

Door mw. Dr. F.T. Russchen, arts.

Wij moeten ons mee ontwikkelen met de ontwikkelingen in de wetenschap

Ook in de antioxidanten paradox

Als orthomoleculaire behandelaars zijn we er ons allemaal van bewust dat vrije radicalen producten zijn van levensprocessen. En ook dat het organisme zich zo heeft ontwikkeld dat vrije radicalen nuttige functies vervullen in het lichaam. Bijvoorbeeld dat zij nuttig zijn bij de bestrijding van micro organismen die ons ziek kunnen maken. Bij infecties met lichaamsvreemde eenheden zoals bacteriën, virussen, schimmels en protozoën. En we weten dat het niet uitgesloten is dat ook een kankercel, die weliswaar zijn identiteit als lichaamsvreemd zo veel mogelijk uitschakelt, niet in het lichaam hoort. Het ligt dus voor de hand dat vrije radicalen in zekere mate ingeschakeld zullen worden om ook kankercellen uit te schakelen.

Maar niet alleen dat.

We zijn ons er ook van bewust dat calorie beperking, periodes met vasten en lichamelijke inspanning, allemaal vormen van stress, levensverlengend werken. Kijk naar de longevity theorieën. Stress zet aan tot overleven, houdt het systeem flexibel zou je kunnen zeggen. En stress, ja, dat vertaalt zich voor een deel in vrije radicalen, de boodschappers naar het aan zetten van overlevingsmechanismen.

(zie ook abstracts 1 & 2 in de bijlage)

Deze twee zaken, bestrijding door vrije radicalen van schadelijke lichaamsvreemde eenheden en overleven door stress, zijn de thema's van de twee recente onderzoeken die door professor Katan naar voren gehaald worden om het slikken van antioxidanten te hekelen.

Hier onder volgt een vertaling van de samenvattingen van beide onderzoeken zoals gepubliceerd op PubMed. Het eerste abstract is echt een biochemisch verhaal en voor velen waarschijnlijk niet makkelijk te lezen. Het tweede heeft een zekere schoonheid door eenvoud. Van het tweede onderzoek is het volledige artikel in het Engels in de bijlage gevoegd.

Het eerste onderzoek:

Herstel van metabole effecten, ontstaan door verlies van binding aan de matrix, door antioxidanten en oncogenen.

Normale epitheel cellen hebben bevestiging aan een matrix nodig om te overleven. De mogelijkheid voor tumor cellen om te overleven buiten hun natuurlijke extracellulaire matrix (ECM) niches is afhankelijk van het verwerven van een onafhankelijkheid van de verankering. Hoewel apoptose het snelste mechanisme om cellen zonder passende ECM verbinding te elimineren, suggereert recent onderzoek dat niet-apoptotische doodprocessen overleving voorkomen wanneer apoptose geblokkeerd is in de cellen zonder matrix. Hier tonen wij aan dat het loslaten van mamma epitheelcellen van de ECM een ATP tekort veroorzaakt door het verlies van glucose transport. Overexpressie van ERBB2 herstelt het ATP tekort door de glucose opname te herstellen door stabilisering van EGFR en fosfatidylinositol-3-OH kinase (PI(3)K) activering. Dit herstel is afhankelijk van glucose-gestimuleerde flux door de antioxidanten-genererende pentose phosphate route. Opvallend is dat wij vonden dat het ATP tekort hersteld kon worden door behandeling met antioxidanten (N-acetyl-cysteine en Trolox, een vitamine E derivaat) zonder dat hiermee de glucose opname werd hersteld. Dit herstel bleek afhankelijk van stimulatie van vetzuur oxidatie, die geremd wordt door reactive oxygen species (ROS) die vrijkomen bij het losmaken.

De significantie van deze bevindingen wordt ondersteund door aanwijzingen voor een toename van ROS in cellen die ontdaan zijn van hun matrix in het lumen van de mamma acini, en de ontdekking dat antioxidanten de overleving van deze cellen bevorderen en de anker-onafhankelijke kolonie vorming versterken. Deze resultaten laten zowel het belang zien van matrix bevestiging in de regulering van de metabole activiteit en een niet voorzien mechanisme voor overleving van de cel in veranderde matrix omgevingen door antioxidant herstel van de ATP generering.

Schafer ZT, Grassian AR, Song L, Jiang Z, Gerhart-Hines Z, Irie HY, Gao S, Puigserver P, Brugge JS.. Nature. 2009 Sep 3;461(7260):109-13.

Het komt er op neer dat, in een kweekbakje, antioxidanten het normale afsterven van cellen die los laten van de hun omgevende matrix, voorkomen. Antioxidanten doen dit door er voor te zorgen dat de cellen van energie winning uit glucose, die niet mogelijk is zonder matrix, over kunnen schakelen op energie winning uit vetzuren. En daardoor overleven de los gekomen cellen. Antioxidanten bevorderen dus de vorming van metastasen zou je hier uit af kunnen leiden. In elk geval in een kweekbakje en met de, deels niet natuurlijke, antioxidanten die gebruikt werden.

