	11	2.04 ± 0.65	0.83	0.09	0.13
AC (cm)	74	93.76 ± 15.91	38	90.17 ± 14.99	5	90.02 ± 8.47	0.29	0.61	0.98
WC (cm)	37	91.83 ± 8.72	37	93.79 ± 12.17	5	82.65 ± 9.8	0.84	**0.04**	**0.04**
% BF	51	40.72 ± 7.76	33	37.88 ± 7.57	4	38.75 ± 3.37	0.14	0.55	0.75
FM (kg)	46	29.37 ± 13.47	31	26.81 ± 10.72	4	26.08 ± 6.84	0.41	0.69	0.86
% LBM	34	59.03 ± 6.48	23	62.19 ± 7.5	3	60.77 ± 3.96	0.2	0.5	0.81
LBM (kg)	46	43.41 ± 11.72	31	45.3 ± 10.9	4	41.68 ± 12.81	0.53	0.63	0.52
TL (mg/dl)	39	540.13 ± 99.49	20	532.91 ± 119.3	5	556.89 ± 120.71	0.61	0.51	0.48
TC (mg/dl)	109	161.23 ± 34.43	68	162.85 ± 39.83	10	154.57 ± 27.95	0.91	0.66	0.64
VLDL-C (mg/dl)	42	19.72 ± 8.72	20	19.18 ± 9.26	5	24.98 ± 12.69	0.66	0.35	0.26
HDL-C (mg/dl)	124	48.38 ± 14.28	79	48.42 ± 10.22	10	52.15 ± 11.07	0.75	0.22	0.22
LDL-C (mg/dl)	109	91.08 ± 25.34	68	92.51 ± 30.23	10	78.93 ± 17.79	0.91	0.1	0.17
TG (mg/dl)	124	105.58 ± 54.26	79	105.23 ± 54.17	10	117.56 ± 52.21	0.97	0.39	0.42
Glucose (mg/dl)	126	86.18 ± 9.9	77	85.94 ± 11.46	11	87.14 ± 11.72	0.87	0.76	0.75
Glucose 120 (mg/dl)	41	95.34 ± 21.44	21	95.19 ± 12.58	2	83 ± 2.83	0.98	0.43	0.19
Insulin (uUI/ml)	117	14.11 ± 12.52	75	16.52 ± 12.11	9	10.4 ± 5.51	0.06	0.42	0.13
Insulin 120 (uUI/ml)	27	37.89 ± 29.84	18	44.04 ± 40.88	2	11 ± 5.66	0.61	0.11	**0.04**
HOMA-IR	52	1.78 ± 1.35	33	2.03 ± 1.39	4	1.44 ± 0.82	0.4	0.74	0.39
QUICKI	46	0.36 ± 0.07	31	0.35 ± 0.06	3	0.39 ± 0.04	0.82	0.23	0.16

VARIABLES	Overweight and obese								
	After								
	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	59	2.73 ± 1.3	45	2.56 ± 0.77	5	2.38 ± 0.53	0.85	0.57	0.66
AC (cm)	34	96.72 ± 13.01	21	93.19 ± 13.28	3	90.6 ± 9.87	0.32	0.52	0.97
WC (cm)	29	91.57 ± 9.66	26	94.42 ± 13.07	1	90.53 ± 0	0.36	0.92	0.77
% BF	42	37.15 ± 7.89	29	35.43 ± 8.42	3	30.67 ± 5.78	0.38	0.17	0.35
FM (kg)	30	28.77 ± 10.51	15	23.09 ± 11.19	3	19.73 ± 3.16	0.07	0.14	0.91
% LBM	9	56.96 ± 9.84	4	62.35 ± 7.8			0.25		
LBM (kg)	30	47.09 ± 14.03	15	43.04 ± 8.35	3	48.67 ± 23.46	0.31	0.86	0.45
TL (mg/dl)	15	558.59 ± 129.24	11	550.08 ± 128.12	2	484.3 ± 173.52		0.71	0.77
TC (mg/dl)	61	157.77 ± 38.07	46	158.76 ± 31.54	4	123.75 ± 24.77	0.83	**0.04**	**0.03**
VLDL-C (mg/dl)	15	19.69 ± 8.68	11	22.66 ± 10.06	2	24.65 ± 15.63	0.43	0.49	0.81
HDL-C (mg/dl)	60	50.6 ± 17.75	46	45.56 ± 9.67	4	37.35 ± 7.02	0.19	0.06	0.09
LDL-C (mg/dl)	61	90.14 ± 28.74	46	90.44 ± 29.83	4	64.83 ± 10.94	0.78	0.06	**0.04**
TG (mg/dl)	61	100.07 ± 48.36	46	110.73 ± 62.31	4	102.9 ± 52.82	0.39	0.82	0.73
Glucose (mg/dl)	60	85.02 ± 7.26	45	82.82 ± 7.15	4	79.3 ± 7.08	0.12	0.13	0.35
Glucose 120 (mg/dl)	21	89.55 ± 16.08	13	91.69 ± 9.98	1	94 ± 0	0.67	0.79	0.83
Insulin (uUI/ml)	56	12.1 ± 7.59	43	15.99 ± 9.32	3	6.6 ± 3.08	**0.01**	0.1	0.05
Insulin 120 (uUI/ml)	21	31.91 ± 20.21	13	26.87 ± 14.01			0.57		
HOMA-IR	30	1.55 ± 1.44	18	1.95 ± 1.28	2	0.83 ± 0.38	0.17	0.28	0.15
QUICKI	25	0.36 ± 0.04	17	0.35 ± 0.04	1	0.42 ± 0	0.35	0.1	0.12

VARIABLES	Normal weight								
	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score (kg)/(m2)	87	-0.22 ± 0.83	63	-0.25 ± 0.9	5	0.03 ± 0.8	0.91	0.49	0.54
AC (cm)	66	68.26 ± 7.23	47	67.17 ± 6.52	3	67.9 ± 1.91	0.6	0.69	0.84
WC (cm)	31	68.26 ± 6.54	24	66.33 ± 4.64	2	63 ± 1.13	0.29	0.21	0.27
TL (mg/dl)	16	518.89 ± 74.52	9	487.34 ± 42.42			0.18		
TC (mg/dl)	46	162.25 ± 31.02	28	148.47	2	148 ± 57.98	**0.04**	0.74	0.93
VLDL-C (mg/dl)	35	18.67 ± 7.64	20	14.84 ± 4.58			**0.04**		
HDL-C (mg/dl)	81	46.55 ± 11.68	58	44.15 ± 7.67	5	46.54 ± 11.54	0.22	0.98	0.62
LDL-C (mg/dl)	46	95.9 ± 28.68	28	86.92 ± 25.49	2	83.94 ± 39.51	0.13	0.7	0.97
TG (mg/dl)	81	79.04 ± 35.21	57	70.31 ± 27.95	5	72.92 ± 10.45	0.21	0.99	0.7
Glucose (mg/dl)	85	91.77 ± 11.91	62	92.07 ± 12.06	5	100.86 ± 12.34	0.88	0.1	0.12
Insulin (uUI/ml)	81	5.79 ± 3.73	58	5.28 ± 3.85	5	6.6 ± 6.55	0.89	0.77	
HOMA-IR	61	1.17 ± 0.76	47	1.12 ± 0.88	4	1.6 ± 1.74	0.59	0.84	0.85
QUICKI	38	0.41 ± 0.07	30	0.42 ± 0.1	3	0.44 ± 0.1	0.92	0.56	0.51

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p: GG vs AG; p*: GG vs AA; p**: AG vs AA. Variables that did not have enough data for analysis were not presented.

