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Associations of MC3R Polymorphisms With Physical Activity in South African Adolescents

Yandiswa Y. Yako, Mogamat S. Hassan, Rajiv T. Erasmus, Lize van der Merwe, Susan Janse van Rensburg, and Tandi Edith Matsha

Background: There is evidence demonstrating that the contribution of sedentary behavior and effect of physical activity on metabolic phenotypes is mediated by polymorphisms in genes. ***Methods***: The type and frequency of physical activity was assessed by means of structured questionnaires in 1555 South African school learners. Anthropometric measurements, blood pressure, fasting blood glucose and lipids were measured using standard procedures. The effect of different types and frequency of physical activity on obesity-related traits was assessed in relation to MC3R T6K and V81I genotypes in 430 of the learners. ***Results***: Levels of total cholesterol were significantly lower in learners carrying the MC3R T6K and V81I minor alleles, after adjusting for age, race, gender, and each specific physical activity category. An activity-by-genotype interaction was also detected: learners heterozygous for the V81I polymorphism and performed house chores often had reduced total cholesterol. Though no association was observed between frequency of physical activity and BMI, television viewing was significantly associated with an increase in height, weight and marginally with waist circumference. ***Conclusion***: Our findings suggest that physical activity even in the form of house chores has a positive effect on metabolic traits and this effect is further enhanced in the presence of MC3R polymorphisms.

K**eywords**: total cholesterol, obesity

Globally the prevalence of childhood overweight and obesity has increased tremendously becoming one of the leading causes of early onset cardiovascular diseases and related mortality in adulthood. It is widely accepted that weight gain can be reversed by increasing energy expenditure and reducing energy intake. Physical activity, therefore, has been identified as one of the interventions for obesity management in both adults and children, and several studies have reported the effectiveness of physical activity in reducing weight and associated cardiovascular risk factors. Despite the small number of South African studies demonstrating the relationship between physical inactivity and obesity, it is acknowledged that physical inactivity is a major determinant of obesity in both adults and children.[1–5] Urbanization in South African children has introduced a sedentary lifestyle, and hence lower physical activity, possibly due to high crime rates in urban areas and consequent fear of playing outside.[2,6,7] However, there is interindividual variation on the effect of physical inactivity on body weight that may be caused in part by the presence of susceptibility polymorphisms.

Most studies have investigated genotype-dependent responses of cardiovascular risk factors such as plasma lipoprotein-lipid levels, type 2 diabetes, blood pressure, and obesity to exercise training.[8,9] For example, polymorphisms in apoliprotein E and cholesteryl ester transfer protein genes have been extensively studies and shown to affect exercise training responses on plasma lipoprotein levels.[10–16] Similarly, several studies have found signifi genotype-dependent effects for blood pressure changes with exercise training, and the most studied genes include endothelial nitric oxide synthase, angiotensin-converting enzyme (ACE) and angiotensinogen.[17–21] Polymorphisms in genes such as beta adrenergic receptor, and peroxisome proliferators-activated receptor delta and gamma have been demonstrated to affect type 2 diabetes–related trait responses to exercise.[22–30] In relation to obesity-related traits, most frequently studied polymorphisms associated with individual's responses to exercise include those in genes that lie within the adrenergic receptor (AR) pathway.[31–34] Genes involved in appetite regulation have also been shown to affect responses of physical activities on metabolic diseases, and these include melanocortin receptor 4 (MC4R), leptin receptor, and ghrelin genes.[35–38] Recently, cross-sectional studies have consistently demonstrated that physical activity levels alter the association of polymorphisms in the fat mass and obesity-associated (FTO) gene with obesity-related traits.[39–42]

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Although there is large amount of data demonstrating genotype-independent response of cardiovascular risk factors to physical activity, other studies failed to identify the effects of polymorphisms on these risk factors. For example, the ACE II genotype was found to increase insulin sensitivity more with exercise than the D allele, but 2 studies could not demonstrate the genotype-independent effect on type 2 diabetes traits.[43,44] In the context of obesity-related traits, Phares and coworkers[33] demonstrated that the beta2-AR Gln27Glu, beta3-AR Trp64Arg, and alpha2b-AR Glu12/Glu9 polymorphisms independently and interactively affected responses of total body and trunk fat to exercise, but the authors could not replicate these fi   in a larger HERITAGE cohort.