Een mens is natuurlijk geen kweekbakje, maar er zijn meerdere onderzoeken gepubliceerd met vergelijkbare resultaten. Aan de andere kant zijn er ook veel onderzoeken die een positief effect van antioxidanten in kweekbakjes laten zien (bv abstract 4). Het is wel zorgwekkend dat reviews van onderzoeken die kijken naar de effecten van antioxidanten op het voorkomen van kanker bij gezonde mensen in de recente literatuur nogal eens tot de conclusie komen dat ze geen goed doen. De overall mortaliteit lijkt soms zelfs groter bij suppletie (bv abstract 5). Andere studies, (bv Abstract 6 &7), laten wel een positief resultaat voor antioxidanten zien in de preventie van verschillende vormen van kanker. De bestudeerde populaties zijn in beide laatste studies minder gezond.

Het tweede onderzoek:

Antioxidanten voorkomen gezondheidsbevorderende effecten van lichamelijke inspanning bij mensen.

Inspanning bevordert de levensduur en verbetert type 2 diabetes mellitus en insuline resistentie. Maar, inspanning verhoogt ook de mitochondriële vorming van de, verondersteld, schadelijke reactieve zuurstof deeltjes (ROS). Antioxidanten worden wijd en zijd gebruikt als supplementen, maar of zij de gezondheidsbevorderende effecten van inspanning aantasten, is onbekend. Wij onderzochten de effecten van een combinatie van vitamine C (1000 mg/dag) en vitamine E (400 IU/day) op de insuline gevoeligheid, gemeten als glucose infusie snelheid (GIR) tijdens een hyperinsulinemische, euglycemische clamp bij zowel vooraf ongetrainde (n = 19) en voorgetrainde (n = 20) gezonde jonge mannen. Voor, en 4 weken na de interventie, bestaande uit fysieke inspanning, werd de GIR bepaald, werden spierbiopten genomen voor gen expressie analyses en werd plasma afgenomen om veranderingen ten opzichte van de uitgangssituatie en de potentiële invloed van vitamines op het effect van inspanning te bepalen. Inspanning verhoogde de parameters voor insuline gevoeligheid (GIR en plasma adiponectine) alleen bij afwezigheid van antioxidanten, zowel in vooraf ongetrainde ($P < 0.001$) als in voorgetrainde ($P < 0.001$) individuen. Dit liep parallel aan een toegenomen expressie van ROS-gevoelige transcriptie regulators van insuline gevoeligheid en ROS verdedigings capaciteit, peroxisoom-proliferator-activated receptor gamma (PPARgamma), en de PPARgamma coactivators PGC1alpha en PGC1beta alleen bij afwezigheid van antioxidanten ($P < 0.001$ voor allemaal). Ook moleculaire mediators van endogene ROS verdediging (superoxide dismutases 1 en 2; glutathione peroxidase) werden geïnduceerd door inspanning.

Dit effect werd eveneens geblokkeerd door antioxidanten suppletie. Consistent met het concept van mitohormesis, verbetert inspannings- geïnduceerde oxidatieve stress insuline resistentie en veroorzaakt het een adaptieve respons die de endogene antioxidant verdedigings capaciteit bevordert. Suppletie met antioxidanten verhindert dus mogelijk de gezondheidsbevorderende effecten van inspanning bij mensen.

We moeten natuurlijk niet over het hoofd zien dat er verschillende soorten antioxidanten zijn. De vitamine antioxidanten, aminozuur gerelateerde antioxidanten (bv N-acetyl-cysteine) en anderen, antioxidant enzymen (en de er mee verbonden mineralen) en de polyfenolen in planten. In de twee onderzoeken waar het hier om draait komen de polyfenolen niet aan de orde. Er zijn meerdere studies die zowel op het gebied van de preventie van kanker als diabetes 2 positieve resultaten vermelden bij gebruik van groenten en fruit en bij gebruik van kruiden of kruiden extracten (bv abstract 8 & 9).

Mogelijk is dit een ander hoofdstuk. Niet het hoofdstuk van de antioxidant werking van de betreffende stoffen, maar van een ander mechanisme.(bv abstract 10).

Hoe verder?

Het standpunt dat antioxidanten suppletie slecht is, lijkt dus, op grond van de beschikbare literatuur, absoluut ongenueanceerd.

Ik denk dat onze genuanceerdere insteek veilig is wanneer we er van uit gaan dat:

- suppletie van antioxidanten, naast een voeding rijk aan groenten, fruit, kruiden en specerijen, niet nodig, en mogelijk zelfs niet verantwoord is bij jonge gezonde mensen.
- Suppletie van antioxidanten bij ouderen en bij mensen met een aandoening wel degelijk gunstig kan zijn.
- Hierbij moet voor elke situatie wel goed overwogen worden welke antioxidanten wel en niet geïndiceerd zijn en zal, naast een gezonde voeding, suppletie met niet-vitamine voedingsantioxidanten veelal de eerste keus zijn.
 - Bij het maken van die keuze zal het een rol spelen of er bij het ziekteproces overmatig vrije radicalen vrijkomen,
 - en of dat lokaal gebeurt, en zo ja waar, of systemisch.
 - Of ontstekingsprocessen een belangrijke rol spelen in het ziekte verloop.

