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SHORT COMMUNICATION

Circulating β-carotene levels and type 2 diabetes—cause or effect?

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Abstract
Aims/hypothesis Circulating β-carotene levels are inversely associated with risk of type 2 diabetes, but the causal direction of this association is not certain. In this study we used a Mendelian randomisation approach to provide evidence for or against the causal role of the antioxidant vitamin β-carotene in type 2 diabetes.
Methods We used a common polymorphism (rs6564851) near the *BCMO1* gene, which is strongly associated with circulating β-carotene levels ($p=2\times10^{-24}$), with each G allele associated with a 0.27 standard deviation increase in levels. We used data from the InCHIANTI and Uppsala Longitudinal Study of Adult Men (ULSAM) studies to estimate the association between β-carotene levels and type 2 diabetes. We next used a triangulation approach to estimate the expected effect of rs6564851 on type 2 diabetes risk and compared this with the observed effect using data from 4549 type 2 diabetes patients and 5579 controls from the Diabetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium.
Results A 0.27 standard deviation increase in β-carotene levels was associated with an OR of 0.90 (95% CI 0.86–0.95)

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for type 2 diabetes in the InCHIANTI study. This association was similar to that of the ULSAM study (OR 0.90 [0.84–0.97]). In contrast, there was no association between rs6564851 and type 2 diabetes (OR 0.98 [0.93–1.04], p=0.58); this effect size was also smaller than that expected, given the known associations between rs6564851 and β-carotene levels, and the associations between β-carotene levels and type 2 diabetes.

Conclusions/interpretation Our findings in this Mendelian randomisation study are in keeping with randomised controlled trials suggesting that β-carotene is not causally protective against type 2 diabetes.

Keywords β-Carotene · Mendelian randomisation · Type 2 diabetes

Abbreviations

DGI	Diabetes Genetics Initiative
DIAGRAM	Diabetes Genetics Replication And Meta-analysis
FUSION	Finland–United States Investigation of NIDDM Genetics
SNP	Single nucleotide polymorphism
ULSAM	Uppsala Longitudinal Study of Adult Men
WTCCC	Wellcome Trust Case Control Consortium

Introduction

Circulating β-carotene levels are associated with type 2 diabetes, but the causal direction of this association is disputed. Recently, Ärnlöv et al. reported results of a longitudinal community-based study, Uppsala Longitudinal Study of Adult Men (ULSAM), assessing effect of serum and dietary β-carotene on the incidence of type 2 diabetes [1]. This study observed a strong association between increased baseline serum levels of β-carotene at age 50 years and reduced type 2 diabetes incidence during 27 years of follow-up. For a 1 SD increase in serum β-carotene, the authors observed a protective effect with an OR of 0.68 (95% CI 0.53–0.89). They also reported that a 1 SD increase in β-carotene levels at age 50 years was associated with improved insulin sensitivity at age 70 years in non-diabetic individuals. Ärnlöv et al. argued that these associations support the importance of impaired antioxidant status for the development of insulin resistance and type 2 diabetes. They also suggested that antioxidants could be involved early in the pathological processes leading to diabetes and that it takes a long period of exposure to low antioxidant levels before metabolic factors are affected. These findings are consistent with some but not all observational epidemiological reports on the role of β-carotene levels in type 2 diabetes. Previous studies have provided evidence that β-carotene is not causally associated with type 2 diabetes [2, 3]. Notably three placebo-controlled trials, the Physicians' Health Study [4], the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study [5] and the Women's Antioxidant Cardiovascular Study [6] have all reported null effects of mega-dose β-carotene supplementation on adverse metabolic effects, including type 2 diabetes.

A caveat to observational epidemiological studies is that associations between risk factors and disease incidence many years later do not necessarily strengthen the case that the risk factor is causal. Disease processes can begin many years before disease diagnosis, with adverse metabolic effects being reported as early as the first decade of life [7]. Confounding factors may also result in a misleading association between antioxidant vitamins and adverse metabolic outcomes such as diabetes. We note that the association between β-carotene levels and type 2 diabetes in the ULSAM study was stronger before correcting for BMI, self-reported physical activity and smoking status [1].

