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Increasing insulin resistance accentuates the effect of triglyceride-associated loci on serum triglycerides during 5 years^S

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Abstract Blood concentrations of triglycerides are influenced by genetic factors as well as a number of environmental factors, including adiposity and glucose homeostasis. The aim was to investigate the association between a serum triglyceride weighted genetic risk score (wGRS) and changes in fasting serum triglyceride level over 5 years and to test whether the effect of the wGRS was modified by 5 year changes of adiposity, insulin resistance, and lifestyle factors. A total of 3,474 nondiabetic individuals from the Danish Inter99 cohort participated in both the baseline and 5 year follow-up physical examinations and had information on the wGRS comprising 39 genetic variants. In a linear regression model adjusted for age, sex, and baseline serum triglyceride, the wGRS was associated with increased serum triglyceride levels over 5 years [per allele effect = 1.3% (1.0–1.6%); $P = 1.0 \times 10^{-17}$]. This triglyceride-increasing effect of the wGRS interacted with changes in insulin resistance ($P_{\text{interaction}} = 1.5 \times 10^{-6}$). This interaction indicated that the effect of the wGRS was stronger in individuals who became more insulin resistant over 5 years. **Conclusion** In conclusion, our findings suggest that increased genetic risk load is associated with a larger

increase in fasting serum triglyceride levels in nondiabetic individuals during 5 years of follow-up. This effect of the wGRS is accentuated by increasing insulin resistance.—Justesen, J. M., E. A. Andersson, K. H. Allin, C. H. Sandholt, T. Jørgensen, A. Linneberg, M. E. Jørgensen, T. Hansen, O. Pedersen, and N. Grarup. **Increasing insulin resistance accentuates the effect of triglyceride-associated loci on serum triglycerides during 5 years.** *J. Lipid Res.* 2016. 57: 2193–2199.

Supplementary key words genetic association • genetic risk score • interaction

A high level of circulating triglycerides is a risk factor for type 2 diabetes, myocardial infarction, ischemic heart disease, and all-cause mortality (1–4). A recent Mendelian randomization study supports a causal effect of triglycerides on coronary heart disease risk (5). Regulation of blood levels of triglycerides involves a complex interplay implicating both the liver and pancreas. Some of the important players in this regard are body adiposity, insulin resistance, and glucose homeostasis (6). In addition, circulating triglyceride levels are heritable, as shown in a recent population-based study of twins ($H^2 = 0.59$; $h^2 = 0.33$) (7). Genetic association studies have identified more than 40 SNPs

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Abbreviations: GRS, genetic risk score; HOMA-IR, homeostatic model assessment of insulin resistance; IQR, interquartile range; OGTT, oral glucose tolerance test; wGRS, weighted genetic risk score.

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influencing levels of triglycerides (8–10), which together explain up to 9.3–9.6% of the variance in fasting serum triglyceride concentrations estimated from a random sample of adults from the FINRISK cohort (8) and in the Framingham Heart study (9).

It is increasingly acknowledged that prevention and treatment of metabolic diseases are not equally efficacious in all people and thus the concept called “precision medicine” has lately received a lot of attention. In order to fulfill the requirement of tailored treatment and prevention, one important first step is to understand the pathogenic mechanism of genetic variants and their complex interplay with the environmental factors that each individual is continuously facing.

While many studies have consolidated the effect of genetic risk variants in cross-sectional studies, fewer studies have focused on prospective analyses (11–13) and interactions with metabolic and lifestyle factors (14–17). A few studies based on genetic risk scores (GRSs) with 32 and 40 triglyceride-associated SNPs, respectively, have found that a higher genetic load associates with increased concentration of triglycerides over 9 and 10 years of follow-up (12, 13). Very few interactions with metabolic and lifestyle factors have been investigated in prospective studies for the full triglyceride GRS. One study performed prospective age-GRS interaction analyses and found that the effect of the triglyceride GRS on levels of triglycerides was stronger in younger (age: 45–54 years) participants than in older (age: 55–64 years) (12). Another study tested a combined LDL-cholesterol, HDL-cholesterol, and triglyceride GRS of 32 SNPs for a differential effect on lipid levels during one year in response to lifestyle or metformin treatment in the Diabetes Prevention Program (18). No genetic modulation of the triglyceride response was observed in this study for the combined lipid GRS.