- **Bijlage**

Abstract 1

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 2007;39(1):44-84

Reactive oxygen species (ROS) and reactive nitrogen species (RNS, e.g. nitric oxide, NO(*)) are well recognised for playing a dual role as both deleterious and beneficial species. ROS and RNS are normally generated by tightly regulated enzymes, such as NO synthase (NOS) and NAD(P)H oxidase isoforms, respectively. Overproduction of ROS (arising either from mitochondrial electron-transport chain or excessive stimulation of NAD(P)H) results in oxidative stress, a deleterious process that can be an important mediator of damage to cell structures, including lipids and membranes, proteins, and DNA. In contrast, beneficial effects of ROS/RNS (e.g. superoxide radical and nitric oxide) occur at low/moderate concentrations and involve physiological roles in cellular responses to noxia, as for example in defence against infectious agents, in the function of a number of cellular signalling pathways, and the induction of a mitogenic response. Ironically, various ROS-mediated actions in fact protect cells against ROS-induced oxidative stress and re-establish or maintain "redox balance" termed also "redox homeostasis". The "two-faced" character of ROS is clearly substantiated. For example, a growing body of evidence shows that ROS within cells act as secondary messengers in intracellular signalling cascades which induce and maintain the oncogenic phenotype of cancer cells, however, ROS can also induce cellular senescence and apoptosis and can therefore function as anti-tumourigenic species. This review will describe the: (i) chemistry and biochemistry of ROS/RNS and sources of free radical generation; (ii) damage to DNA, to proteins, and to lipids by free radicals; (iii) role of antioxidants (e.g. glutathione) in the maintenance of cellular "redox homeostasis"; (iv) overview of ROS-induced signaling pathways; (v) role of ROS in redox regulation of normal physiological functions, as well as (vi) role of ROS in pathophysiological implications of altered redox regulation (human diseases and ageing). Attention is focussed on the ROS/RNS-linked pathogenesis of cancer, cardiovascular disease, atherosclerosis, hypertension, ischemia/reperfusion injury, diabetes mellitus, neurodegenerative diseases (Alzheimer's disease and Parkinson's disease), rheumatoid arthritis, and ageing. Topics of current debate are also reviewed such as the question whether excessive formation of free radicals is a primary cause or a downstream consequence of tissue injury.

Abstract 2

[Sheikh-Ali M](#), [Chehade JM](#), [Mooradian AD](#). The Antioxidant Paradox in Diabetes Mellitus *Am J Ther.* 2009 Sep 21.

There is ample empiric evidence to indicate that oxidative stress contributes to the pathogenesis of coronary artery disease and has a key role in the onset and progression of diabetes and its complications. Diabetes leads to depletion of the cellular antioxidant defense system and is associated with an increase in the production of free radicals. Oxidative stress can be the result of multiple pathways. Some of these are related to substrate-driven overproduction of mitochondrial reactive oxygen species, advanced glycation end product formation, glucose autoxidation, and depletion of micronutrients and cellular elements with antioxidative properties. There are numerous observational studies in the literature showing a beneficial outcome of the consumption of antioxidant vitamins. However, the interventional trials portray a different picture. The divide between the robust experimental evidence of the pathogenetic role of increased oxidative load in diabetes and the overwhelming failure of antioxidants to show any health benefits in clinical trials may well be characterized as the "antioxidant paradox."

Abstract 3

Een review waarin overproductie van vrije radicalen een rol blijkt te spelen in de pathogenese van diabetes 2

Rolo AP, Palmeira CM Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicol Appl Pharmacol.* 2006 Apr 15;212(2):167-78.

Hyperglycemia resulting from uncontrolled glucose regulation is widely recognized as the causal link between diabetes and diabetic complications. Four major molecular mechanisms have been implicated in hyperglycemia-induced tissue damage: activation of protein kinase C (PKC) isoforms via de novo synthesis of the lipid second messenger diacylglycerol (DAG), increased hexosamine pathway flux, increased advanced glycation end product (AGE) formation, and increased polyol pathway flux. Hyperglycemia-induced overproduction of superoxide is the causal link between high glucose and the pathways responsible for hyperglycemic damage. In fact, diabetes is typically accompanied by increased production of free radicals and/or impaired antioxidant defense capabilities, indicating a central contribution for reactive oxygen species (ROS) in the onset, progression, and pathological consequences of diabetes. Besides oxidative stress, a growing body of evidence has demonstrated a link between various disturbances in mitochondrial functioning and type 2 diabetes. Mutations in mitochondrial DNA (mtDNA) and decreases in mtDNA copy number have been linked to the pathogenesis of type 2 diabetes. The study of the relationship of mtDNA to type 2 diabetes has revealed the influence of the mitochondria on nuclear-encoded glucose transporters, glucose-stimulated insulin secretion, and nuclear-encoded uncoupling proteins (UCPs) in beta-cell glucose toxicity. This review focuses on a range of mitochondrial factors important in the pathogenesis of diabetes. We review the published literature regarding the direct effects of hyperglycemia on mitochondrial function and suggest the possibility of regulation of mitochondrial function at a transcriptional level in response to hyperglycemia. The main goal of this review is to include a fresh consideration of pathways involved in hyperglycemia-induced diabetic complications.