Table 6. Comparisons of means variations (initial – final) of variables between overweight/obese children and adolescents stratified according to a co-dominance model for rs1800977 (*ABCA1*).

Variables	TT		CT		CC		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score variation	21	-0.04 ± 0.35	57	0.12 ± 0.28	55	0.06 ± 0.46	**0.04**	**0.04**	0.94
AC variation	8	0.14 ± 5.34	38	0.35 ± 5.33	35	1.38 ± 5.83	0.36	0.28	0.96
WC variation	12	-1.5 ± 7.02	20	1.11 ± 2.78	22	0.83 ± 3.43	0.06	0.16	0.75
% BF variation	10	4.18 ± 5.53	43	3.19 ± 4.7	37	3.58 ± 5.21	0.73	0.62	0.67
FM variation	7	0.43 ± 4.38	30	1.65 ± 3.16	29	2.94 ± 7.38	0.47	0.34	0.5
% LBM variation			4	6.83 ± 14.29	6	-1.32 ± 2.48			0.29
LBM variation	7	-4.41 ± 2.63	30	-3.08 ± 6.04	29	-2.51 ± 4.87	0.06	0.2	0.6
TL variation	7	-15.4 ± 74.89	10	-6.39 ± 62.25	12	14.4 ± 56.71	0.79	0.34	0.42
TC variation	22	7.62 ± 22.98	57	5.89 ± 25.87	55	7.86 ± 21.18	0.78	0.97	0.66
VLDL-C variation	7	-3.42 ± 5.99	10	-0.21 ± 8.97	12	0.33 ± 7.11	0.42	0.26	0.88
HDL-C variation	21	5.96 ± 9.67	57	2.54 ± 11.66	55	0.88 ± 10.6	0.32	0.09	0.36
LDL-C variation	22	1.23 ± 25.85	57	5.43 ± 20.98	55	5.76 ± 22.16	0.46	0.44	0.94
TG variation	21	-1.14 ± 42.3	57	-9.29 ± 58.06	55	5.99 ± 38.14	0.91	0.25	0.16
Glucose variation	22	1.96 ± 8.52	57	0.47 ± 8.2	54	4.04 ± 8	0.48	0.32	**0.02**
Glucose 120 variation	5	-3.2 ± 15.04	15	3.63 ± 15.49	19	6.05 ± 17.38	0.41	0.24	0.97
Insulin variation	17	0.37 ± 4.27	42	2.02 ± 6.2	47	4.98 ± 11.32	0.58	0.15	0.36
Insulin 120 variation	2	-0.25 ± 4.03	14	6.05 ± 16.53	15	6.73 ± 12.21	0.58	0.41	0.88
HOMA-IR variation	5	-0.05 ± 0.82	22	0.33 ± 0.74	26	0.6 ± 1.28	0.19	0.26	0.58
QUICKI variation	5	0.02 ± 0.03	19	-0.01 ± 0.02	23	-0.03 ± 0.03	**0.02**	**0.02**	0.28

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p:TT vs CT; p*: TT vs CC; p**: CT vs CC. Variables that did not have enough data for analysis were not presented.

Table 7. Comparisons of means variations (initial – final) of variables between overweight/obese children and adolescents stratified according to a co-dominance model for rs2230806 (*ABCA1*).

Variables	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score variation	68	0.15 ± 0.36	64	0.1 ± 0.51	22	0.34 ± 0.38	0.53	0.07	**0.04**
AC variation	44	1.91 ± 6.11	42	1.52 ± 5.85	20	0.49 ± 3.75	0.88	0.36	0.42
WC variation	22	0.35 ± 5.92	23	0.51 ± 3.08	5	0.2 ± 2.4	0.55	0.57	
% BF variation	46	4.12 ± 4.96	44	2.54 ± 4.96	21	3.83 ± 2.43	0.21	0.63	0.07
FM variation	32	2.1 ± 3.66	40	1.99 ± 6.3	17	3.04 ± 2.47	0.61	0.22	0.09
% LBM variation	3	-1.53 ± 2.97	5	-1.12 ± 2.87	3	8.8 ± 16.65	0.88	0.38	0.55
LBM variation	32	-2.25 ± 4.42	40	-1.94 ± 3.2	17	-4.57 ± 7.36	0.68	0.26	0.26
TL variation	13	-1.67 ± 67.22	15	5 ± 60.41			0.78		
TC variation	68	8.29 ± 22.32	64	2.71 ± 23.58	23	8.57 ± 22.13	0.16	0.96	0.3
VLDL-C variation	13	-2.24 ± 5.64	15	0.56 ± 8.97			0.34		
HDL-C variation	68	1.87 ± 10.15	63	0.13 ± 12.31	23	-0.28 ± 8.98	0.4	0.39	0.99
LDL-C variation	68	5.04 ± 20.84	64	1.34 ± 21.35	23	9.07 ± 22.91	0.32	0.44	0.15
TG variation	66	7.64 ± 49.95	64	8.01 ± 48.52	23	-9.7 ± 60.57	0.71	0.2	0.24
Glucose variation	70	2.08 ± 8.7	64	1.2 ± 7.63	21	1.57 ± 9.29	0.54	0.82	0.86
Glucose 120 variation	28	4.29 ± 15.7	23	5.72 ± 13.1	11	14.82 ± 20.81	0.98	0.2	0.26
Insulin variation	58	1.72 ± 5.54	52	4.6 ± 10.69	16	2.89 ± 7.24	0.33	0.66	0.78
Insulin 120 variation	25	13.47 ± 19.28	19	9.09 ± 19.28	9	23.26 ± 31.7	0.71	0.63	0.55
HOMA-IR variation	34	0.23 ± 0.8	28	0.59 ± 1.19	14	0.4 ± 0.8	0.35	0.72	0.62
QUICKI variation	33	-0.01 ± 0.03	24	-0.01 ± 0.03	13	-0.03 ± 0.03	0.88	0.25	0.22

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p: GG vs AG; p*: GG vs AA; p**: AG vs AA.

Table 8. Comparisons of means variations (initial – final) of variables between overweight/obese children and adolescents stratified according to a co-dominance model for rs2279796 (*ABCA7*).

Variables	CC		CT		TT		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score variation	31	0.04 ± 0.36	58	0.12 ± 0.28	32	0.1 ± 0.23	0.61	0.88	0.54
AC variation	13	0.25 ± 4.86	19	0.78 ± 4.53	19	1.28 ± 5.74	0.52	0.55	0.77
WC variation	20	0.74 ± 5.08	21	1.15 ± 3.37	12	1.16 ± 4.51	0.97	0.37	0.37
% BF variation	15	4.73 ± 5.04	45	3.69 ± 4.55	21	2.22 ± 4.13	0.23	0.2	0.49
FM variation	7	2.95 ± 3.69	34	1.71 ± 4.61	15	1.03 ± 2.23	0.53	0.29	0.36
% LBM variation			7	2.63 ± 11.44	2	1.3 ± 0.85			0.38
LBM variation	7	-4.02 ± 3.91	34	-3.4 ± 6.4	15	-1.83 ± 2.24	0.38	0.16	0.69
TL variation	10	13.38 ± 65.29	8	8.96 ± 59.39	10	-15.21 ± 64.92	0.88	0.34	0.43
TC variation	33	7.79 ± 27.65	58	5.23 ± 18.32	30	6.68 ± 20.77	0.6	0.86	0.74
VLDL-C variation	10	2.42 ± 8.54	8	-1.11 ± 7.48	10	-3.6 ± 6.07	0.37	0.09	0.45
HDL-C variation	33	1.38 ± 12.26	57	0.76 ± 9.63	30	5 ± 12.78	0.82	0.53	0.27
LDL-C variation	33	5.08 ± 25.3	58	5.24 ± 19.33	30	0.73 ± 19.21	0.97	0.45	0.3
TG variation	33	4.13 ± 46.91	58	-2.51 ± 53.22	30	4.49 ± 41.03	0.94	0.91	0.78
Glucose variation	34	2.01 ± 9.94	57	1.74 ± 7.51	30	2.42 ± 9.36	0.88	0.86	0.71
Glucose 120 variation	9	3.39 ± 22.68	24	4.58 ± 12.98	8	4.88 ± 10.7	0.63	0.6	0.7
Insulin variation	33	2.85 ± 7.31	50	4.57 ± 10.42	25	0.32 ± 5.53	0.69	0.29	0.18
Insulin 120 variation	7	5.27 ± 15.66	19	6.33 ± 14.49	7	4.29 ± 10.23	0.39		0.73
HOMA-IR variation	12	0.15 ± 0.43	29	0.6 ± 1.25	14	0.15 ± 0.93	0.16	0.57	0.46
QUICKI variation	12	-0.01 ± 0.02	25	- 0.02 ± 0.04	12	-0.006 ± 0.04	0.47		0.43