We and others have reported interactions with triglyceride GRS in the cross-sectional setting, including interactions with BMI and insulin resistance using homeostatic model assessment of insulin resistance (HOMA-IR) (14–17, 19). Taken together these cross-sectional results point toward a bigger effect of the triglyceride GRS on triglycerides in obese individuals compared with lean individuals. However, because obesity and insulin resistance often coincide and are associated with lifestyle-related parameters, it is uncertain which of these factors is more relevant for the interactions observed. Moreover, a recent study has suggested that the interaction between triglyceride GRS and BMI is stronger in women (17).

The aim of this study was to investigate whether the complex interplay between blood triglyceride levels and insulin resistance and obesity could be modified by genetic factors. With the present analyses, we sought to investigate interactions between a GRS for triglyceride and obesity/insulin resistance modeled in a dynamic setting with changes occurring over 5 years.

Using the prospective Inter99 cohort, we specifically aimed to evaluate the effect of a triglyceride weighted GRS (wGRS) on changes in fasting serum triglyceride levels during a 5 year follow-up period and to assess whether the effect of the wGRS on changes in triglycerides was modified

by changes in adiposity, insulin resistance, or the lifestyle factors (physical activity, dietary habits, smoking habits, and alcohol intake).

MATERIALS AND METHODS

Inter99 study population

The Inter99 study (clinicaltrials.gov identification number: NCT00289237) is a population-based nonpharmacological intervention study for ischemic heart disease conducted at the Research Centre for Prevention and Health in Glostrup, Denmark (<https://www.regionh.dk/rcph/population-based-epidemiology/Pages/The-Inter99-Study.aspx>) (20). A random sample of 13,016 individuals living in Copenhagen County (aged 30–60 years) was drawn from the Civil Registration System, and further prerandomized into high-intensity (90%) and low-intensity (10%) intervention groups. The baseline health examination was attended by 6,784 (52%) (median age 45 years). All participants received individual lifestyle counseling at the baseline examination, focused on habits of smoking, physical activity, diet, and use of alcohol. The high intensity group was, in addition, offered group-based lifestyle counseling if they were considered at high risk for ischemic heart disease. Follow-up examinations were conducted after 5 years with a participation rate of 66% ($n = 4,511$) (21, 22). This intervention program was of rather low intensity with examinations and individual tailored lifestyle counseling up to four times over 5 years. Those at high risk of ischemic heart disease based on predefined criteria were, in addition, offered six sessions of group-based lifestyle counseling. The intervention had no effect on primary and secondary endpoints: ischemic heart disease, stroke, and all-cause mortality after 10 years (23).

A total of 3,474 individuals had information on the wGRS and fasting serum triglyceride levels at baseline and follow-up. Descriptions of study participants are shown in supplemental Table S1. All participants were Danes by self-report. Written informed consent was obtained from all participants and the study was approved by the Scientific Ethics Committee of the Capital Region of Denmark (Inter99: KA98155) and was in accordance with the principles of the Declaration of Helsinki II.

Biochemical and anthropometric measures

At the baseline and 5 year follow-up health examination, body weight (in kilograms) and height (to the nearest 0.5 cm) were measured in light indoor clothing and without shoes. BMI was defined as body weight in kilograms divided by height in meters squared (kg/m^2). Blood samples were drawn in the morning after a 12 h overnight fast. All participants without previously diagnosed diabetes were characterized by a standardized 75 g oral glucose tolerance test (OGTT) with plasma glucose and serum insulin measured at fasting and 30 and 120 min after the oral glucose load. A description of assays for measurements for glucose, insulin, and lipids has been published (20, 21) and the assays were similar at baseline and at follow-up. LDL-cholesterol was calculated by the Friedewald equation if triglycerides were <4.56 mmol/l (24). Individuals receiving lipid-lowering treatment (of 3,474 individuals: baseline, $n = 28$; follow-up, $n = 123$; combined, $n = 125$) had lipid levels corrected by the statin constants proposed by Wu et al. (25): HDL-cholesterol, -0.059 mmol/l; LDL-cholesterol, 1.279 mmol/l; total cholesterol, 1.336 mmol/l; and triglyceride, 0.207 mmol/l. The participants did not give information on the type of lipid lowering medication, but Danish guidelines state that statins should be the first-line drug of choice (26). Sensitivity analyses were performed by excluding individuals

receiving lipid lowering medication ($n = 125$ in the present analyses). HOMA-IR was calculated as: [fasting plasma glucose (mmol/l) \times fasting serum insulin (pmol/l)]/135 (27).