Abstract 4

Park IJ, Lee YK, Hwang JT, Kwon DY, Ha J, Park OJ. Green tea catechin controls apoptosis in colon cancer cells by attenuation of H₂O₂-stimulated COX-2 expression via the AMPK signaling pathway at low-dose H₂O₂. *Ann N Y Acad Sci.* 2009 Aug;1171:538-44

This study investigated the apoptotic regulation by green tea catechin epigallocatechin-3-gallate (EGCG) on colon cancer cells in the presence of low-dose H₂O₂ known to exert the activation of signal pathways leading to cell proliferation. In the presence of low-dose H₂O₂, EGCG induced apoptosis and abolished the cell-proliferative effect exhibited by low-dose H₂O₂. This reduction of growth was accompanied by an activation of AMP-activated kinase (AMPK), a decrease in cyclooxygenase-2 (COX-2) expression and prostaglandin E₂ (PGE₂) levels, and the induction of apoptotic markers such as p53 and poly(ADP-ribose) polymerase (PARP) cleavage. The low-dose H₂O₂ stimulated COX-2 expression, and treating cells with synthetic AMPK activator AICAR (5-aminoimidazole-4-carboxamide-1-beta-d-ribofuranoside) resulted in greater suppression of COX-2 expression and PGE₂. By treating cells with high concentrations of the reactive oxygen species (ROS) scavenger NAC (N-acetyl-L-cysteine), the apoptotic effect of EGCG was abolished and led to suppression of AMPK and COX-2, indicating that the liberation of excessive ROS might be the upstream signal of the AMPK-COX-2 signaling pathway even in the presence of low-dose H₂O₂.

Abstract 5

Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database Syst Rev.* 2008 Apr 16;(2):

BACKGROUND: Animal and physiological research as well as observational studies suggest that antioxidant supplements may improve survival. **OBJECTIVES:** To assess the effect of antioxidant supplements on mortality in primary or secondary prevention randomised clinical trials. **SEARCH STRATEGY:** We searched The Cochrane Library (Issue 3, 2005), MEDLINE (1966 to October 2005), EMBASE (1985 to October 2005), and the Science Citation Index Expanded (1945 to October 2005). We scanned bibliographies of relevant publications and wrote to pharmaceutical companies for additional trials. **SELECTION CRITERIA:** We included all primary and secondary prevention randomised clinical trials on antioxidant supplements (beta-carotene, vitamin A, vitamin C, vitamin E, and selenium) versus placebo or no intervention. Included participants were either healthy (primary prevention trials) or had any disease (secondary prevention trials). **DATA COLLECTION AND ANALYSIS:** Three authors extracted data. Trials with adequate randomisation, blinding, and follow-up were classified as having a low risk of bias. Random-effects and fixed-effect meta-analyses were performed. Random-effects meta-regression analyses were performed to assess sources of intertrial heterogeneity. **MAIN RESULTS:** Sixty-seven randomised trials with 232,550 participants were included. Forty-seven trials including 180,938 participants had low risk of bias. Twenty-one trials included 164,439 healthy participants. Forty-six trials included 68111 participants with various diseases (gastrointestinal, cardiovascular, neurological, ocular, dermatological, rheumatoid, renal, endocrinological, or unspecified). Overall, the antioxidant supplements had no significant effect on mortality in a random-effects meta-analysis (relative risk [RR] 1.02, 95% confidence interval [CI] 0.99 to 1.06), but significantly increased mortality in a fixed-effect model (RR 1.04, 95% CI 1.02 to 1.06). In meta-regression analysis, the risk of bias and type of antioxidant supplement were the only significant predictors of intertrial heterogeneity. In the trials with a low risk of bias, the antioxidant supplements significantly increased mortality (RR 1.05, 95% CI 1.02 to 1.08).

When the different antioxidants were assessed separately, analyses including trials with a low risk of bias and excluding selenium trials found significantly increased mortality by vitamin A (RR 1.16, 95% CI 1.10 to 1.24), beta-carotene (RR 1.07, 95% CI 1.02 to 1.11), and vitamin E (RR 1.04, 95% CI 1.01 to 1.07), but no significant detrimental effect of vitamin C (RR 1.06, 95% CI 0.94 to 1.20). Low-bias risk trials on selenium found no significant effect on mortality (RR 0.91, 95% CI 0.76 to 1.09). **AUTHORS' CONCLUSIONS:** We found no evidence to support antioxidant supplements for primary or secondary prevention. Vitamin A, beta-carotene, and vitamin E may increase mortality. Future randomised trials could evaluate the potential effects of vitamin C and selenium for primary and secondary prevention. Such trials should be closely monitored for potential harmful effects. Antioxidant supplements need to be considered medicinal products and should undergo sufficient evaluation before marketing.