FTO is predicted to have a slight DNA demethylase[45] and nonheme dioxygenase activities,[46] however, the reported association studies are suggestive of a role in energy intake and expenditure. Energy intake and expenditure are mainly regulated by the leptin-melanocortin pathway. The pathway also plays a role in glucose homeostasis, and insulin secretion. Neuropeptides in the melanocortin system regulate food intake and energy balance by binding to melanocortin receptors, particularly MC4R, and to a lesser extent MC3R. There is also evidence supporting the role of the melanocortin receptors, particularly the melanocortin 4 and 3 receptor genes (MC4R and MC3R), in the development of common polygenic obesity.[47,48] Mutations in MC4R are the most common cause of early onset morbid obesity, accounting for up to 6% of the condition, particularly in European populations. It is only recently that morbid obesity-related mutations (Ala293Thr, Ile335Ser, Ter361Ser, Ile183Asn, Ala70Thr, and Met134Ile) have been identified in the MC3R.[48,49] Similar to MC4R polymorphisms, common variants in MC3R show significant frequency variation among different populations accompanied by variable effects on obesity-related traits.[48,50–54] We recently demonstrated that the minor alleles of the MC3R T6K and V81I polymorphisms are associated with decreasing obesity indices, total cholesterol, as well as blood pressure in South African Mixed Ancestry learners.[54] In this study we wanted to establish whether the effect of these genotypes could have been infl   by physical fi   of learners as assessed by physical activity questionnaires. Another aim was to investigate the effect of physical activities on obesity and metabolic traits. To our knowledge, the combined effect of the MC3R polymorphisms and physical activity on obesity has not been investigated in South African populations.

Materials and Methods

Study Population and Data Collection

The school setting provided an ideal social context and almost ready-made sampling frame (gender, age, education level, geographical area, etc.) to obtain information, making it the most appropriate sampling frame for this study.[55] The study was approved by the Research Ethics Committees of the University of Stellenbosch (Project number: N07/ 07/ 160) and Cape Peninsula University of Technology (Project Reference number: CPUT/ HAS-REC 0016), and conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). Permission to conduct the 'parent' research project was granted by the Western Cape Department of Education, school governing bodies and principal. Written consent was obtained from the parent or guardian of each learner, and oral assent from learners was obtained on the sampling day. The current study was part of an investigation that aimed to elucidate obesity, diabetes, hypertension, and the metabolic syndrome in children and adolescents attending schools at the peri-urban areas of the Western Cape. The study population of the 'parent' research project consisted of a total of 1555 learners age 8–18 years that were recruited through a proportionally stratifi multistage random sampling technique from government funded primary and secondary schools using a list of 107 schools obtained from the Western Cape Education Department as described by Matsha et al.[56] The study population was of both male and female learners from 3 South African racial groups, namely Caucasians, Black, and Mixed Ancestry.

Anthropometric Measurements

Anthropometric and biochemical parameters of interest that were measured are as follows: body weight and height; waist, hip, and mid-upper-arm circumferences (WC, HC, and MUAC); waist-hip ratio; blood pressure; blood glucose; and plasma lipid levels. Body weight and height measurements were used to calculate the BMI. Professional nurses and field workers who were trained in the use of prescribed standardized techniques conducted anthropometric measurements and biochemical analyses. These standardized techniques and associated data-collection methods were piloted, and used in a research project that aimed at investigating the prevalence of obesity among learners attending schools in Belhar, Delf, and Mfuleni in the Western Cape Province.[57] In a pilot study, careful selection of instruments with adequate detection limits and sensitivity was done to enhance the accuracy and validity of results. Statistical measures in the form of repeated measures were used to ensure inter- and intravalidity. Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, using a stadiometer and a balanced Sunbeam EB710 digital bathroom electronic scale to calculate BMI as weight per square meter (kg/m^2). Learners were classifi according to their weight status as obese, overweight and normal using the International reference gender-and age-based cut-off points provided by the IOTF as developed by Cole and coworkers.[58] Due to the nonrepresentative nature of the CDC growth charts, the International Obesity Task Force (IOTF) developed BMI reference values that defined childhood obesity and overweight based on the data pooled from different countries such as Brazil, Britain, Hong Kong, Netherlands, Singapore,

and the United States. These reference values were developed based on adult cut-off points that were linked to BMI centiles (85th and 95th percentiles) of children and adolescents.[58] MUAC measurements were done to the nearest 0.2 cm on the left arm midway between the acromion and radial points, with both arms loosely and comfortably at the sides. WC and HC were measured using an anthropometric fiberglass tape measure to the nearest 0.1 cm. The WC was measured at the narrowest part of the torso as seen from the anterior view, and in obese learners, at the smallest circumference measured in the area between the ribs and the iliac crest. The HC was measured with each participant assuming a relaxed stand, the gluteal muscles (buttocks) relaxed, arms folded across the thorax and feet together. The hip or girth was considered as the widest part of the thighs and the greatest posterior protuberance (apex) of the buttocks. Measurements were taken 3 times at the end of normal expiration and averages were recorded.

Fasting Blood Glucose and Lipid Measurements

Fasting blood samples obtained through finger pricking were used for glucose and lipid measurements. Before screening, the fasting state was determined by interview on the morning of examination. Blood glucose levels were measured using the Accutrend GCT glucometer. Capillary glucose measurements are as suitable as venous glucose measurements in the diagnosis and detection of type 2 diabetes mellitus in epidemiological studies.[59,60] The commercial glucometer used in this study had a mean imprecision of < 5%, with a range of 1.1–33.3 mmol/L on capillary whole blood. Total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and triglycerides (TG) were measured using CardioCheck P.A analyzer (Polymer Technology Systems, Inc. USA) according to the manufacturer's instructions. CardioChek PA complied with the National Cholesterol Education Program Expert Panel guidelines for TC and high density lipoprotein-cholesterol (HDL-C) as tested in a study conducted by Panz and coworkers.[61] According to this study, lipid levels measured by the CardioChek PA analyzer agreed satisfactorily with the laboratory results, and instrument therefore could be used for screening purposes. For quality control purposes, 1 control sample was included everyday during sample collection.