Lifestyle measures

Dietary habits, alcohol consumption, physical activity, and smoking were all estimated from questionnaire data and calculated into discrete score variables. A three-point dietary score was derived from a validated food frequency questionnaire. The scores were constructed as: 1, unhealthy; 2, moderately healthy; and 3, healthy. A higher dietary score was related to a lower intake of saturated fat and a higher intake of vegetables, fruit, and fish (28). The self-reported total intake of alcoholic beverages per week was calculated into units of ethanol (1 unit = 12 g) and divided into a score from 1 to 4; 1, abstinent; 2, less than 14/21 (women/men) units per week; 3, more than 14/21 (women/men) units per week and less than 35 units per week; and 4, more than 35 units per week. The level of physical activity was estimated from time spent actively commuting (minutes per week) and time spent on leisure time physical activity (minutes per week) (21). Subsequently, a physical activity score from 1 to 4 was created: 1, 0–113 min/week; 2, 143–225 min/week; 3, 255–340 min/week; and 4, 450–720 min/week (29). Smoking habits were divided into a score from 1 to 4; 1, habitual/daily smoker; 2, occasional smoker; 3, former smoker; and 4, nonsmoker (30). From the scores described above, 5 year changes in each lifestyle measure were individually defined into three classes as follows: class 1, healthier if the 5 year lifestyle score was calculated to be better than at baseline; class 2, no change if the 5 year lifestyle score was the same as the baseline; or class 3, unhealthier if the 5-year lifestyle score was calculated to be worse than the baseline.

SNP selection and genotyping

The SNPs were selected based on Teslovich et al. (9) and Willer et al. (10) that have confirmed 40 loci associating with circulating fasting levels of triglycerides at genome-wide significance ($P < 5 \times 10^{-8}$). Of the identified triglyceride risk variants, one variant (*CTF1* rs11649653) was not present and was not captured by any proxies ($r^2 \geq 0.80$) on Illumina Cardio-Metabochip or Human Exome BeadChip. Hence, we included 39 loci, of which 4 were proxies ($r^2 \geq 0.97$). Proxy search was performed based on Hap-Map and 1000 Genomes Pilot 1 data for linkage disequilibrium estimation using SNP annotation proxy search (SNAP; available from <http://www.broadinstitute.org/mpg/snap/>). An overview of variants used in the present study is given in supplemental Table S2.

Individuals from Inter99 were genotyped using the Cardio-Metabochip (31) and Human Exome BeadChip on an Illumina HiScan system (Illumina, San Diego, CA). Quality control and genotype calling have previously been described (14).

GRS

The effect of multiple genetic risk loci was studied by constructing a wGRS as previously described (32). We used reported effect sizes (9, 10) for each SNP to weight the contribution of each risk allele, which was defined as the allele reported to be associated with increased levels of fasting triglyceride. Proxies were weighted using the reported effect sizes for lead SNPs, because these reflect the same association signal (supplemental Table S2). Analyses were performed using the wGRS on a continuous scale. Individuals with more than two missing genotypes were excluded ($n = 20$). For individuals with one ($n = 73$) or two ($n = 25$) missing genotypes, genotypes were imputed by assessing the most frequent genotype in Inter99 for the specific variant. The mean number of risk alleles was 38.5 ranging from 24 to 53.