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p: CC vs TC; p*: CC vs TT; p**: TC vs TT.

Table 9. Comparisons of means variations (initial – final) of variables between overweight/obese children and adolescents stratified according to a co-dominance model for rs692383 (*ABCG1*).

Variables	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score variation	13	0.03 ± 0.25	52	0.04 ± 0.3	38	0.08 ± 0.24	0.7	0.86	0.42
AC variation	7	-0.2 ± 6.66	30	0.26 ± 4.25	14	1.16 ± 4.57	0.42	0.41	0.85
WC variation	7	1.51 ± 4.64	25	-0.06 ± 4.44	21	0.81 ± 4.31	0.7	0.67	0.29
% BF variation	8	3.51 ± 3.68	21	3.69 ± 4.8	21	3.72 ± 5.54	0.96	0.61	0.71
FM variation	4	0.35 ± 4.37	25	0.61 ± 3.73	10	-0.43 ± 5.63	0.7	0.4	0.15
% LBM variation	2	-2.5 ± 1.13	7	3.71 ± 11.01			0.19		
LBM variation	4	-1.28 ± 1.21	25	-4.65 ± 6.96	10	-2.43 ± 2.93	0.19	0.29	0.57
TL variation	3	3.1 ± 30.5	10	-18.1 ± 30.5	15	15 ± 74.96	0.47	0.79	0.22
TC variation	14	7.9 ± 26.34	52	6.1 ± 19.3	36	3.86 ± 24.26	0.78	0.61	0.63
VLDL-C variation	3	4.58 ± 10.69	10	-1.92 ± 6.29	15	-1.02 ± 7.92	0.2	0.3	0.77
HDL-C variation	14	-1.64 ± 10.8	52	3 ± 10.62	35	2.57 ± 13.85	0.31	0.32	0.9
LDL-C variation	14	7.3 ± 23.31	52	3.51 ± 19.94	36	3.82 ± 23.69	0.54	0.64	0.95
TG variation	14	13.77 ± 27.43	52	-1.76 ± 35.98	36	-11.93 ± 64.79	0.27	0.13	0.53
Glucose variation	13	3.75 ± 9.86	53	0.91 ± 8.99	35	1.82 ± 8.36	0.32	0.5	0.63
Glucose 120 variation	5	9.4 ± 19.11	16	4.06 ± 16.43	10	2.55 ± 17.67	0.71	0.58	0.58
Insulin variation	13	1.03 ± 6.57	48	3.56 ± 9.21	35	4.11 ± 9.12	0.22	0.21	0.8
Insulin 120 variation	3	17.2 ± 22.33	13	6.08 ± 14.4	8	-1.16 ± 5.82	0.5	0.13	0.12
HOMA-IR variation	7	0.27 ± 0.86	24	0.57 ± 1.29	13	0.46 ± 0.86	0.64	0.72	0.73
QUICKI variation	7	-0.005 ± 0.04	20	-0.02 ± 0.03	11	-0.02 ± 0.03	0.42	0.79	0.35

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p: GG vs AG; p*: GG vs AA; p**: AG vs AA.

Table 10. Comparisons of means variations (initial – final) of variables between overweight/obese children and adolescents stratified according to a co-dominance model for rs3827225 (*ABCG1*).

Variables	GG		AG		AA		p	p*	p**
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD			
BMI Z-score variation	59	0.04 ± 0.32	45	0.08 ± 0.2	5	0.08 ± 0.16	0.38	0.66	
AC variation	33	0.78 ± 4.04	21	0.76 ± 5.43	3	0 ± 2.61	0.66	0.59	0.93
WC variation	27	1.54 ± 4.2	26	-0.23 ± 4.14			0.09		
% BF variation	39	4.2 ± 4.83	27	1.7 ± 4.23	3	8 ± 4.86	0.12	0.15	0.06
FM variation	27	1.15 ± 4.99	14	1.05 ± 1.87	3	5.93 ± 5.16	0.92	0.13	0.05
% LBM variation	7	2.86 ± 11.37	3	-0.2 ± 1.85			0.91		
LBM variation	27	-3.56 ± 6.47	27	-2.17 ± 2.94	3	-7.33 ± 8.16	0.51	0.35	0.23
TL variation	15	-10.86 ± 58.05	11	13.57 ± 72.23	2	33.46 ± 18.7	0.35	0.31	0.72
TC variation	60	2.19 ± 20.81	46	10.23 ± 22.26	4	9.45 ± 6.13	0.06	0.49	0.95
VLDL-C variation	15	-1.15 ± 8.87	11	-0.81 ± 6.56	2	2.7 ± 2.07	0.92	0.56	0.48
HDL-C variation	59	0.87 ± 13.03	46	3.24 ± 10.06	4	5.28 ± 5.99	0.32	0.37	0.64
LDL-C variation	60	1.88 ± 22.51	46	8.04 ± 19.77	4	4.98 ± 2.79	0.14	0.79	0.76
TG variation	60	-3.68 ± 47.81	46	-3.07 ± 48.86	4	1.47 ± 16.27	0.66	0.82	0.9
Glucose variation	60	1.44 ± 8	45	1.88 ± 10.10	4	5.2 ± 7.16	0.8	0.36	0.53
Glucose 120 variation	21	5.4 ± 18.25	13	4.69 ± 15.88			0.86		
Insulin variation	56	3.93 ± 10.43	43	2.53 ± 6.02	3	5.53 ± 4.34	0.9	0.33	0.23
Insulin 120 variation	15	10.16 ± 24.73	11	1.6 ± 5.77			0.24		
HOMA-IR variation	29	0.49 ± 1.19	18	0.4 ± 0.82	2	0.76 ± 0.91	0.79	0.52	0.41
QUICKI variation	25	-0.02 ± 0.04	17	-0.01 ± 0.02			0.33		

BMI: Body Mass Index; AC: Abdominal Circumference; WC: Waist Circumference; % BF: Body Fat Percentage; FM: Fat Mass; % LBM: Lean Body Mass Percentage; LBM: Lean Body Mass; TL: Total lipids; TC: Total Cholesterol; VLDL-C: Cholesterol of Very Low Density Lipoprotein; HDL-C: Cholesterol of High Density Lipoprotein; LDL-C: Cholesterol of Low Density Lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; QUICKI: Quantitative Insulin Sensitivity Check Index; p: GG vs AG; p*: GG vs AA; p**: AG vs AA.