Blood Pressure

Blood pressure measurements were performed according to WHO guidelines (1999). Measurements were performed using a semiautomatic digital blood pressure monitor (Rossmax PA, USA) on the right arm in sitting and relaxed position with the learner not having ingested coffee or smoked for 30 minutes before measurement. The cuff was placed at a point midway between the olecranon and acromion to ensure accurate measurement. After a 10-minute rest period, 3 readings were taken at 5 minutes interval and the lowest of the 3 readings was taken as the blood pressure.

Physical Activity Assessment

The Physical Activity and Energy expenditure was assessed in the form of questionnaire that were developed based on the validated questionnaire designed by Arvidsson and coworkers.[62] The questionnaire was modified for local conditions, and was used to qualitatively assess physical activity of 1555 participating school learners. This set of questionnaire contained indicators of sporting as well as leisure activities, and assessed the frequency and type of activities learners participated in. All questionnaires were developed in English, and then translated into Afrikaans and Xhosa for participants to fully understand the procedure. A pilot study was conducted among 22 school learners that were randomly selected from a school with similar characteristics as the current study population. The questionnaire was further used in a research study that aimed at investigating the prevalence of obesity among learners attending schools in Belhar, Delf, and Mfuleni in the Western Cape Province.[57] On the sampling day, each learner was asked about the frequency at which they performed activities during the week such as walking, sport, cycling, household chores, and time spent on watching television. Furthermore, learners were asked if their schools offered physical education, and if yes, the number of days per week they attended the session. Each activity was then categorized according to the frequency of performing each activity as follows: not at all or physically inactive (0 days per week), occasional (1–2 days per week), and often (3–5 days per week).

Genotyping

From the original study population of 1555 learners, 430 (226 obese-overweight, and 204 normal) individuals consented for genetic analysis. Genomic DNA was extracted from either whole blood collected in vacutainer ethylenediamine tetra-acetic acid (EDTA) tubes using a salting out procedure63 or from capillary blood collected onto Whatman FTA Cards (Merck Laboratories, UK) as follows: from each sample spot on the Whatman FTA card, a disc was cut using a Uni-Core Punch and placed in a labeled 0.2-ml PCR tube. The sample disc was washed 3 times by a FTA purifi   reagent, discarding the used reagent after a 5-minute incubation period at room temperature. The disc was then washed with a 1× tris ethylenediamine tetra acetic acid (TE) buffer 2 times discarding the used TE buffer after a 5-minute incubation period at room temperature. The disc was allowed to dry at room temperature for about 1 hour or at 56°C for 10 minutes. Learners were genotyped for MC3R T6K (rs3746619) and V81I (rs3827103) polymorphisms by digesting the amplicons (polymerase chain reaction protocol is available on request) with Mae III and BseD 1 restriction enzymes, respectively.[64] The C allele of the T6K variant abolished the Mae III restriction enzyme

recognition site, while the G allele of the V81I polymorphism created a second restriction recognition site for the BseD 1. Digested amplicons were separated along with a negative control (undigested amplicon) and a 100-bp ladder in 3% agarose gels and visualized with GelRed under ultraviolet light.

Statistical Analyses

The following guided our genotype sample size calculation: If the "risk" allele frequency in the general population (controls) is 40%, then a study with 226 cases and 204 controls have 80% power to detect, at a 5% significance level, a "risk" allele frequency of 57% in cases. Anthropometric and metabolic parameters were each modeled as a function of age, race and gender, and p-values are from the joint model, which means that p-values are adjusted for the other factors in the model. Frequency of participation (categorized as "often," "occasional," or "not at all") in various physical activities (Cycling, walking, and others) and sedentary behavior in the form of television viewing was compared between race groups and also gender, using Fisher exact tests. To assess associations, anthropometric and metabolic parameters were modeled (linear) as function of frequency of participation in each activity, while adjusting for age, race and gender, by including them as fixed effects in the models. Linear models were used to assess the effect of the MC3R genotypes on anthropometric and metabolic parameters while adjusting for frequency of participation in each activity, age, race and gender. Finally linear models were used to assess the effect of the interaction between the MC3R genotypes and frequency of participation in various physical activities on anthropometric and metabolic parameters while adjusting for, age, race and gender. Results with p-values below 5% are described as signifi   No adjustment was made for multiple testing. The effect sizes and 95% confi nce intervals reported in the results and tables were calculated from the specific models yielding significant results. Data were analyzed using the freely available programming language R (www.r-project.org) and specifi package genetics (genotype association).