Statistical analyses

The statistical analyses were performed using the statistical software R, version 3.2.1 (<http://www.r-project.org/>). Individuals were included in the analyses only if they had nondiabetic glucose values in the fasting condition and 2 h following an OGTT according to World Health Organization 1999 criteria (33) both at the baseline and the follow-up examination. A total of 504 individuals were excluded. Paired *t*-tests were used to test for differences in serum triglyceride from baseline to follow-up. We applied three linear regression models to test the effect of the wGRS on 5 year changes in serum triglyceride levels: Model 1, a simple model including only the wGRS, sex, age, baseline triglyceride, and intervention program of Inter99: 5 year triglyceride = $\beta_0 + \beta_1$ wGRS + β_2 age + β_3 sex + β_4 baseline triglyceride + β_5 intervention + ϵ . Model 2, as model 1 and also including baseline BMI, baseline HOMA-IR, 5 year BMI, and 5 year HOMA-IR. Model 3, a fully adjusted model as model 2 and also including baseline waist circumference, baseline HDL-cholesterol, baseline LDL-cholesterol, 5 year waist circumference, 5 year HDL-cholesterol, 5 year LDL-cholesterol, and baseline and 5 year lifestyle scores for smoking habits, alcohol intake, diet, and physical activity. Values of serum triglyceride, serum total cholesterol, serum HDL-cholesterol, serum LDL-cholesterol, and HOMA-IR were logarithmically transformed to approach a normal distribution. Additive interactions between wGRS and changes in metabolic and lifestyle traits were tested by introducing interaction terms (wGRS \times baseline trait value and wGRS \times 5 year trait value) in the association model 1: 5 year triglyceride = $\beta_0 + \beta_1$ wGRS + β_2 age + β_3 sex + β_4 baseline triglyceride + β_5 intervention + β_6 baseline trait value + β_7 5 year trait value + (β_8 wGRS \times baseline trait value) + (β_9 wGRS \times 5 year trait value) + ϵ . Sensitivity analyses were performed by doing inverse normal transformation for the levels of serum triglyceride values and HOMA-IR to ensure that results were not driven by artifacts arising from skewed distributions. A *P* value was considered significant if below the Bonferroni corrected threshold of $P = 0.006$ (corrected for eight tests).

RESULTS

Genetic associations with 5 year changes in fasting serum triglyceride level

A total of 3,474 individuals had information on the wGRS and fasting serum triglyceride levels at baseline and follow-up. A description of study participants is shown in supplemental Table S1. During the 5 years, these participants, on average, increased in fasting serum triglyceride [5.4% (4.0; 6.7), $P = 9.0 \times 10^{-15}$]. Furthermore, on average, the BMI, total cholesterol, and HDL-cholesterol increased, while LDL-cholesterol and HOMA-IR decreased. Descriptions of average changes in the population are shown in supplemental Tables S3–S5.

The distribution of the wGRS in the study population is shown in supplemental Fig. S1. The wGRS was positively associated with changes in triglyceride levels over a 5 year period (Table 1). In a model adjusted for age, sex, intervention, and baseline triglycerides, each additional weighted risk allele was associated with an increase in serum triglyceride level of 1.3% (1.0–1.6%), $P = 1.0 \times 10^{-17}$ ($n = 3,474$) (Table 1), this corresponds to 0.009–0.018 mmol/l for the interquartile range (IQR) of the distribution. The association increased in strength and magnitude

TABLE 1. Associations between the wGRS and changes in serum triglyceride level over 5 year follow-up in the Inter99 cohort

	Model 1	Model 2	Model 3
Effect of wGRS (95% CI)	1.3% (1.0–1.6)	1.5% (1.2–1.8)	1.2% (0.9–1.4)
Effect in mmol/l IQR	0.009–0.018	0.01–0.02	0.008–0.017
<i>P</i>	1.0×10^{-17}	1.0×10^{-27}	1.4×10^{-15}
<i>n</i>	3,474	3,352	2,638

Data is effect per weighted risk allele given in percent (95% CI) and the mean effect translated into millimoles per liter for IQR of the baseline distribution and *P* values of three different models. Three models were analyzed: model 1, a simple model including only the GRS, sex, age, intervention, and baseline triglyceride; model 2, a model including GRS, sex, age, intervention, baseline triglyceride, baseline and 5 year BMI, and baseline and 5 year HOMA-IR; model 3, a fully adjusted model including GRS, sex, age, intervention, baseline triglyceride, baseline and 5 year BMI, baseline and 5 year HOMA-IR, baseline and 5 year waist circumference, baseline and 5 year HDL-cholesterol, baseline and 5 year LDL-cholesterol, baseline and 5 year smoking habits, baseline and 5 year alcohol intake, baseline and 5 year dietary score, and baseline and 5 year physical activity.

when the model was additionally adjusted for baseline and 5 year BMI and baseline and 5 year HOMA-IR [1.5% (1.2; 1.8), $P = 1.0 \times 10^{-27}$ ($n = 3,352$)] (Table 1). A fully adjusted model, additionally including baseline and 5 year waist circumference, baseline and 5 year HDL-cholesterol, baseline and 5 year LDL-cholesterol, baseline and 5 year alcohol intake, baseline and 5 year smoking habits, baseline and 5 year physical activity, and baseline and 5 year dietary score, resembled the results of model 1 [1.2 (0.9–1.4), $P = 1.4 \times 10^{-15}$ ($n = 2,638$)] (Table 1). Excluding individuals treated with lipid lowering medication produced similar results (data not shown).