6 DISCUSSÃO GERAL

Foram analisados SNPs em genes envolvidos com obesidade e dislipidemias, visto que essas doenças possuem uma grande prevalência nos dias de hoje, especialmente entre crianças e adolescentes. A interação entre esses polimorfismos e a prática controlada de exercícios físicos também foi considerada nesse trabalho, visto que se sabe que o mesmo programa de exercícios físicos apresenta variação quanto ao seu efeito, em partes devido à composição genética individualizada.

Variantes alélicas nos genes *FTO*, *ABCA7* e *ABCG1* não influenciaram na resposta a exercícios físicos. Entretanto, os SNPs rs1800977 e rs2230806 do gene *ABCA1* afetaram a resposta aos exercícios, sendo que o SNP rs1800977 foi associado à maior redução do IMC escore-Z e maior aumento de QUICKI e o SNP rs2230806 foi associado à maior ganho de MM.

Os SNPs dos genes analisados também influenciaram as variáveis na análise transversal. Com relação ao gene *FTO,* o alelo A do SNP rs9939609 foi associado ao aumento de HOMA-IR e insulina e redução de QUICKI em crianças e adolescentes obesos, o que sugere que pode estar envolvido no metabolismo da glicose. O SNP rs1800977 do gene *ABCA1* foi associado à maior IMC escore-Z, CA, GC e insulina 120 e redução de QUICKI, demonstrando uma possível influencia na adiposidade e no metabolismo da glicose. O SNP rs2230806 do gene *ABCA1* foi associado à maior IMC escore-Z e CA e menor %MM, o que sugere que pode afetar a adiposidade. O SNP rs2279796 do gene *ABCA7* foi associado ao aumento no IMC escore-Z, sugerindo que também pode estar envolvido com o ganho de peso. O SNP rs692383 do gene *ABCG1* foi associado à maior IMC escore-Z, CA, HDL-C, glicose, insulina e HOMA-IR, o que aponta uma possível influência na adiposidade, metabolismo de lipídeos e da glicose. Por fim, o SNP rs3827225 do gene *ABCG1* foi associado ao aumento de VLDL-C e glicose, indicando sua possível relação com o metabolismo lipídico e da glicose.

Alguns resultados encontrados por outros pesquisadores não foram replicados nesse estudo, como a associação do alelo A do SNP rs9939609 do gene *FTO* com obesidade (FRAYLING *et al*., 2007)*,* o aumento dos níveis de HDL-C causado pelo alelo T do SNP rs1800977 do gene *ABCA1* (PORCHAY *et*

al., 2006), e o aumento dos níveis de HDL-C em indivíduos magros e redução dos níveis dessa lipoproteína em obesos gerados pelo alelo A do SNP rs2230806 do gene *ABCA1* (PORCHAY *et al*., 2006). Algumas das possíveis razões para esses resultados discrepantes seriam a miscigenação da população brasileira, uma vez que outros polimorfismos específicos presentes no background genético das diferentes populações podem influenciar de modo diferente a relação genótipo/fenótipo de variantes específicas, e relativo número amostral reduzido, o que pode ter contribuído para a não detecção de possíveis efeitos. Além disso, a amostra é constituída por crianças e adolescentes, que possuem um metabolismo mais acelerado.

A maioria das associações nesse trabalho foi observada no grupo de indivíduos com excesso de peso ou obesidade e não no grupo eutrófico, o que pode ter ocorrido devido a uma influência do estado metabólico. A condição de obesidade gera um desequilíbrio metabólico, que pode modular a relação genótipo/fenótipo.

Com relação aos SNPs dos genes *ABCA7* e *ABCG1*, esse é um dos poucos estudos que analisa o efeito desses SNPs em variáveis antropométricas e bioquímicas em humanos, além de verificar a interação desses genótipos com exercício físico.

Nesse trabalho nós verificamos os efeitos dos polimorfismos analisados em variáveis relacionadas ao metabolismo (adiposidade, metabolismo da glicose e de lipídeos). Esses efeitos podem influenciar a resposta dos indivíduos a tratamentos ou a exercícios físicos, visto que cada paciente responde de uma maneira diferente de acordo com sua composição genética. Dessa maneira, estudos desse tipo podem ajudar na determinação de tratamentos mais individualizados no futuro.

7 CONCLUSÕES

1. O alelo A do SNP rs9939609 do gene *FTO* foi associado a um aumento dos valores de HOMA-IR e insulina e uma redução de QUICKI em crianças e adolescentes obesos.

2. O SNP rs1800977 (alelo C) do gene *ABCA1* foi associado a aumento de IMC escore-Z, CA, GC e insulina 120 e redução de QUICKI.

3. O SNP rs2230806 (alelo A) do gene *ABCA1* foi associado a aumento de IMC escore-Z e CA e redução de %MM.

4. O SNP rs2279796 (alelo C) do gene *ABCA7* foi associado a aumento de IMC escore-Z.

5. O SNP rs692383 do gene *ABCG1* foi associado a aumento de IMC escore-Z, CA, HDL-C, glicose, insulina e HOMA-IR.

6. O SNP rs3827225 (alelo G) do gene *ABCG1* foi associado a aumento de VLDL-C e glicose.

7. Variantes alélicas dos genes *FTO, ABCA7* e *ABCG1* não apresentaram interação com exercícios físicos.

8. Os SNPs rs1800977 e rs2230806 do gene *ABCA1* alteraram a resposta aos exercícios físicos, sendo que o SNP rs1800977 foi associado à maior redução de IMC escore-Z e maior aumento de QUICKI e o SNP rs2230806 foi associado à maior ganho de MM.

9. De forma geral, foi possível verificar o efeito dos SNPs investigados em variáveis relacionadas ao metabolismo (adiposidade, metabolismo da glicose e metabolismo lipídico).

REFERÊNCIAS

AADAHL, M.; KJÆR, M.; JØRGENSEN, T. Associations between overall physical activity level and cardiovascular risk factors in an adult population. **European Journal of Epidemiology**, v. 22, n. 6, p. 369–378, 2007.

ABDEL-AZIZ, E. A. *et al*. Health related quality of life and psychological problems in Egyptian children with simple obesity in relation to body mass index. **Egyptian Journal of Medical Human Genetics**, v. 15, n. 2, p. 149–154, 2014.

BAIRDAIN, S. *et al*. A Single Institution's Overweight Pediatric Population and Their Associated Comorbid Conditions. **ISRN obesity**, v. 2014, p. 517694, 2014.

BERULAVA, T.; HORSTHEMKE, B. The obesity-associated SNPs in intron 1 of the FTO gene affect primary transcript levels. **European Journal of Human Genetics**, v. 18, n. 9, p. 1054–1056, 2010.

BIENERTOVÁ-VASKŮ, J. *et al*. Genotype x nutrient association of common polymorphisms in obesity-related genes with food preferences and time structure of energy intake. **The British journal of nutrition**, v. 103, n. 3, p. 352–9, 2010.

BRUCE, C. **Anthropometric Indicators Measurement Guide.** Food and Nutrition Technical Assistance Project. Academy for Educational Development, Washington, D.C., 2003.

BULBUL, T.; HOQUE, M. Prevalence of childhood obesity and overweight in Bangladesh: findings from a countrywide epidemiological study. **BMC pediatrics**, v. 14, n. 1, p. 86, 2014.

CALABRESI, L.; FRANCESCHINI, G. Lecithin: Cholesterol acyltransferase, high-density lipoproteins, and atheroprotection in humans. **Trends in Cardiovascular Medicine**, v. 20, n. 2, p. 50–53, 2010.