Abstract 6

Sato R, Helzlsouer KJ, Alberg AJ, Hoffman SC, Norkus EP, Comstock GW. Prospective study of carotenoids, tocopherols, and retinoid concentrations and the risk of breast cancer. *Cancer Epidemiol Biomarkers Prev.* 2002 May;11(5):451-7

Previous prospective studies have raised the possibility that the antioxidant properties of carotenoids and vitamin E (alpha-tocopherol) and the role of vitamin A (retinol) in cellular differentiation may be associated with a reduced risk of subsequent breast cancer. To investigate the association between serum and plasma concentrations of retinol, retinyl palmitate, alpha-carotene, beta-carotene, beta-cryptoxanthin, lutein, lycopene, total-carotenoids, alpha-tocopherol, and gamma-tocopherol with subsequent development of breast cancer, a nested case control study was conducted among female residents of Washington County, Maryland, who had donated blood for a serum bank in 1974 or 1989. Cases (n = 295) and controls (n = 295) were matched on age, race, menopausal status, and date of blood donation, and the analyses were stratified by cohort participation. Median concentrations of beta-carotene, lycopene, and total carotene were significantly lower in cases compared with controls in the 1974 cohort (13.1, 12.5, and 7.9% difference; P = 0.01, 0.04, and 0.04, respectively) and for lutein in the 1989 cohort (6.7% difference; P = 0.02). The risk of developing breast cancer in the highest fifth was approximately half of that of women in the lowest fifth for beta-carotene [odds ratio (OR) = 0.41; 95% confidence interval (CI) 0.22-0.79; P trend = 0.007], lycopene (OR = 0.55; 95% CI 0.29-1.06; P trend = 0.04), and total carotene (OR = 0.55; 95% CI 0.29-1.03; P trend = 0.02) in the 1974 cohort. There was generally a protective association for other micronutrients in both cohorts, although none reached statistical significance. The results suggest that carotenoids may protect against the development of breast cancer.

Abstract 7

Bardia A, Platz EA, Yegnasubramanian S, De Marzo AM, Nelson WG. Anti-inflammatory drugs, antioxidants, and prostate cancer prevention. *Curr Opin Pharmacol.* 2009

Aug;9(4):419-26

Prostate cancer may be the most common preventable cancer among men in the United States (US) and the rest of the developed world. Emerging insights into the molecular pathogenesis of prostate cancer suggest that damage to the prostate epithelium, potentially inflicted by a variety of exposures, triggers procarcinogenic inflammatory processes to promote disease development. In this milieu, the damaged epithelium may generate proliferative inflammatory atrophy (PIA) lesions, which may progress to prostatic intraepithelial neoplasia (PIN) or to prostate cancer. To attenuate prostatic carcinogenesis driven by chronic or recurrent prostate inflammation, rational chemoprevention has thus far featured anti-inflammatory drugs and antioxidants. Results from clinical trials of these approaches have been mixed, emphasizing the need for mechanistic studies of the contribution of inflammation to prostatic carcinogenesis, more extensive analyses of the pharmacology, including distribution of drugs into target tissue, and, rational development of biomarkers to identify patients that are most likely to respond to anti-inflammatory drugs and antioxidants (targeted chemoprevention), alone, or in combination (combination chemoprevention).

Abstract 8

[Lee JE](#), [Männistö S](#), [Spiegelman D](#), [Hunter DJ](#), [Bernstein L](#), [van den Brandt PA](#), [Buring JE](#), [Cho E](#), [English DR](#), [Flood A](#), [Freudenheim JL](#), [Giles GG](#), [Giovannucci E](#), [Håkansson N](#), [Horn-Ross PL](#), [Jacobs EJ](#), [Leitzmann MF](#), [Marshall JR](#), [McCullough ML](#), [Miller AB](#), [Rohan TE](#), [Ross JA](#), [Schatzkin A](#), [Schouten LJ](#), [Virtamo J](#), [Wolk A](#), [Zhang SM](#), [Smith-Warner SA](#). Intakes of fruit, vegetables, and carotenoids and renal cell cancer risk: a pooled analysis of 13 prospective studies. *Cancer Epidemiol Biomarkers Prev.* 2009 Jun;18(6):1730-9