Results

Table 1 summarizes the anthropometric and metabolic parameters of the study group, stratified by, gender and race. The study group consisted of 146 Caucasians, 537 Black Africans, and 872 Mixed Ancestry learners. Generally, both anthropometric and metabolic parameters signifi   increased with age except fasting blood glucose (FBG) and low density lipoprotein-cholesterol (LDL-C). Significant differences across race and gender were noted for all clinical characteristics except the diastolic blood pressure, fasting blood glucose (FBG), and triglycerides (TG). The Mixed Ancestry learners weighed the least. Though Black Africans weighed significantly less than the Caucasians, they were significantly shorter,

consequently the BMI did not differ signifi   between the 2 racial groups. Waist circumference was signifi smaller in Black Africans than in Caucasians. While no gender differences were observed, Mixed Ancestry learners had higher FBG compared with the other 2 racial groups. TC was signifi   lower in Black Africans than in Caucasian and Mixed Ancestry learners (Figure 1).

Table 2 shows the number of learners who participated in different physical activity categories. The levels of activity differed highly significantly between racial groups for all activities investigated. Gender differences were highly significant for physical education, sport and household chores only. More than 80% of learners reported watching television often. Across all racial groups, more than 70% of learners participated in physical education. Although proportionally more girls than boys participated in physical education lessons, more boys participated in sport compared with their female counterparts. While the majority of learners walked often, less than 40% participated in sports or cycled often. Compared with Mixed Ancestry and Black African learners, more Caucasians reported participation in sport activities, including cycling. The house chores were performed mostly by the girls (Figure 2).

We modeled the association between frequency of activities and anthropometric and metabolic parameters; adjusted for age, gender, and race. Learners who watched television often had higher weight (4.87 kg; 95% CI: 1.24–8.5), height (5.57 cm; 95% CI: 3.01–8.13), and hip circumference (3.88 cm; 95% CI: 0.85–6.91:) measurements compared with those who did not watch television at all. Learners who walked occasionally and often, were taller than those who never engaged in the activity by 2.01 cm (95% CI: 0.42–3.61) and 2.35 cm (95% CI: 0.90–3.80), respectively. Playing sport was also positively associated with height; with those who played sport often 1.26 cm (95% CI: 0.23–2.30) taller than their inactive counterparts. Cycling occasionally increased systolic blood pressure by 1.81 mmHg (95% CI: 0.21–3.41) among learners who participated in the activities compared with those who never cycled, but the effect of cycling often was not statistically significant. Doing household chores (occasionally or often) reduced TC by 0.187 mmol/L (95% CI: 0.042–0.332), on average, compared with those who did not do the duties. None of the other associations were significant.

Out of 1555 learners who were included in the parent study, 430 participants who consented to genetic analyses and thus were genotyped for the MC3R T6K (rs3746619) and V81I (rs3827103) polymorphisms. Previously we reported association of the T6K and V81I minor alleles with obesity-related traits (MUAC, HC and BMI), blood pressure, and TC.[54] In the present, we wanted to establish whether the effect of the 2 MC3R polymorphisms was influenced by physical activeness of participants as measured by questionnaires. Of the anthropometric and metabolic parameters that were investigated, only the TC levels showed a significant association with MC3R genotypes when adjusted for age, gender, and race, and

Table 1 Anthropometric and Metabolic Parameters of 1555 Learners

	Black African		Mixed ancestry		Caucasian		*P*-values		
	Male	Female	Male	Female	Male	Female	Age	Race	Gender
Number of learners	197	340	354	518	66	80			
Age (years)	13.4 ± 2.6	12.6 ± 2.3	12.5 ± 2.6	12.8 ± 2.6	14.3 ± 2.0	13.8 ± 2.0			
Weight (kg)	48.0 ± 13.7	48.9 ± 13.3	43.8 ± 15.8	47.0 ± 15.6	62.7 ± 13.3	55.1 ± 15.8	<0.0001	<0.0001	0.0014
Height (m)	1.6 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.5 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	<0.0001	<0.0001	<0.0001
BMI (kg/m^2)	19.7 ± 3.5	21.3 ± 4.3	18.5 ± 4.0	20.5 ± 4.9	21.5 ± 3.3	21.1 ± 4.7	<0.0001	0.0008	<0.0001
WC (cm)	66.4 ± 8.2	70.0 ± 9.9	63.8 ± 10.1	67.1 ± 11.0	74.4 ± 9.0	72.0 ± 11.7	<0.0001	<0.0001	<0.0001
HP (cm)	83.6 ± 10.2	87.5 ± 11.7	80.2 ± 12.0	86.4 ± 13.3	92.1 ± 8.4	90.0 ± 12.4	<0.0001	0.0003	<0.0001
WHR	0.80 ± 0.04	0.79 ± 0.05	0.80 ± 0.05	0.78 ± 0.06	0.81 ± 0.05	0.80 ± 0.05	<0.0001	<0.0001	<0.0001
SBP (mmHg)	107 ± 15	107 ± 13	105 ± 14	105 ± 13	109 ± 13	102 ± 14	<0.0001	0.0066	0.3351
DBP (mmHg)	65 ± 12	66 ± 11	64 ± 10	65 ± 9	66 ± 10	65 ± 10	<0.0001	0.5026	0.0288
FBG (mmol/L)	3.9 ± 0.8	3.8 ± 0.72	4.2 ± 0.83	4.1 ± 0.71	4.0 ± 0.85	3.9 ± 0.81	0.1103	<0.0001	0.0543
TC (mmol/L)	3.3 ± 0.75	3.6 ± 0.8	3.7 ± 5.2	3.8 ± 0.9	3.6 ± 0.9	3.9 ± 0.8	<0.0001	<0.0001	<0.0001
TG (mmol/L)	0.79 ± 0.55	0.83 ± 0.44	0.72 ± 0.37	0.81 ± 0.59	0.83 ± 0.41	0.78 ± 0.26	0.0025	0.4657	0.0143
HDL-C (mmol/L	1.06-0.32	1.2 ± 0.38	1.0 ± 0.40	1.12 ± 0.39	0.87 ± 0.04	1.05 ± 0.36	<0.0001	0.0023	<0.0001
LDL-C (mmol/L)	2.00 ± 0.66	2.18 ± 0.70	2.25 ± 0.65	2.39 ± 0.70	2.64 ± 0.81	2.64 ± 0.69	0.1264	<0.0001	0.0062