CAVELIER, C. *et al*. Lipid efflux by the ATP-binding cassette transporters ABCA1 and ABCG1. **Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids**, v. 1761, n. 7, p. 655–666, 2006.

CHO, Y. S. *et al*. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. **Nature genetics**, v. 41, n. 5, p. 527–534, 2009.

CHURCH, C. *et al*. Overexpression of Fto leads to increased food intake and results in obesity. **Nature genetics**, v. 42, n. 12, p. 1086–1092, 2010.

CLEE, S. M. *et al*. Common genetic variation in ABCA1 is associated with altered lipoprotein levels and a modified risk for coronary artery disease.

Circulation, v. 103, n. 9, p. 1198–1205, 2001.

DA SILVA, C. F. *et al*. Association between a frequent variant of the FTO gene and anthropometric phenotypes in Brazilian children. **BMC medical genetics**, v. 14, n. 1, p. 34, 2013.

DÂMASO, A. R. et al. Aerobic plus resistance training was more effective in improving the visceral adiposity, metabolic profile and inflammatory markers than aerobic training in obese adolescents. **Journal of sports sciences**, v. 32, n. June, p. 1–11, 2014.

DI ANGELANTONIO, E. *et al*. Major Lipids , Apolipoproteins , and Risk of Vascular Disease. **Jama**, v. 302, n. 18, p. 1993–2000, 2012.

FAWCETT, K. A.; BARROSO, I. The genetics of obesity: FTO leads the way. **Trends in Genetics**, v. 26, n. 6, p. 266–274, 2010.

FERGUSON, M. A et al. Effects of four different single exercise sessions on lipids, lipoproteins, and lipoprotein lipase. **Journal of applied physiology**, v. 85, n. 3, p. 1169–1174, 1998.

FISCHER, J. *et al*. Inactivation of the Fto gene protects from obesity. **Nature**, v. 458, n. 7240, p. 894–898, 2009.

FRAYLING, T. M. *et al*. A Common Variant in the FTO Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. **Science,** v. 316, p. 889–895, 2007.

FRIEDEWALD, W. T.; LEVY, R. I.; FREDRICKSON, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. **Clinical Chemistry**, v. 18, n. 6, p. 499–502, 1972.

GARCÍA-HERMOSO, A. et al. Endocrinology and adolescence: Aerobic exercise reduces insulin resistance markers in obese youth: A meta-analysis of randomized controlled trials. **European Journal of Endocrinology**, v. 171, n. 4, p. R163–R171, 2014.

GARCÍA-HERMOSO, A. et al. Is high-intensity interval training more effective on improving cardiometabolic risk and aerobic capacity than other forms of exercise in overweight and obese youth? A meta-analysis. **Obesity Reviews**, v. 17, n. 6, p. 531–540, 2016.

GERKEN, T. *et al*. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. **Science (New York, N.Y.)**, v. 318, n. 5855, p. 1469–1472, 2007.

GIBALA, M. J. et al. Physiological adaptations to low-volume , high-intensity interval training in health and disease. **Journal of Physiology**, v. 5, n. March 2012, p. 1077–1084, 2012.

GIULIANO, I. C. B. *et al*. I Diretriz de Prevenção da Aterosclerose na Infância e na Adolescência. **Arquivos Brasileiros de Cardiologia**, v. 85, n. 6, 2005.

GORDON, B.; CHEN, S.; DURSTINE, J. L. The effects of exercise training on the traditional lipid profile and beyond. **Current sports medicine reports**, v. 13, n. 4, p. 253–9, 2014.

GRANT, S. F. A *et al*. Association analysis of the FTO gene with obesity in children of Caucasian and African ancestry reveals a common tagging SNP. **PLoS ONE**, v. 3, n. 3, p. 1–6, 2008.

HARDY, R. *et al*. Life course variations in the associations between FTO and MC4R gene variants and body size. **Human Molecular Genetics**, v. 19, n. 3, p. 545–552, 2009.

IIDA, A. *et al*. Catalog of 605 single-nucleotide polymorphisms (SNPs) among 13 genes encoding human ATP-binding cassette transporters: ABCA4, ABCA7, ABCA8, ABCD1, ABCD3, ABCD4, ABCE1, ABCF1, ABCG1, ABCG2, ABCG4, ABCG5, and ABCG8. **Journal of Human Genetics**, v. 47, n. 6, p. 285–310, 2002.

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE). Pesquisa de Orçamentos Familiares 2008-2009. Antropometria e estado nutricional de crianças, adolescentes e adultos no Brasil. 2010. Disponível em: < http://www.ibge.gov.br/home/estatistica/populacao/condicaodevida/pof/2008_2009/POFpublicacao.pdf>. Acesso em: 12/06/2015.

JAHANGIR, E.; SCHUTTER, A. DE; LAVIE, C. J. The relationship between obesity and coronary artery disease. **Translational research : the journal of laboratory and clinical medicine**, p. 1–9, 2014.

JIA, G. *et al*. Oxidative demethylation of 3-methylthymine and 3-methyluracil in single-stranded DNA and RNA by mouse and human FTO. **FEBS Letters**, v. 582, n. 23-24, p. 3313–3319, 2008.

JIA, G. *et al*. N6-Methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. **Nature Chemical Biology**, v. 7, n. 12, p. 885–887, 2011.

JORGE, M. L. M. P. et al. The effects of aerobic, resistance, and combined exercise on metabolic control, inflammatory markers, adipocytokines, and muscle insulin signaling in patients with type 2 diabetes mellitus. **Metabolism: Clinical and Experimental**, v. 60, n. 9, p. 1244–1252, 2011.

JUNG, U. J.; CHOI, M.-S. Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease. **International journal of molecular sciences**, v. 15, n. 4, p. 6184–223, 2014.

KAMINSKI, W. E. *et al*. Identification of a novel human sterol-sensitive ATP-binding cassette transporter (ABCA7). **Biochemical and biophysical research communications**, v. 273, n. 2, p. 532–538, 2000.

KAMINSKI, W. E.; PIEHLER, A.; WENZEL, J. J. ABC A-subfamily transporters: Structure, function and disease. **Biochimica et Biophysica Acta - Molecular Basis of Disease**, v. 1762, n. 5, p. 510–524, 2006.

KANG, H.-S. et al. Physical training improves insulin resistance syndrome markers in obese adolescents. **Medicine and science in sports and exercise**, v. 34, n. 30, p. 1920–1927, 2002.

KARRA, E. *et al*. A link between FTO, ghrelin, and impaired brain food-cue responsivity Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases. **The Journal of Clinical Investigation**, v. 123, n. 8, p. 3539–3551, 2013.

KATZ, A. et al. Quantitative insulin sensitivity check index: A simple, accurate method for assessing insulin sensitivity in humans. **Journal of Clinical Endocrinology and Metabolism**, v. 85, n. 7, p. 2402–2410, 2000.

KELLEY, G. A.; KELLEY, K. S. Impact of progressive resistance training on lipids and lipoproteins in adults: A meta-analysis of randomized controlled trials. **Preventive Medicine**, v. 48, n. 1, p. 9–19, 2009.

KELLEY, G. A.; KELLEY, K. S.; TRAN, Z. V. Aerobic exercise, lipids and lipoproteins in overweight and obese adults: a meta-analysis of randomized controlled trials. **Int J Obes (Lond)**, v. 29, n. 8, p. 881–893, 2005.

KOBAYASHI, A. *et al*. Efflux of sphingomyelin, cholesterol, and phosphatidylcholine by ABCG1. **Journal of Lipid research,** v. 47, 2006.