Fruit and vegetable consumption has been hypothesized to reduce the risk of renal cell cancer. We conducted a pooled analysis of 13 prospective studies, including 1,478 incident cases of renal cell cancer (709 women and 769 men) among 530,469 women and 244,483 men followed for up to 7 to 20 years. Participants completed a validated food-frequency questionnaire at baseline. Using the primary data from each study, the study-specific relative risks (RR) were calculated using the Cox proportional hazards model and then pooled using a random effects model. We found that fruit and vegetable consumption was associated with a reduced risk of renal cell cancer. Compared with <200 g/d of fruit and vegetable intake, the pooled multivariate RR for ≥ 600 g/d was 0.68 [95% confidence interval (95% CI) = 0.54-0.87; P for between-studies heterogeneity = 0.86; P for trend = 0.001]. Compared with <100 g/d, the pooled multivariate RRs (95% CI) for ≥ 400 g/d were 0.79 (0.63-0.99; P for trend = 0.03) for total fruit and 0.72 (0.48-1.08; P for trend = 0.07) for total vegetables. For specific carotenoids, the pooled multivariate RRs (95% CIs) comparing the highest and lowest quintiles were 0.87 (0.73-1.03) for alpha-carotene, 0.82 (0.69-0.98) for beta-carotene, 0.86 (0.73-1.01) for beta-cryptoxanthin, 0.82 (0.64-1.06) for lutein/zeaxanthin, and 1.13 (0.95-1.34) for lycopene. In conclusion, increasing fruit and vegetable consumption is associated with decreasing risk of renal cell cancer; carotenoids present in fruit and vegetables may partly contribute to this protection.

Abstract 9

Bishayee A *Cancer Prev Res (Phila Pa)*. 2009 May;2(5):409-18. **Cancer prevention and treatment with resveratrol: from rodent studies to clinical trials.**

Resveratrol (3,4',5-trihydroxy-trans-stilbene) is a dietary polyphenol derived from grapes, berries, peanuts, and other plant sources. During the last decade, resveratrol has been shown to possess a fascinating spectrum of pharmacologic properties. Multiple biochemical and molecular actions seem to contribute to resveratrol effects against precancerous or cancer cells. Resveratrol affects all three discrete stages of carcinogenesis (initiation, promotion, and progression) by modulating signal transduction pathways that control cell division and growth, apoptosis, inflammation, angiogenesis, and metastasis. The anticancer property of resveratrol has been supported by its ability to inhibit proliferation of a wide variety of human tumor cells *in vitro*. These *in vitro* data have led to numerous preclinical animal studies to evaluate the potential of this drug for cancer chemoprevention and chemotherapy. This review provides concise, comprehensive data from preclinical *in vivo* studies in various rodent models of human cancers, highlighting the related mechanisms of action. Bioavailability, pharmacokinetic, and potential toxicity studies of resveratrol in humans and ongoing interventional clinical trials are also presented. The conclusion describes directions for future resveratrol research to establish its activity and utility as a human cancer preventive and therapeutic drug.

Abstract 10

Koltover VK. *Toxicol Ind Health*. **Bioantioxidants: the systems reliability standpoint.** *Toxicol Ind Health* 2009 May-Jun;25(4-5):295-9

The antioxidant power of the so-called antioxidants is negligible because their rate constants and concentrations are too small to compete with the specialized defense enzymes, like superoxide dismutase (SOD), for the reactive oxygen species. In this short review, we present a number of experimental data of our group, along with the relevant literature data, to show that *in-vivo* antioxidants increase the systems reliability in other tacks. For example, butylated hydroxytoluene can prevent production of O₂⁻ in mitochondria, whereas flavonoids can induce expression of antioxidant enzymes, SOD and catalase. We suggest that the timely introduction of antioxidants can provide the beneficial physiological effects through the prophylactic reliability maintenance against reactive forms of oxygen via the hormonal system.

Antioxidants prevent health-promoting effects of physical exercise in humans

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ABSTRACT

Exercise promotes longevity and ameliorates type 2 diabetes mellitus and insulin resistance. However, exercise also increases mitochondrial formation of presumably harmful reactive oxygen species (ROS). Antioxidants are widely used as supplements but whether they affect the health-promoting effects of exercise is unknown. We evaluated the effects of a combination of vitamin C (1000 mg/day) and vitamin E (400 IU/day) on insulin sensitivity as measured by glucose infusion rates (GIR) during a hyperinsulinemic, euglycemic clamp in previously untrained ($n = 19$) and pretrained ($n = 20$) healthy young men. Before and after a 4 week intervention of physical exercise, GIR was determined, and muscle biopsies for gene expression analyses as well as plasma samples were obtained to compare changes over baseline and potential influences of vitamins on exercise effects. Exercise increased parameters of insulin sensitivity (GIR and plasma adiponectin) only in the absence of antioxidants in both previously untrained ($P < 0.001$) and pretrained ($P < 0.001$) individuals. This was paralleled by increased expression of ROS-sensitive transcriptional regulators of insulin sensitivity and ROS defense capacity, peroxisome-proliferator-activated receptor gamma (PPAR γ), and PPAR γ coactivators PGC1 α and PGC1 β only in the absence of antioxidants ($P < 0.001$ for all). Molecular mediators of endogenous ROS defense (superoxide dismutases 1 and 2; glutathione peroxidase) were also induced by exercise, and this effect too was blocked by antioxidant supplementation. Consistent with the concept of mitohormesis, exercise-induced oxidative stress ameliorates insulin resistance and causes an adaptive response promoting endogenous antioxidant defense capacity. Supplementation with antioxidants may preclude these health-promoting effects of exercise in humans.