Genetics studies may be able to help dissect the causal directions of disease biomarker associations. Genotypes cannot be influenced by disease status or any other trait, making them much less likely than non-genetic factors to be confounded or to be the result of reverse causation. This principle of 'Mendelian randomisation' has been applied before to indicate that C-reactive protein is unlikely to have a causal role in the development of various metabolic traits [8]. More recently it has also been applied to examine the possible associations between a range of inflammatory proteins and type 2 diabetes, with the authors finding no evidence of a causal role of inflammatory or autoimmune factors, including interleukin 18, on type 2 diabetes risk, [9].

Here, we have used a Mendelian randomisation approach to help dissect the causal role of β-carotene in type 2 diabetes risk (Fig. 1). To do this we used: (A) a common polymorphism (rs6564851) near the *BCMO1* gene, recently identified as strongly associated with circulating β-carotene levels; (B) an estimate of the association between β-carotene levels and type 2 diabetes using two studies; (C) an estimate of the expected effect of rs6564851 on type 2 diabetes risk given (A) and (B); and finally (D) a large case–control study to assess the observed effect of the β-carotene-associated single nucleotide polymorphism (SNP) on type 2 diabetes.

Methods

SNP–β-carotene association We recently reported results from a genome-wide association study that identified a

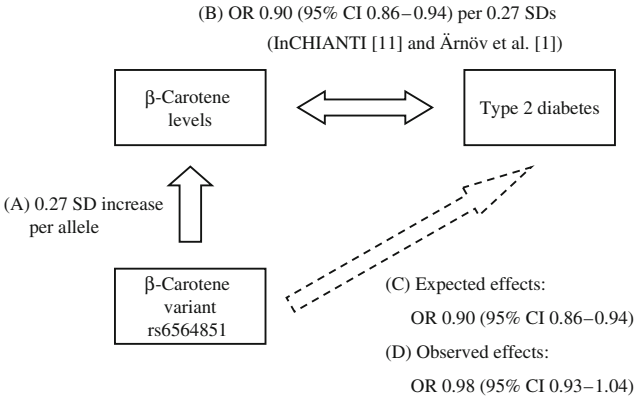

Fig. 1 Triangulation of β-carotene levels and risk of type 2 diabetes. Associations between: the SNP rs6564851 and β-carotene levels; β-carotene levels and type 2 diabetes; and the expected and observed effects of rs6564851 on type 2 diabetes. Odds ratios for the association between β-carotene and type 2 diabetes were estimated for a 0.27 SD increase in β-carotene

polymorphism near the *BCMO1* gene as robustly associated with fasting serum β-carotene levels (rs6564851, $p=2\times10^{-24}$, 0.15 mmol/l per allele effect) [10]. The finding was consistent across three studies including individuals from across the adult age range. Using discovery and replication data combined, each G allele at rs6564851 was associated with a 0.27 SD increase in β-carotene levels.

β-Carotene–type 2 diabetes association To obtain an estimate of the association between circulating β-carotene and type 2 diabetes, we used data from Ärnlöv et al. [1] and unpublished data from the InCHIANTI study [11]. For InCHIANTI, age- and sex-adjusted *z* scores were produced for fasting serum β-carotene levels ($n=1191$). Of these 1191 individuals, 112 had clinically defined type 2 diabetes. Linear regression was used to estimate the correlation between β-carotene levels and type 2 diabetes risk. Within the InCHIANTI cohort, a 1 SD increase in circulating β-carotene was associated with reduced type 2 diabetes risk (OR 0.68 [95% C.I 0.56–0.82]). This was similar to the findings of Ärnlöv et al. in their combined (lifestyle and metabolic covariates) model (OR 0.68 [0.53–0.89]) [1].

Estimated SNP–type 2 diabetes association Given the common polymorphism (rs6564851) near the *BCMO1* gene and our estimate of the association between β-carotene levels and type 2 diabetes using two studies, we calculated that, if circulating β-carotene levels were causally involved in type 2 diabetes, a SNP with a 0.27 SD increase in circulating levels should give a reduced type 2 diabetes risk of approximately OR 0.90 (95% CI 0.86–0.94).