KRAUS, W. E. et al. Effects of the amount and intensity of exercise on plasma lipoproteins. **The New England journal of medicine**, v. 347, n. 19, p. 1483–1492, 2002.

LAHIRI, D.K., NURNBERGER JR., J.I. A rapid non-enzymaticmethod for the reparation of HMW DNA from blood for RFLP studies. **Nucleic Acids Res**., v. 19, p. 5444, 1991.

LARDER, R. *et al*. Where to go with FTO? **Trends in endocrinology and metabolism: TEM**, v. 22, n. 2, p. 53–59, 2011.

LEE, H.-J. *et al*. Effects of common FTO gene variants associated with BMI on dietary intake and physical activity in Koreans. **Clinica chimica acta,** 411, n. 21-22, p. 1716–1722, 2010.

LEITE, N. *et al*. Associação entre o perfil lipídico e medidas antropométricas indicadoras de adiposidade em adolescentes. **Rev Bras Cineantropom Desempenho Hum**, v. 11, n. 2, p.127-133, 2009.

LEITE, N. *et al*. Efeitos de exercícios aquáticos e orientação nutricional na composição corporal de crianças e adolescentes obesos. **Revista Brasileira de Cineantropometria e Desempenho Humano**, v. 12, n. 4, p. 232–238, 2010.

LEOŃSKA-DUNIEC, A.; AHMETOV, I.; ZMIJEWSKI, P. Genetic variants influencing effectiveness of exercise training programmes in obesity – an overview of human studies. **Biology of Sport**, v. 33, n. 3, p. 207–214, 2016.

LEWIS, G. F.; RADER, D. J. New insights into the regulation of HDL metabolism and reverse cholesterol transport. **Circulation Research**, v. 96, n. 12, p. 1221–1232, 2005.

LIMA, E. S.; COUTO, R. D. Estrutura, metabolismo e funções fisiológicas da lipoproteína de alta densidade. **Jornal Brasileiro de Patologia e Medicina Laboratorial**, v. 42, n. 3, p. 169–178, 2006.

LOOS, R. J. F.; BOUCHARD, C. FTO: The first gene contributing to common forms of human obesity. **Obesity Reviews**, v. 9, n. 3, p. 246–250, 2008.

LOPES, W. A. et al. Effects of 12 weeks of combined training without caloric restriction on inflammatory markers in overweight girls. **Journal of Sports Sciences**, v. 34, n. 0, p. 1902–12, 2016.

LOURENÇO, B. H. *et al*. FTO genotype, vitamin D status, and weight gain during childhood. **Diabetes**, v. 63, n. 2, p. 808–814, 2014.

MA, X. Y.; LIU, J. P.; SONG, Z. Y. Associations of the ATP-binding cassette transporter A1 R219K polymorphism with HDL-C level and coronary artery disease risk: A meta-analysis. **Atherosclerosis**, v. 215, n. 2, 2011.

MANN, S.; BEEDIE, C.; JIMENEZ, A. Differential effects of aerobic exercise, resistance training and combined exercise modalities on cholesterol and the lipid profile: review, synthesis and recommendations. **Sports Medicine**, v. 44, n. 2, p. 211–221, 2014.

MATTHEWS, D. R. et al. Homeostasis model assessment: IR and beta-cell function from fasting plasma glucose and insulin concentration in man. **Diabetologia**, v. 28, p. 412–9, 1985.

MEDEIROS *et al*. Cardiovascular risk assessment of dyslipidemic children: analysis of biomarkers to identify monogenic dyslipidemia. **J. Lipid Res**, v. 55, p. 947-955, 2014.

MERKESTEIN, M. *et al*. Changes in gene expression associated with FTO overexpression in mice. **PLoS ONE**, v. 9, n. 5, p. 1–11, 2014.

MIHALACHE, L. *et al*. Effects of ghrelin in energy balance and body weight homeostasis. **Hormones**, v. 15, n. 2, p. 186–196, 2016.

MILANO, G. E. **Consumo máximo de oxigênio em adolescentes obesos e não- obesos em esteira e bicicleta ergométrica no método convencional e alométrico**. 89f. Dissertação (Mestrado em Educação Física) – Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, 2008.

MILANO, G. E. *et al*. Atividade da butirilcolinesterase e fatores de risco cardiovascular em adolescentes obesos submetidos a um programa de exercícios físicos. **Arq Bras Endocrinol Metab**., v.57, n.7, 2013.

MINISTÉRIO DA SAÚDE. Vigitel Brasil 2015: Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico. 2016. Disponível em: <http://bvsms.saude.gov.br/bvs/publicacoes/vigitel_brasil_2015.pdf>. Acesso em: 11/02/2017.

MOKUNO, J. *et al*. ATP-binding cassette transporter A1 (ABCA1) R219K (G1051A, rs2230806) polymorphism and serum high- density lipoprotein cholesterol levels in a large Japanese population: cross-sectional data from the Daiko Study Junichiro. **Endocrine Journal,** v. 62, n. 6, p. 543–549, 2015.

MURRAY, R. K.; GRANNER, D. K.; RODWELL, V. W. **Harper, Bioquímica Ilustrada.** 27ª edição. Rio de Janeiro: McGraw-Hill International, 2007.

MUST, A; ANDERSON, S. E. Body mass index in children and adolescents: considerations for population-based applications. **International journal of obesity (2005)**, v. 30, n. 4, p. 590–594, 2006.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION (NCBI). Short genetic Variations (dbSNP). 2015. Disponível em: <http://www.ncbi.nlm.nih.gov/projects/SNP/>. Acesso em: 02/08/2015.

NELSON, D. L.; COX, M. M. **Princípios de Bioquímica de Lehninger.** 5ª edição. Porto Alegre: Artmed, 2011.

NETO, O. D. A. *et al*. Fatores associados à dislipidemia em crianças e adolescentes de escolas públicas de Salvador, Bahia. **Rev Bras Epidemiol**, v. 15, n. 2, p. 335–345, 2012.

PETERS, T.; AUSMEIER, K.; RÜTHER, U. Cloning of Fatso (Fto), a novel gene deleted by the fused toes (Ft) mouse mutation. **Mammalian Genome**, v. 10, n. 10, p. 983–986, 1999.

PIZZI, J. **Efeito do treinamento intervalado nos fatores de risco cardiovasculares e genéticos de crianças e adolescentes obesos.** Francisco Beltrão: UNIPAR, 2014. 18p. (APEC. Efeito do exercício físico intervalado nos fatores de risco cardiovasculares de crianças e adolescentes obesos). Projeto concluído.

PORCHAY, I. *et al*. ABCA1 single nucleotide polymorphisms on high-density lipoprotein-cholesterol and overweight: the D.E.S.I.R. study. **Obesity (Silver Spring, Md.)**, v. 14, n. 11, p. 1874–1879, 2006.

QUAZI, F.; MOLDAY, R. S. Lipid transport by mammalian ABC proteins. **Essays in Biochemistry**, v. 50, n. 1, p. 265–90, 2011.

RACIL, G. et al. Greater effects of high- compared with moderate-intensity interval training on cardio-metabolic variables , blood leptin concentration and ratings of perceived exertion in obese ado ... Greater effects of high- compared with moderate-intensity interval tr. **Biology of Sport**, v. 33, n. 2, p. 145–152, 2016.

RANG, H. P*. et al*. **Rang & Dale: Farmacologia**. 7ª edição. Rio de Janeiro: Elsevier, 2011.

REILLY, J. J. Childhood obesity: An overview. **Children and Society**, v. 21, n. 5, p. 390–396, 2007.