Note. Summary statistics: mean ± SD. *P*-values are from joint linear model of association with age, race, and gender, respectively.

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, high density lipoprotein-cholesterol; HP, hip circumference; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHR, waist hip ratio.
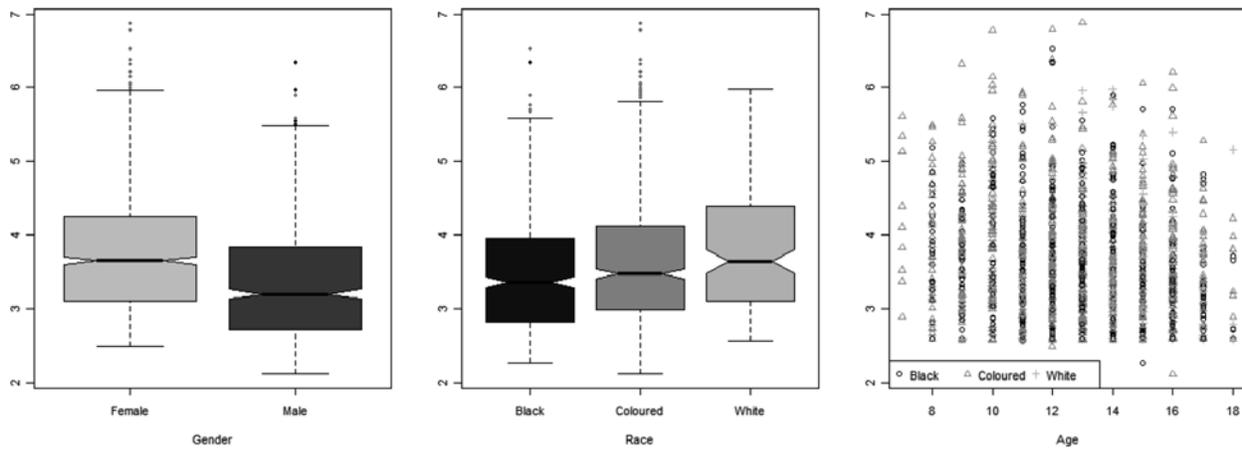
Figure 1 — Illustration of observed significant differences in total cholesterol (in mmol/L) in 1555 learners; stratified by gender, age, and race.

separately for the frequency of each physical activity. For all 5 activities, TC levels were significantly lower in learners who were homozygous for either the T6K or V81I minor alleles, compared with the respective wild type homozygotes. The estimated TC levels ranged from 0.292 mmol/L (P = .0388; 95% CI: −0.529 to −0.055) for television viewing and T6K to 0.316 mmol/L (P = .0207; 95% CI: −0.555 to −0.078) for sport and T6K. A single interaction between polymorphism and activity on metabolic outcome was detected. The only activity by genotype interaction detected in the current study was on TC between the frequency of doing house chores and the V81I polymorphism (Figure 3). Learners heterozygous for the V81I polymorphism and performed house chores often had an extra 0.358 mmol/L (95% CI: 0.137–0.580) reduced TC while AA carriers had 0.353 mmol/L (95% CI: 0.083–0.624) lower TC, compared with GG. In learners doing house chores occasionally, the estimated effect of AG versus GG was in the opposite direction, increasing TC by 0.358 mmol/L (95% CI: 0.023–0.693).

Discussion

In this study we provide further evidence that the minor A allele of both T6K and V81I reduces TC, and that these polymorphisms interact with physical activity for an added reduction in TC. TC was decreased in learners who carried T6K and V81I minor A alleles after adjusting for each physical activity category, further confirming our initial finding that demonstrated an association of the minor alleles with decreased TC levels (54). Learners homozygous for the V81I minor A allele had twice lower TC levels (0.299 mmol/L) compared with individuals with 1 A allele (0.134 mmol/L). Doing house chore further reduced TC in A allele carriers from 0.203 mmol/L to 0.355 mmol/L compared with wild type homozygotes. The type of house chores performed by adolescent learners in our study was not specifi   to explain the difference