These estimated ORs and CIs were calculated by meta-analysis of the two effect estimates of a 1 SD increase in β-carotene levels on type 2 diabetes risk from the InCHIANTI [11] and ULSAM studies [1]. To estimate a 0.27 SD effect, we then multiplied 0.27 by the 1 SD OR effect sizes on the natural log scale, e.g. $\exp(0.27\times\ln[0.68])=0.90$.

Observed SNP–type 2 diabetes association We used data from the published dataset of 4549 type 2 diabetes patients and 5579 controls from the Diabetes Genetics Replication And Meta-analysis (DIAGRAM) consortium [12] to calculate an observed effect of rs6564851 on type 2 diabetes risk (for details of the three cohorts: Wellcome Trust Case Control Consortium (WTCCC), Finland–United States Investigation of NIDDM Genetics (FUSION), Diabetes Genetics Initiative (DGI), see Electronic supplementary material [ESM] Table 1 and Zeggini et al. [12]). Within the DIAGRAM meta-analysis data, rs6564851 was directly genotyped in one of three studies (FUSION) and passed all imputation-QC criteria (minor allele frequency ~ 45%, DGI r^2hat=0.76, WTCCC average_maximum_posterior_call=0.96) in the two studies which imputed it.

Results

We did not observe any association between the β-carotene SNP rs6564851 and type 2 diabetes risk (OR 0.98 [95% CI 0.93–1.04], $p=0.58$). Each β-carotene-raising allele of the SNP was associated with a point estimate effect size (OR 0.98) outside the effect range predicted from the circulating levels estimate (0.86–0.94). The individual effect estimates for each of the three DIAGRAM studies is presented in Fig. 2.

Discussion

Our data provide evidence that life-long exposure to modestly lower β-carotene levels does not increase the risk of type 2 diabetes. Our results are in keeping with the negative results from randomised controlled trials [4–6].

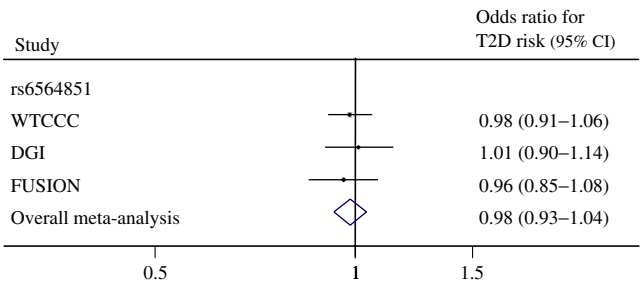

Fig. 2 Three study DIAGRAM [12] results for effect of rs6564851 on type 2 diabetes (T2D) risk. Odds ratio effect based on β-carotene-raising G allele

We suggest that the associations between β-carotene and type 2 diabetes are more likely to be confounded or the consequence of diabetes disease processes rather than aetiological. It is well accepted that observational epidemiological studies can be confounded even when they account for multiple covariates. Imperfect measurement of known and no measurement of unknown confounding factors can often result in spurious associations. It is also now well-known that disease processes can begin long before diagnosis and that metabolic disease processes clearly cause many secondary metabolic changes [7]. A build-up of disease processes over many years could mean that long-term prospective studies are not immune from reverse causation. These factors could explain the difference in results between many of the observational epidemiology studies [13–16] and the randomised controlled trials and genetic studies [4–6].

There are limitations to our Mendelian randomisation approach [17]. The main one is that the approach tests the effects of life-long altered exposure to modest differences in levels, which could mean the body adapts early to the altered state, which then has no adverse effect. Studies of common gene variants that alter LDL-cholesterol and have subtle, life-long effects on LDL-cholesterol, while also altering the risk of coronary heart disease [18, 19], suggest this is not necessarily a concern. Importantly, the weakness of a Mendelian randomisation approach may also be a strength, depending on the disease mechanism, since Mendelian randomisation is likely to be testing the effects of small changes over a longer time compared with randomised controlled trials, which compare the effects of a larger change over a much shorter time. It is also possible that altered intra-cellular levels, which are not accounted for by Mendelian randomisation approaches, could have a disease effect. A further limitation is that the association between β-carotene levels and type 2 diabetes is based on a relatively small number of cases and controls with wide confidence intervals. However, the fact that two studies have very similar results suggests that the point estimate of the uncorrected association between β-carotene levels and type 2 diabetes is a good approximation of the real association in the whole population.