RIBAS, S. A.; SILVA, L. C. S. DA. Fatores de risco cardiovascular e fatores associados em escolares do Município de Belém, Pará, Brasil. **Cadernos de Saúde Pública**, v. 30, n. 3, p. 577–586, 2014.

RIDKER, P. M. LDL cholesterol: controversies and future therapeutic directions. **The Lancet**, v. 384, n. 9943, p. 607–617, ago. 2014.

RIEDL, I. et al. Regulation of skeletal muscle transcriptome in elderly men after 6weeks of endurance training at lactate threshold intensity. **Experimental Gerontology**, v. 45, n. 11, p. 896–903, 2010.

RIFFO, B. *et al*. FTO gene is related to obesity in Chilean Amerindian children and impairs HOMA-IR in prepubertal girls. **Pediatric Diabetes**, v. 13, n. 5, p. 392–399, 2012.

SCUTERI, A. *et al*. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. **PLoS Genetics**, v. 3, n. 7, p. 1200–1210, 2007.

SHAWKY, R. M.; SADIK, D. I. Genetics of obesity. **Egyptian Journal of Medical Human Genetics**, v. 13, n. 1, p. 11–17, 2012.

SHEHZAD, A. *et al*. Adiponectin: Regulation of its production and its role in human diseases. **Hormones-International Journal of Endocrinology and Metabolism**, v. 11, n. 1, p. 8–20, 2012.

SIGAL, R. J. et al. Effects of aerobic training, resistance training, or both on percentage body fat and cardiometabolic risk markers in obese adolescents: the healthy eating aerobic and resistance training in youth randomized clinical trial. **JAMA pediatrics**, v. 168, n. 11, p. 1006–14, 2014.

SINGARAJA, R. R. *et al*. Efflux and atherosclerosis: The clinical and biochemical impact of variations in the ABCA1 gene. **Arteriosclerosis, Thrombosis, and Vascular Biology**, v. 23, n. 8, p. 1322–1332, 2003.

SOCIEDADE BRASILEIRA DE CARDIOLOGIA. Consenso Brasileiro Sobre Dislipidemias. Detecção - Avaliação - Tratamento. **Arq Bras Cardiol**, v.67, p. 113–128, 1996.

SOVIO, U. *et al*. Association between common variation at the FTO locus and changes in body mass index from infancy to late childhood: The complex nature of genetic association through growth and development. **PLoS Genetics**, v. 7, n. 2, 2011.

STUNKARD, A. *et al*. The Body mass index of twins who have been reared apart. **The New England Journal of Medicine**, v.322, n.21, p. 1483-1487, 1990.

SUPERKO, H. R. et al. High-density lipoprotein subclasses and their relationship to cardiovascular disease. **Journal of Clinical Lipidology**, v. 6, n. 6, p. 496–523, 2012.

TARLING, E. J.; DE AGUIAR VALLIM, T. Q.; EDWARDS, P. A. Role of ABC transporters in lipid transport and human disease. **Trends Endocrinol Metab**, v. 24, n. 7, p. 342–50, 2013.

TONKIN, A.; BYRNES, A. Treatment of dyslipidemia. **F1000prime reports**, v. 6, n. February, p. 17, 2014.

UEHARA, Y.; SAKU, K. High-density lipoprotein and atherosclerosis: Roles of lipid transporters. **World J Cardiol**, v. 6, n. 10, p. 1049–1059, 2014.

UUSITUPA, M. Gene-diet interaction in relation to the prevention of obesity and type 2 diabetes: Evidence from the Finnish Diabetes Prevention Study. **Nutrition, Metabolism and Cardiovascular Diseases**, v. 15, n. 3, p. 225–233, 2005.

WANG, N. et al. Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. **Journal of Biological Chemistry**, v. 275, n. 42, p. 33053–33058, 2000.

WANG, N. *et al*. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. **PNAS,** v. 2004, 2004.

WARDLE, J. *et al*. The FTO gene and measured food intake in children. **International journal of obesity (2005)**, v. 33, n. 1, p. 42–45, 2009.

WILKENS, S. Structure and mechanism of ABC transporters. **Current Opinion in Structural Biology**, v. 14, n. 4, p. 426–431, 2015.

WORLD HEALTH ORGANIZATION (WHO). Growth reference 5-19 years. 2007. Disponível em: <http://www.who.int/topics/obesity/en/>. Acesso em: 20/06/2015.

WORLD HEALTH ORGANIZATION (WHO). Obesity. 2015. Disponível em: <http://www.who.int/topics/obesity/en/>. Acesso em: 15/05/2015.

WORLD HEALTH ORGANIZATION (WHO). Physical activity recommendations. 2016. Disponível em: <http://www.who.int/dietphysicalactivity/factsheet_recommendations/en/>. Acesso em: 10/12/2016.

XAVIER *et al*. V Diretriz Brasileira de Dislipidemias e Prevenção da Aterosclerose. **Arquivos Brasileiros de Cardiologia**, v. 101, n. 4, 2013.

YEO, G. S. H. The role of the FTO (Fat Mass and Obesity Related) locus in regulating body size and composition. **Molecular and Cellular Endocrinology**, v. 397, n. 1-2, p. 34–41, 2014.

YEO, G. S. H.; O'RAHILLY, S. Uncovering the biology of FTO. **Molecular Metabolism**, v. 1, n. 1-2, p. 32–36, 2012.

APÊNDICE – EFETIVIDADE DO TREINO

COMPARAÇÃO ENTRE OS TIPOS DE TREINO

Foi realizada uma comparação entre os quatro diferentes tipos de treino, dois a dois, a fim de verificar qual treino foi mais efetivo. Os resultados foram apresentados na TABELA 1A (as variáveis para as quais não são mostrados resultados não possuíam dados com relação aos treinos comparados).

TABELA 1A – COMPARAÇÃO DOS EFEITOS DOS QUATRO DIFERENTES TIPOS DE TREINO

Variáveis	N1	Média 1 ± DP	N2	Média 2 ± DP	N3	Média 3 ± DP	N4	Média 4 ± DP	p1 (1x2)	p2 (1x3)	p3 (1x4)	p4 (2x3)	p5 (2x4)	p6 (3x4)
Variação IMC escore-Z	84	0,24 ± 0,40	28	0,04 ± 0,19	33	0,19 ± 0,61	23	0,04 ± 0,33	**0,009**	0,66	0,21	**0,02**	0,06	0,07
Variação CA	82	1,74 ± 4,88			33	0,10 ± 6,74				0,21				
Variação CC			26	0,09 ± 5,65			29	0,80 ± 2,65					0,66	
Variação %G	72	3,60 ± 3,47			33	2,89 ± 5,33	19	2,53 ± 6,08		0,1	0,44			
Variação G	65	1,89 ± 3,67			33	2,62 ± 6,22				0,58				
Variação MM	65	2,68 ± 5,03			33	1,60 ± 3,49				0,39				
Variação CT	77	1,36 ± 21	29	1,72 ± 18,95	34	17,29 ± 24,23	29	14,10 ± 21,92	0,94	**0,0007**	**0,007**	**0,007**	**0,03**	0,59
Variação HDL-C	76	3,45 ± 8,06	29	3,30 ± 16,75	34	5,56 ± 6,42	29	5,14 ± 9,61	**0,02**	**0**	**0**	0,77	0,85	0,94
Variação LDL-C	77	1,49 ± 20,10	29	0,83 ± 22,61	34	11,94 ± 20,94	29	10,99 ± 18,16	0,61	**0,01**	**0,03**	**0,02**	**0,03**	0,85
Variação TG	76	16,98 ± 52,26	29	3,81 ± 37,37	33	2,36 ± 49,03	29	11,14 ± 49,19	**0,006**	**0,02**	**0,004**	0,9	0,73	0,82
Variação GLI	78	1,59 ± 8,64	29	3,45 ± 7,85	34	2,79 ± 6,76	27	2,93 ± 8,76	0,31	0,47	**0,02**	0,72	**0,006**	**0,006**
Variação INS	79	2,91 ± 9,13	29	1,52 ± 4,93			27	3,81 ± 8,79	0,71		0,75		0,71	

Nota: 1 = Aeróbico Terrestre; 2 = Treinamento Combinado; 3 = Exercícios Aquáticos; 4 = Treinamento Intervalado de Alta Intensidade (HIIT).
As médias das variáveis paramétricas (variação de CT, LDL-C e glicose) foram comparadas pelo teste T e as médias das variáveis não paramétricas foram comparadas pelo teste de Mann Whitney (variação de IMC escore-Z, CA, CC, %GC, GC, MM, HDL-C, TG e insulina).