in TC levels observed in the presence of MC3R genotypes. Generally, several physical activity intervention studies done on school children and adolescents reported statistically significant positive effects on mean blood TC,[65–67] consistent with findings in adults.[68,69] There are currently no findings that directly link MC3R on lipid metabolism, but our findings are suggestive of its dual role on energy expenditure and lipid metabolism through physical activity. Animal studies have supported the role of MC3R in energy expenditure, in which MC3R knockout mice exhibited diminished physical activity.[70,71] MC3R, Leptin and leptin receptor genes, and MC4R are part of the central melanocortin pathway that controls nutrient intake, energy expenditure, and balance of substrate oxidation. The melanocortin system has also been shown to play a role in lipid metabolism. In their study, Nogueiras et al[72] demonstrated that inhibition of the melanocortin system in mice by pharmacological, genetic or endocrine mechanisms increased circulating HDL-C by reducing its uptake by the liver independent of food intake or body weight status. It is also possible that the 2 MC3R polymorphisms investigated in the current study are in linkage disequilibrium with sequence variants in genes such as apoliprotein E and cholesteryl ester transfer protein that have been extensively studies and shown to affect exercise training responses on plasma lipoprotein levels.[10–16]

Apart from the above mentioned functions, other studies have demonstrated an expression of MC3R on peritoneal[73,74] and knee joint macrophages,[75] suggesting a role in modulating the host infl   response. Subsequent to these reports, several studies have demonstrated the association of MC3R polymorphisms to tuberculosis susceptibility.[77] It is therefore important when interpreting findings of the current study to consider the influence of inflammatory response that were not accounted for.

Table 2   Numbers and Percentages of Frequencies at Which Learners Participated in Various Physical Activities and Sedentary Behavior in the Form of Television Viewing; _P_-values Are Unadjusted