Keywords: aging, hormesis, insulin resistance, oxidative stress, reactive oxygen species

Type 2 diabetes mellitus is increasing worldwide at epidemic rates and is associated with both microvascular and macrovascular complications (1). Type 2 diabetes mellitus is caused by a combination of insulin resistance involving a number of peripheral tissues, including skeletal muscle (2, 3), and an inadequate β -cell response despite normal or even increased amounts of circulating insulin.

Physical exercise exerts numerous favorable effects on general health (4) and specifically has been shown to improve glucose metabolism in the insulin-resistant state (5). This effect may be independent of exercise-related changes in body mass (6). Moreover, physical exercise has been shown to be effective in preventing type 2 diabetes in high risk individuals (7, 8) and may be even more effective than the most widely used anti-diabetic drug, metformin (9).

These beneficial effects of physical exercise on insulin resistance involve multiple mechanisms, including enhanced expression of glucose transporters and translocation of glucose transporters to the plasma membrane independent of insulin (10). Exercise, as well as weight loss, has been linked to activation of mitochondrial metabolism, and reduced mitochondrial metabolism has been functionally connected with type 2 diabetes (11). Mitochondria, however, are also the main source of reactive oxygen species (ROS), which are inevitable by-products of oxidative glucose metabolism. Muscle is also known to generate free radicals, especially during contraction and physical exercise (12).

It has been suggested that ROS may mediate some health-promoting effects, at least in nonprimate model systems (13–17).

We here evaluated the possibility that ROS are required for the insulin-sensitizing capabilities of physical exercise in healthy humans and that commonly used antioxidants, such as vitamin C and vitamin E, may abrogate the health-promoting effects of both physical exercise and oxidative stress in humans.

RESULTS

Baseline Characteristics.

Of the 40 individuals included in the present study, 20 were known to be previously trained, and 20 were previously untrained. Study subject characteristics in the preinterventional state are given in Table 1. No significant differences in age, height, body mass index, fat free mass, or VO₂ maximum were observed within the groups and no significant differences in age, height and body mass index were observed between untrained and pretrained groups. Not surprisingly, pretrained individuals had a significantly higher fat free mass ($P = 0.03$) and VO₂ maximum ($P < 0.001$).

Table 1.

Baseline characteristics of study subjects (n.a. = not applicable.)

	Previously untrained study group					Pretrained study group				
	No supplements		Vitamin C/Vitamin E		p-Value	No supplements		Vitamin C/Vitamin E		p-Value
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Number/sex	10/male	n.a.	10/male	n.a.	n.a.	10/male	n.a.	10/male	n.a.	n.a.
Age, years	26.70	4.34	27.44	3.02	0.69	25.40	2.15	26.00	1.95	0.54
Height, cm	179.20	4.98	182.32	8.83	0.38	182.96	3.87	177.91	7.16	0.08
Body Mass Index, kg/m ²	24.37	2.53	24.19	1.84	0.87	24.33	1.31	23.33	1.36	0.13
Fat free mass, kg	60.64	5.88	66.20	7.28	0.10	69.71	3.85	65.84	5.31	0.09
VO ₂ max, ml/min·kg ⁻¹	45.85	5.46	45.21	7.03	0.84	54.42	4.90	54.31	5.10	0.96

Half of the previously untrained and previously trained groups were randomly assigned to either antioxidant supplementation as described in *Methods* or to no supplementation (creating 4 groups of 10 each) (supporting information (SI) Fig. S1). All subjects underwent a 4 week exercise training program irrespective of antioxidant supplementation and previous training status. One untrained individual withdrew during the study for personal reasons unrelated to the experimental protocol.

Induction of Oxidative Stress by Short-Term Exercise.

It is well-established that physical exercise increases ROS formation in skeletal muscle (12); however, it is not known if the health-promoting effects of exercise are partly due to this effect. To replicate the ROS-inducing capacity of exercise in our specific experimental set-up, we subjected previously untrained individuals to 3 days of exercise with muscle biopsy before (Fig. S1, “pre”) and after this short-term intervention (Fig. S1, “early”).

We measured concentrations of thiobarbituric acid-reactive substances (TBARS), a well-established marker of overall oxidative stress reflecting oxidized lipids and thus ROS formation in mammals, within skeletal muscle of these previously untrained individuals in the presence or absence of antioxidant treatment. As expected (12), we observed a more than 2-fold increase in oxidative stress, as reflected by TBARS levels, following physical exercise in the absence of antioxidants (Fig. S2, Left pair of bars, $P = 0.008$). By contrast, those individuals taking antioxidant supplements showed no significant increase in muscle TBARS levels after exercise (Fig. S2, Right pair of bars, $P = 0.19$) resulting in significantly reduced TBARS formation after 3 days of exercise in comparison to untreated individuals (Fig. S2, shaded bars, $P = 0.03$). Thus, consistent with previous findings (12), these observations suggest that short-term physical exercise induces skeletal muscle ROS formation and that antioxidant supplements reduce this formation, at least during the first 3 days.