O treino aeróbico terrestre gerou uma resposta mais efetiva do que o treinamento combinado com relação ao IMC escore-Z (p = 0,009), HDL-C (p = 0,02) e TG (p = 0,006). Em comparação com o programa de exercícios aquáticos, o aeróbico terrestre foi mais efetivo com relação aos níveis de HDL-C ($p = 10^{-4}$) e TG (p = 0,02). Por fim, o treino aeróbico terrestre gerou melhor resposta do que HIIT nos níveis de HDL-C ($p = 10^{-4}$), TG (p = 0,004) e glicose (p = 0,02).

O treinamento combinado foi mais efetivo apenas do que HIIT, e em relação aos níveis de glicose (p = 0,006).

O programa de exercícios aquáticos gerou melhor resposta do que o treino aeróbico terrestre com relação aos valores de CT (p = 0,0007) e LDL-C (p = 0,01), melhor resposta do que o treinamento combinado em relação ao IMC (p = 0,02), CT (p = 0,007) e LDL-C (p = 0,02), e foi mais efetiva que HIIT em relação à glicose (p = 0,006).

Por fim, HIIT foi mais efetivo do que o treinamento aeróbico terrestre em relação à CT (p = 0,007) e LDL-C (p = 0,03), e mais efetivo do que o treinamento combinado em relação aos níveis de CT (p = 0,007) e LDL-C (p = 0,03).

De uma maneira geral, todos os tipos de treinamento foram efetivos, sendo que algumas variáveis responderam melhor a um determinado treino.

Apesar das diferenças entre os treinos, para as análises genéticas não foi feita separação entre os treinamentos, pois isso promoveria uma redução significativa do N amostral.

ANEXOS

ANEXOS

ANEXO 1 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

TÍTULO DO PROJETO: Adolescentes obesos submetidos a tratamento físico multidisciplinar: Avaliações físicas multidisciplinares e estudos de associação com variantes genéticas
INVESTIGADOR: Prof. Dra. Lupe Furtado Alle

LOCAL DA PESQUISA: Departamento de Genética e Departamento de Educação Física

Telefone (41) 3361 1730

Você está sendo convidado (a) para participar de uma pesquisa. Este termo de consentimento livre e esclarecido tem informações para ajudá-lo a decidir se irá permitir que seu filho (a) participe deste estudo.

O objetivo deste estudo é investigar fatores de predisposição genética à obesidade em adolescentes submetidos a sessões de exercícios físicos e de orientação nutricional.

Caso o seu filho participe da pesquisa, será necessário fazer exames de rotina médica, bioimpedância, testes cardiorrepiratórios em esteira e bicicleta ergométrica, avaliação do estágio puberal e eletrocardiograma.

Como em qualquer tratamento seu filho (a) poderá experimentar alguns desconfortos, principalmente relacionados ao uso de máscara na calorimetria, ao utilizar o bucal e o clamp nasal para respiração exclusivamente oral e dores musculares e articulares após os testes ergométricos máximos.

Os riscos que envolvem a avaliação de seu filho (a) são dores musculares e articulares após o teste ergométrico.

Para tanto seu filho deverá comparecer no Hospital de Clínicas (HC) para consulta médica, realizar a avaliação puberal, bioimpedância e eletrocardiograma, e ao

Departamento de Educação Física (DEF) da Universidade Federal do Paraná (UFPR) para a realização de testes em esteira e bicicleta ergométrica.

Estão garantidas todas as informações que você queira, antes, durante e após o estudo.

A participação de seu filho (a) é voluntária. Você tem a liberdade de recusar a participar do estudo, ou retirar seu consentimento a qualquer momento.

As informações relacionadas ao estudo poderão ser inspecionadas pelos médicos que executam a pesquisa e pelas autoridades legais, no entanto, se qualquer informação for divulgada em relatório ou publicação, isto será feito sob forma codificada, para que a confidencialidade seja mantida.

Todas as despesas necessárias para a realização da pesquisa **não** são da responsabilidade do paciente ou do seu responsável.

Pela participação do seu filho (a) no estudo, você não receberá qualquer valor em dinheiro.

Quando os resultados forem publicados, não aparecerá o nome de filho (a), e sim um código.

Durante o estudo seu filho (a) não poderá ingerir medicamentos sem informar antecipadamente os pesquisadores responsáveis por este estudo.

Eu, _____ li o texto acima e compreendi a natureza e objetivo de estudo no qual meu filho (a) _____ foi convidado (a) a participar. A explicação que recebi menciona os riscos e benefícios do estudo. Entendi que sou livre para interromper a sua participação no estudo a qualquer momento sem justificar a minha decisão e sem que esta decisão afete o seu tratamento com o seu médico. Eu entendi que não posso fazer durante o estudo e

sei que qualquer problema relacionado ao tratamento será tratado sem custos para mim ou para o meu filho (a).

Eu concordo voluntariamente do (a) meu (minha) filho (a) em participar deste estudo.

ANEXO 2 – APROVAÇÃO DO COMITÊ DE ÉTICA

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Comitê Setorial de Ética em Pesquisa

Projeto: "Adolescentes obesos submetidos a tratamento físico multidisciplinar: Avaliações físicas multidisciplinares e estudos de associação com variantes dos genes *ADBR3*, *BCHE* e *GHRL*".

Pesquisador: Profa. Dra. Lupe Furtado Alle

Protocolo: CEP_05/09

Departamento: Departamento de Genética

Curitiba, 10 de dezembro de 2009

Prezada Profa. Dra. Lupe Furtado Alle

Em relação a projeto acima citado, venho informá-lo de que este foi avaliado pelo CEP-Biológicas, estando de acordo com a Declaração de Helsinque (e suas atualizações) e com a resolução 196/96 do CNS (e resoluções complementares), tendo sido aprovado pelo comitê. Portanto, a partir desta data poderá ser iniciada a execução e a coleta de dados do referido projeto.

Ressalto que, de acordo com a resolução 196/96 que: (a) o pesquisador deve comunicar a este comitê qualquer alteração no protocolo experimental ou no termo de consentimento (nestas circunstâncias a inclusão deve ser temporariamente suspensa até análise do CEP das modificações propostas); (b) comunicar imediatamente ao CEP qualquer evento adverso ocorrido durante o desenvolvimento da pesquisa; (c) os dados individuais de todos indivíduos devem ser mantidos em local seguro por 5 anos para possível auditoria; (d) apresentar relatórios semestrais.

Contando com sua compreensão e apoio, coloco-me à disposição para maiores esclarecimentos, atenciosamente

Prof. Dr. Ricardo Lehtonen R. de Souza
Coordenador do Comitê de Ética em Pesquisa
Setor de Ciências Biológicas – UFPR