Interactions

To explore whether the effect of the wGRS on serum triglycerides was affected by degree of adiposity, insulin resistance, and lifestyle factors, we tested for potential interactions. Main effect analysis is shown in supplemental Table S6. We fitted separate interaction models for the changes in adiposity (BMI and waist circumference), change in insulin resistance (HOMA-IR), and changes in lifestyle factors (physical activity, smoking habits, dietary score, and alcohol intake) (Table 2). In a model adjusted for age, sex, intervention, and baseline triglyceride, we found an interaction between the wGRS and change in HOMA-IR in relation to 5 year changes in fasting serum triglyceride levels ($P_{int} = 1.5 \times 10^{-6}$) (Table 2). An increase in HOMA-IR accentuated the effect of the wGRS on 5 year change in fasting serum triglyceride (Fig. 1). In the lowest quartile of Δ HOMA-IR, the effect of the wGRS was 0.7%

(0.2; 1.3), $P = 0.01$ per risk allele, while it was 1.7% (1.2; 2.3), $P = 2.7 \times 10^{-9}$ in the highest quartile of Δ HOMA-IR (Fig. 1).

The effect of the wGRS on changes in fasting serum triglycerides also showed an interaction with changes in BMI and physical activity $P_{int} < 0.05$, but these were not significant after correction for multiple testing. No other interactions with changes in waist circumference and changes in lifestyle factors were observed ($P_{int} > 0.05$) (Table 2).

As it has previously been suggested that the interaction of the triglyceride GRS with BMI on serum triglycerides is stronger in women (17), we tested our interaction with HOMA-IR in men and women separately. In men, the interaction between wGRS and HOMA-IR on changes in serum triglyceride was only borderline significant ($P_{int} = 0.025$), while this interaction was highly significant in women ($P_{int} = 2.3 \times 10^{-5}$).

The wGRS was not associated with changes in HOMA-IR in a model adjusted for age, sex, intervention, and baseline HOMA-IR ($P = 0.46$). Sensitivity analyses, excluding individuals treated with lipid lowering medication, did not change the results (data not shown). When analyzing the interactions using inverse normal transformation instead of logarithmic transformation for the skewed variables, the interaction between wGRS and HOMA-IR on change in fasting serum triglycerides remained significant ($P_{int} = 6.5 \times 10^{-4}$). Analyses of relevant metabolic factors affecting change in HOMA-IR are shown in supplemental Table S7.

DISCUSSION

In the present study, we have performed prospective analyses of a wGRS comprising 39 triglyceride-increasing variants in the nondiabetic segment of the Danish Inter99 cohort. We have examined how triglyceride-raising loci predict longitudinal changes in fasting serum triglyceride levels over 5 years and some of the factors modifying this relationship. We found that a wGRS of triglyceride-increasing alleles was associated with increased levels of fasting serum triglyceride over 5 years in a middle-aged Danish population. Moreover, in this study, we showed that individuals carrying an increased load of triglyceride-increasing genetic variants have an accentuated effect of this unfavorable genetic predisposition when they increase

TABLE 2. Interactions between wGRS and changes in adiposity, insulin resistance, and lifestyle factors in relation to 5 year changes in serum triglyceride levels in the Inter99 cohort

	<i>P</i> Interaction	<i>n</i>
BMI	0.03	3,473
Waist circumference	0.07	3,474
HOMA-IR	1.5×10^{-6}	3,353
Physical activity	0.03	3,227
Smoking habits	0.20	3,452
Dietary score	0.05	3,339
Alcohol intake	0.96	3,057

Seven separate interaction models were analyzed by introducing the interaction terms wGRS \times baseline trait and wGRS \times 5 year trait into model 1 (including only the wGRS, age, sex, intervention, and baseline serum triglyceride and the interaction terms).