Conclusion

We suggest that the associations between β-carotene and type 2 diabetes are more likely to be confounded or the consequence of diabetes processes rather than aetiological. A combination of randomised supplementation trials and Mendelian randomisation studies together provides a powerful argument that the antioxidant β-carotene is unlikely to be causally involved in the pathogenesis of type 2 diabetes.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Ärnlöv J, Zethelius B, Riserus U et al (2009) Serum and dietary beta-carotene and alpha-tocopherol and incidence of type 2 diabetes mellitus in a community-based study of Swedish men: report from the Uppsala Longitudinal Study of Adult Men (ULSAM) study. Diabetologia 52:97–105
2. Kataja-Tuomola M, Sundell JR, Mannisto S et al (2008) Effect of alpha-tocopherol and beta-carotene supplementation on the incidence of type 2 diabetes. Diabetologia 51:47–53
3. Reunanen A, Knekt P, Aaran RK, Aromaa A (1998) Serum antioxidants and risk of non-insulin dependent diabetes mellitus. Eur J Clin Nutr 52:89–93
4. Liu S, Ajani U, Chae C et al (1999) Long-term beta-carotene supplementation and risk of type 2 diabetes mellitus: a randomized controlled trial. Jama 282:1073–1075
5. The ATBC Cancer Prevention Study Group (1994) The alpha-tocopherol, beta-carotene lung cancer prevention study: design, methods, participant characteristics, and compliance. The ATBC Cancer Prevention Study Group. Ann Epidemiol 4:1–10
6. Song Y, Cook NR, Albert CM, Van Denburgh M, Manson JE (2009) Effects of vitamins C and E and β-carotene on the risk of type 2 diabetes in women at high risk of cardiovascular disease: a randomized controlled trial. Am J Clin Nutr 90:1–9
7. Whincup PH, Gilg JA, Papacosta O et al (2002) Early evidence of ethnic differences in cardiovascular risk: cross sectional comparison of British South Asian and white children. BMJ 324:635
8. Timpson NJ, Lawlor DA, Harbord RM et al (2005) C-reactive protein and its role in metabolic syndrome: Mendelian randomisation study. Lancet 366:1954–1959
9. Rafiq S, Melzer D, Weedon MN et al (2008) Gene variants influencing measures of inflammation or predisposing to autoimmune and inflammatory diseases are not associated with the risk of type 2 diabetes. Diabetologia 51:2205–2213
10. Ferrucci L, Perry JRB, Matteini A et al (2009) Common variation in the beta-carotene 15, 15′-monooxygenase 1 gene affects circulating levels of carotenoids: a genome-wide association study. Am J Hum Genet 84:123–133
11. Ferrucci L, Bandinelli S, Benvenuti E et al (2000) Subsystems contributing to the decline in ability to walk: bridging the gap between epidemiology and geriatric practice in the InCHIANTI study. J Am Geriatr Soc 48:1618–1625
12. Zeggini E, Scott LJ, Saxena R et al (2008) Meta-analysis of genome-wide association data and large-scale replication identifies additional susceptibility loci for type 2 diabetes. Nat Genet 40:638–645
13. Coyne T, Ibiebele TI, Baade PD et al (2005) Diabetes mellitus and serum carotenoids: findings of a population-based study in Queensland, Australia. Am J Clin Nutr 82:685–693
14. Hozawa A, Jacobs DR, Steffes MW et al (2006) Associations of serum carotenoid concentrations with the development of diabetes

and with insulin concentration: interaction with smoking: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Am J Epidemiol 163:929–937
15. Montonen J, Knekt P, Jarvinen R, Reunanen A (2004) Dietary antioxidant intake and risk of type 2 diabetes. Diabetes Care 27:362–366
16. Ylonen K, Alfthan G, Groop L et al (2003) Dietary intakes and plasma concentrations of carotenoids and tocopherols in relation to glucose metabolism in subjects at high risk of type 2 diabetes: the Botnia Dietary Study. Am J Clin Nutr 77:1434–1441
17. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G (2008) Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 27:1133–1163
18. Kathiresan S, Melander O, Anevski D et al (2008) Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 358:1240–1249
19. Willer CJ, Sanna S, Jackson AU et al (2008) Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 40:161–169