| Physical activity category	Black African		Mixed ancestry		Caucasian		_P_-values	
	Male	Female	Male	Female	Male	Female	Race	Gender
Number of learners	197	340	354	518	66	80		
Physical activity education							< 0.0001	0.0014
Yes	140 (71%)	273 (81%)	212 (60%)	340 (66%)	49 (74%)	69 (86%)		
No	56 (29%)	64 (19%)	138 (40%)	172 (34%)	17 (26%)	11 (14%)		
Television viewing							< 0.0001	0.0589
Often	154 (78%)	304 (89%)	282 (80%)	418 (81%)	56 (85%)	71 (89%)		
Occasional Not	33 (17%)	30 (9%)	66 (19%)	85 (16%)	7 (11%)	8 (10%)		
at all	10 (5%)	6 (2%)	5 (1%)	15 (3%)	3 (4%)	1 (1%)		
Cycling							< 0.0001	0.3814
Often	27 (14%)	41 (12%)	111 (31%)	175 (34%)	26 (39%)	28 (35%)		
Occasional	108 (55%)	195 (57%)	156 (44%)	231 (45%)	23 (35%)	42 (52.5%)		
Not at all	62 (32%)	104 (31%)	85 (25%)	111 (21%)	17 (26%)	10 (12.5%)		
Walking							< 0.0001	0.2789
Often	135 (69%)	218 (64%)	245 (69%)	346 (67%)	40 (61%)	38 (48%)		
Occasional	54 (27%)	104 (31%)	75 (21%)	110 (21%)	16 (24%)	32 (40%)		
Not at all	8 (4%)	18 (5%)	34 (10%)	61 (12%)	10 (15%)	10 (12)		
Sport							< 0.0001	0.0003
Often	65 (33%)	98 (29%)	62 (18%)	92 (18%)	22 (33%)	24 (30%)		
Occasional	32 (16%)	58 (17%)	140 (40%)	126 (24%)	21 (32%)	34 (42%)		
Not at all	99 (51%)	183 (54%)	151 (42%)	300 (58%)	23 (35%)	22 (28%)		
Household chores							< 0.0001	< 0.0001
Often	84 (42%)	202 (59%)	203 (57%)	362 (70%)	36 (55%)	54 (67%)		
Occasional Not	87 (44%)	118 (35%)	102 (29%)	127 (25%)	26 (39%)	23 (29%)		
at all	26 (14%)	20 (6%)	48 (14%)	27 (5%)	4 (6%)	3 (4%)		
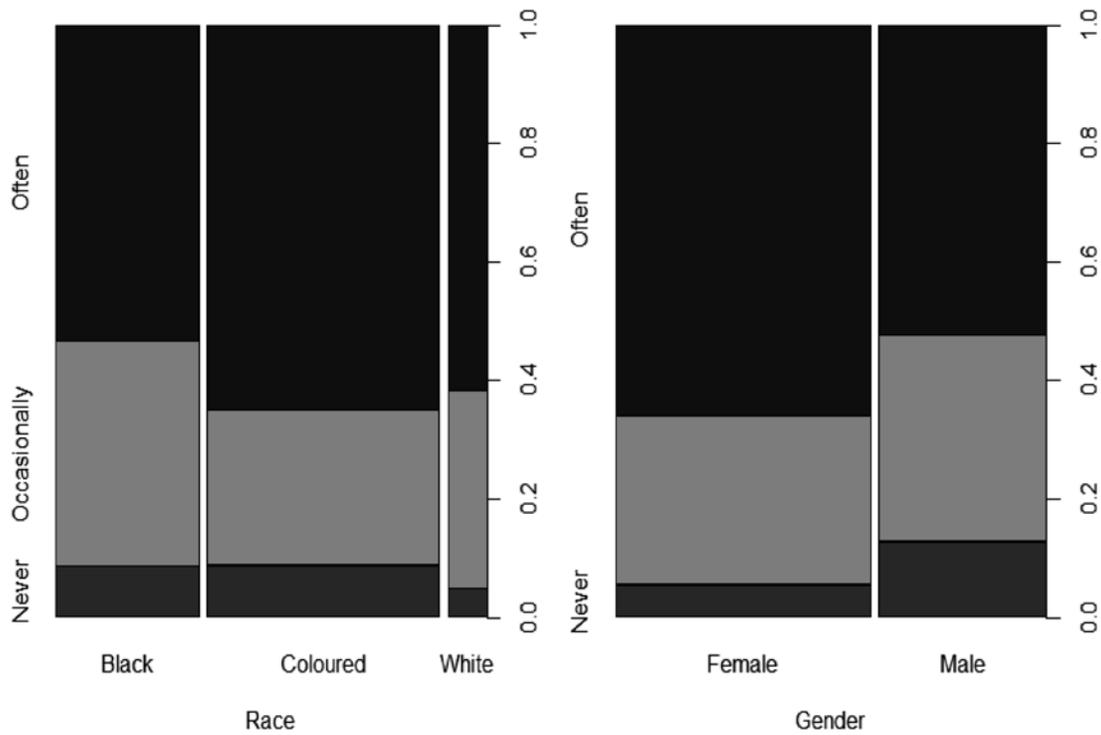
Figure 2 — Illustration of significant differences in frequency of doing house chores by gender and race in study group (n = 1555). Mosaic plots show cumulative proportion on left axis.
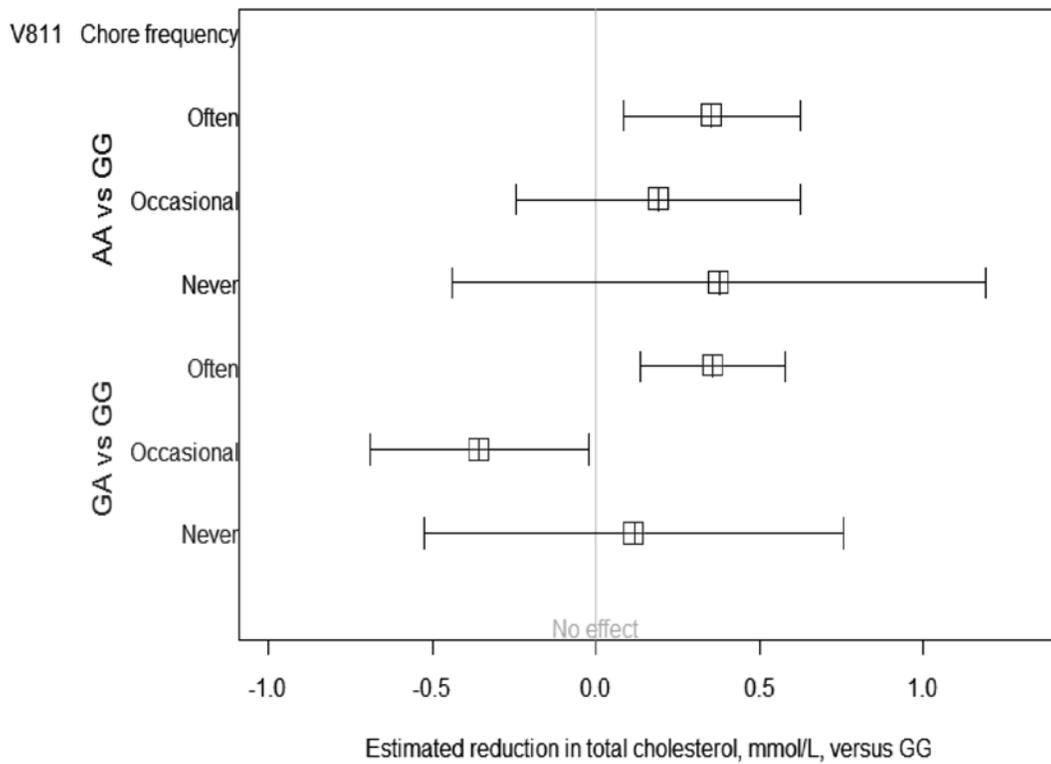

Figure 3 — Illustration of the significant interaction between frequency of doing house chores and V811 polymorphism. Estimated effect (reduction) on total cholesterol (in mmol/L) and 95% confidence interval for V81le AG and AA genotype versus GG, in learners doing house chores often, occasionally, and never.