Effect of the wGRS on 5-year changes in serum triglycerides by quartiles of HOMA-IR change

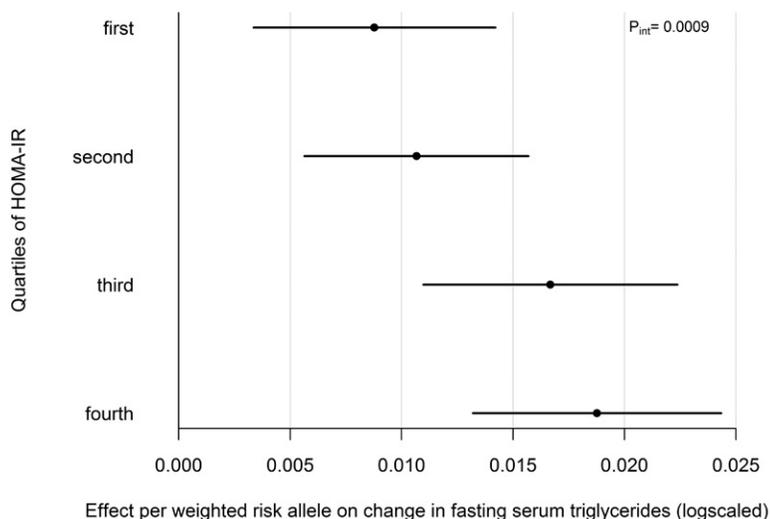


Fig. 1. Visualization of the interaction between the wGRS and change in HOMA-IR levels over 5 years. The effect of the wGRS on changes in serum triglyceride levels is shown by quartiles of HOMA-IR change. Values of HOMA-IR and serum triglyceride were logarithmically transformed before calculating the change and the change is thus calculated as the proportional (percentage) change relative to baseline value. The first quartile comprises individuals having $\geq 35\%$ lower follow-up HOMA-IR compared with baseline. The second quartile includes individuals having between 8% and 35% lower follow-up HOMA-IR. The third quartile comprises the group of individuals having between 8% lower and 31% higher follow-up HOMA-IR. The fourth quartile includes the group of individuals having 31% higher follow-up HOMA-IR. P_{int} denotes the interaction P value expressing the interaction between wGRS and quartiles of Δ HOMA-IR in model 1 (including only the wGRS, age, sex, and baseline serum triglyceride).

their insulin resistance over the same 5 year period of follow-up. In other words, improvement of insulin sensitivity attenuates the effect of the wGRS on levels of serum triglycerides.

Two previous studies have reported a triglyceride-increasing effect of a wGRS over 9 and 10 years in studies involving 9,328 and 3,495 individuals, respectively (12, 13). In the GLACIER study cohort, the reported effect per allele was an increase of 0.02 mmol/l in fasting serum triglyceride over 10 years (13). This per allele effect corresponds to the effect we observe in the present study. In raw values, individuals in the highest quintile (10%) of genetic risk had a mean increase of serum triglycerides of 0.061 mmol/l over 5 years, while those in the lowest quintile had a mean increase of 0.036 mmol/l.

In children, a triglyceride GRS has also been shown to have an effect on serum triglyceride level already at 3–6 years of age, but with a lesser effect than in adulthood (11). This stronger effect in adulthood was not observed for GRSs for serum levels of HDL-cholesterol, LDL-cholesterol, and total cholesterol, indicating that the triglyceride GRS may be a good candidate for gene-environment interactions in the adults. Therefore, we sought to determine whether the genetic predisposition to elevated triglyceride levels was modified by changes in adiposity, insulin resistance, and lifestyle factors. We found that changes in insulin resistance (HOMA-IR) modified the effect of the wGRS. With increasing insulin resistance over 5 years, the effect of the triglyceride wGRS was stronger.

Previous studies of interactions in cross-sectional settings have found comparable interactions suggesting a stronger association between the wGRS and triglycerides in obese or in more insulin-resistant individuals (14–17, 19). In this study, we demonstrate that the interaction with HOMA-IR is also evident in a prospective setting consolidating the results from previous studies using cross-sectional data. We did not observe an interaction with BMI or waist circumference in the prospective setting over 5 years. One explanation may be that adiposity is changing at a slower pace and

that 5 years is not a sufficient follow-up period to observe the dynamic interaction. The previously reported interactions with both BMI and insulin resistance may represent the same underlying interaction because obese individuals are also likely to be insulin resistant. Underlining the complexity, the triglyceride wGRS is reported to associate with decreased risk of type 2 diabetes and with lower levels of HOMA-IR when adjusting for circulating levels of triglycerides (34, 35).