There is evidence supporting an inverse relationship between physical activity, and obesity in South African children and adolescents in children and adolescents,[4,5,78] similar to observations in other countries worldwide.[79–81] In South Africa, more than 37.5% of children and adolescents are engaged in insuffi physical activity regardless of introducing and incorporating physical education at schools.[82] However, not all South African studies were able to observe the association between physical activity, sedentary behavior and obesity.[83,84] Similarly, our study failed to detect significant association between the type and frequency of physical activity and obesity as measured by BMI or waist circumference. The lack of signifi association between physical activity and obesity in this study and others may be due to their design, being cross-sectional. According to the meta-analysis done by Rey-Lopez et al,[85] most cross-sectional studies included failed to identify the effect of physical inactivity on body fatness while longitudinal studies demonstrated a positive association. For example, the Davison et al[86] study included in the meta-analyses did not observe any correlation between TV viewing and obesity when conducted cross-sectionally, but showed a positive association when it was done longitudinally. However, in the current study sedentary lifestyle as measured by the frequency of television viewing was signifi associated with an increase in weight, height, hip circumferences, and weakly with waist circumference.

Though no association was observed between frequency of physical activity and BMI, positive associations between frequencies of walking, playing sport and height were observed. Height is a multifactorial phenotype that responds to both genetic and environmental influences. Environmental factors during various stages of life (pregnancy, childhood, and adolescence) such as maternal smoking, undernutrition during pregnancy and early life, diseases, increased energy consumption, and even psychosocial factors, can contribute to growth velocity and to the final body height.[87] Genetic factors, on the other hand, account for up to 90% of variation in human height.[88] However, in this study, no significant interactions were observed between MC3R polymorphisms and physical activities in raising the height of learners. According to several studies, regular exercise has no effect on rate of growth in height,[89,90] instead it increases bone mineral density.[91] It is, however, not clear whether there is a specifi critical period during the growing years when bones may be most responsive to exercise.[92] The significant association between frequency of physical activity (walking and sport) and height observed in this study may have been the results of an interaction between physical activity and diet, hence warrants further investigation.

The current study also found a significant association between cycling and systolic blood pressure. The systolic blood pressure of learners who reported to cycle occasionally was 1.81 mmHg higher compared with that of learners who did not cycle. It has been reported that any degree of physical activity increases mainly systolic blood pressure.[93] Similarly, Burger et al[94] demonstrated that, in addition to diastolic blood pressure, systolic blood pressure was increased in healthy obese individuals in response to a moderate physical activity. Although other studies observed the enhanced response of systolic blood pressure to daily physical activity in obese individual,[95,96] our analysis did not account for the weight status of learners studied. Furthermore, there are other factors, which were not accounted for in the current study, that may have contributed to the variance in blood pressure observed; and these include genetic and early environmental factors (in utero and early infancy).[97] It has been reported that blood pressure increases in response to moderate physical activity, but with prolonged exercise a positive effect can be observed, particularly in hypertensive individuals.[98–100]

A limitation of our study is the cross-sectional design of our study. Cross sectional studies have been reported to be ineffective in demonstrating the relationship between physical activity and obesity or other metabolic parameters.[86] Another limitation of the current study is that puberty was not accounted for in the statistical analyses, possible affecting the findings observed. Apart from ethnic differences in genetic background, it has been reported that puberty and sex hormones affect lipid profi decreasing HDL-C, low density lipoprotein-cholesterol (LDL-C), and increasing TG levels.[101] Serum lipid levels are also influenced by sex hormones in children and adolescents, with lower HDL-C and LDL-C levels associated with increased testosterone in boys and increased estradiol in girls.[102] In addition, the potential endogeneity of physical activity is a limitation to our study, and should be looked at as an extension for future research. As stated in an editorial by Cawley et al,[103] the use of genes as instrumental variables is problematic due to their possible association with many biological pathways, interaction that cannot be all controlled for in regression analyses.

The use of questionnaires to measure physical activity may have introduced bias. Physical activity questionnaires and diaries are more applicable in large epidemiological studies, but they provide less accurate estimates of physical activity level compared with more objective measures (such as doubly-labeled water, indirect calorimetry, or heart rate calibration equations) as the tool relies on (parental or child/adolescent) self-reported information.[104] The type of house chores performed by adolescent learners was not specifi to estimate the intensity of activity involved. Diet patterns were not accounted for, which would have provided an opportunity to assess whether the increase in height was indeed due to the physical activities.

The main fi of the current study is that the minor alleles of MC3R T6K and V81I polymorphisms were associated with lower levels of TC, and that doing house chores often may further reduce this trait but only in the presence of the V81I minor allele. To our knowledge, this is the first South African study that reports an association between TC and MC3R Val81Ile polymorphism. These findings, however, require further investigation in

a larger homogeneous study population to eliminate the possible effect of population stratification. In addition, different ages of puberty onset may also contribute to the difference in lipid profi and body composition observed among children and adolescents of the same gender and age group, and thus affecting statistical analyses if not accounted for. For this reason, it is also important that statistical analyses are adjusted for puberty when further studies are conducted. The current study also supports previous reports that sedentary lifestyle has deleterious outcomes on obesity-related traits as demonstrated by the increase in weight, height, hip circumferences, and marginally with waist circumference in adolescent learners that often engaged in television viewing.

Acknowledgments

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