We found a borderline interaction with changes in physical activity; i.e., those decreasing their physical activity had a stronger effect of the wGRS on triglyceride levels. This suggestive finding could be a potential false-negative finding because of low power in the statistical analyses. Because peripheral insulin action is strongly linked to physical activity level, the change in physical activity may partly be explanatory for the interaction with both BMI and insulin resistance observed in this and previous studies. It would be of interest to follow-up on this suggestive finding in a larger cohort and preferentially with objectively measured physical activity. Also of notice, it has recently been suggested that the interaction between the triglyceride wGRS and BMI is stronger in women (17). In the present study, we confirm this observation also for the interaction between the wGRS and HOMA-IR.

Our study provides an example that an increase in insulin resistance has a more adverse effect on blood triglycerides for individuals with a specific genetic predisposition to increased triglycerides. From a public health perspective, it could be anticipated that recommendations about staying insulin sensitive, e.g., by doing exercise, would be more strongly encouraged or even prescribed to individuals at a high genetic risk. Exercise has a well-known effect on improving insulin sensitivity and is a factor that is relatively easily modified. Other factors that are suggested to be involved in insulin resistance and which could, in theory, be modifiable are adiposity (modified by both diet and exercise), emotional stress, sleep, and the gut microbiome (36–38). However, at present, genetic studies of complex traits

often suffer from the limitations that variants are of modest effects and together they only explain a small proportion of the variance of the traits examined (39). Estimated from genome-wide association studies, the common genetic variants analyzed in the present study only explain ~8–10% of the total variance of baseline serum triglyceride levels (8, 9). Thus, the translation of our genetic findings into direct clinical application will not be relevant until a sufficient number of genetic variants that can explain a larger proportion of the variance in triglycerides are identified in the future.

Although genetic effects reported in the current study are not currently clinically applicable, it is of importance to understand the complex homeostatic interplay between metabolic factors such as triglyceride levels in blood and insulin resistance. Increased biological understanding will pave the way for more efficient treatment of the metabolic disturbances associated with glucose and lipid metabolism. One suggested pathway of interest, when trying to resolve the underlying biology of the observed pattern of interaction, is resembled by that suggested for mutations in *GCKR*. For these mutations, it is suggested that the process of insulin resistance is associated with increased glucose uptake driving de novo lipogenesis in the liver (40). Further studies are needed to underline other molecular pathways potentially causing a similar interaction pattern.

The major strength of the current study is the longitudinal follow-up in a well-phenotyped homogeneous population. The study includes repeated examinations with OGTTs, anthropometric measurements, and extensive characterization of lifestyle. Some limitations also apply to the current study. Individuals participating in a study such as Inter99 may be a distinctive group of individuals with a higher awareness of their health (41). Moreover, individuals who participated in the follow-up examination after 5 years had a lower body weight and overall a healthier lifestyle at baseline compared with nonattendees (42). Therefore we cannot conclude that the results of the present study are directly transferrable to the general population as a whole. Further, because our sample consisted of middle-aged participants of European ancestry, the results may not be generalizable to populations of different age ranges and to non-Caucasian individuals. In the present study, we only assessed the combined effect of genetic variants and this is, of course, a simplification of the true nature of the genetic susceptibility underlying triglyceride levels. Because the full GRS has a measureable effect from early in life (11, 43), the likely scenario may be that some of the triglyceride-increasing alleles exert their effects at an early age, while others become increasingly important later in life when the burden of other risk factors, such as insulin resistance and obesity, increases. It seems likely that some loci are interacting with insulin resistance, while others are not. Specific loci have been suggested to have this pattern of interaction with obesity and insulin resistance in cross-sectional studies, e.g., *GCKR* and *APOA5* (15, 16, 19). Larger studies are needed to elucidate potential interactions for all loci separately.

In conclusion, in nondiabetic middle-aged people, increased genetic risk is associated with a larger increase in fasting serum triglyceride levels during 5 years. The genetic risk of increased triglycerides during 5 years of follow-up is modified by insulin resistance; i.e., in individuals who become more insulin resistant over 5 years, the triglyceride-increasing effect of the wGRS is accentuated.

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