Antioxidants Prevent Increase of Insulin Sensitivity Following Physical Exercise. Glucose infusion rates.

Physical training has been shown to ameliorate insulin resistance and to improve glucose metabolism (5). Thus, both previously untrained and previously trained individuals were subjected to training with twenty 85 min sessions of defined physical exercise on 5 days per week (with or without addition of antioxidant supplementation) with measurement of insulin sensitivity by glucose infusion rates (GIR) during a hyperinsulinemic euglycemic clamp (18). As expected (5), nonsupplemented individuals showed a significant increase in GIR, i.e., increased insulin sensitivity after 4 weeks of exercise irrespective of previous training status (previously untrained: fig 1; A, Left pair of bars, $P < 0.001$; and previously trained: fig 1, Left pair of bars, $P < 0.001$), confirming previous findings that physical exercise induces an increase in insulin sensitivity. By contrast, neither previously untrained individuals and pretrained individuals who received antioxidants exhibited significant changes in GIR following exercise (fig 1 A, Right pair of bars, $P = 0.07$, and B, Right pair of bars, $P = 0.89$). Thus physical exercise induced an increase in insulin sensitivity only in the absence of antioxidants. The same impaired effect of exercise on GIRs seen in the antioxidant-treated group was also apparent in the previously untrained individuals when the supplement-treated individuals were compared to nonsupplemented individuals (fig 1 A, shaded bars, $P = 0.003$). Finally, analysis of all 39 individuals, irrespective of straining status, demonstrates a highly significant (Fig. S3A, $P < 0.001$ for ANOVA) effect of antioxidant supplementation on the blockage of exercise-induced improvement of GIR. Interestingly, inclusion of the baseline training status in the statistical analyses revealed that the effect of exercise on GIR was independent of the pretraining status of the study participants ($P = 0.58$ for interaction term of pretraining status by ANOVA), indicating that antioxidants can abolish the insulin sensitizing effects of exercise in both untrained and previously well-trained subjects. We additionally measured serum concentrations of TBARS. When comparing these to GIRs in the postinterventional state in all 39 individuals, we observed a significant correlation with TBARS serum levels (Pearson's $r = 0.353$, $P < 0.05$); this suggests that TBARS serum levels, at least to some extent, correlate with insulin sensitivity irrespective of antioxidant supplementation and previous training status, whereas no significant effect of antioxidant supplementation on postinterventional TBARS levels was observed ($P = 0.73$ for ANOVA, and $P = 0.43$ for interaction of vitamins by pretraining status by ANOVA).

Additional plasma markers of insulin sensitivity.

Plasma concentrations of the adipocyte-derived secretory protein adiponectin have been shown to be positively correlated with insulin sensitivity in humans and inversely correlated with type 2 diabetes risk (19). We observe an increase in circulating adiponectin levels following physical exercise in both previously untrained individuals (Fig 1 C, Left pair of bars, $P < 0.001$) and pretrained individuals (Fig 1 D, Left pair of bars, $P < 0.001$).

In contrast, previously untrained individuals and pretrained individuals who received antioxidants did not exhibit any significant change in adiponectin levels following exercise when compared to the preintervention state (Fig 1 C, *Right pair of bars*, $P = 0.14$, and D, *Right pair of bars*, $P = 0.46$), indicating that the exercise-induced increase in adiponectin levels is blocked by antioxidant supplementation. Moreover, comparing postintervention adiponectin levels there was a significantly impaired effect of exercise in the antioxidant-treated group compared to the placebo group (Fig 1 C and D, *shaded bars*, $P < 0.001$ and $P = 0.021$, respectively). Again, as observed for GIR, there was a strong effect of antioxidant supplementation on postintervention adiponectin levels for the entire sample irrespective of training status (Fig. S3B, $n = 39$, $P < 0.001$ for ANOVA). As observed for GIR, additional analyses indicate that previous training status had no impact on the effects of exercise intervention and antioxidant supplementation on serum adiponectin levels ($P = 0.94$ for interaction of vitamins by pretraining-status by ANOVA). Lastly, we compared fasting plasma insulin in individuals receiving antioxidants to those receiving no supplementation. We observed a significant decrease in fasting plasma insulin levels following the exercise intervention in previously untrained and pretrained individuals in the absence of antioxidants ($P = 0.004$ and $P = 0.002$), consistent with improved insulin sensitivity. Once again, antioxidant supplementation completely abrogated this effect of exercise ($P = 0.74$ and $P = 0.94$). As observed for GIR and adiponectin, this effect of antioxidant supplementation on postintervention fasting insulin levels was present for the entire sample ($n = 39$, $P < 0.001$ for ANOVA), and previous training status had no impact on the effects of exercise intervention and antioxidant supplementation on fasting insulin levels ($P = 0.32$ for interaction of vitamins by pretraining-status by ANOVA). These results indicate that antioxidants severely impair the insulin-sensitizing effects of physical exercise as quantified by several measures, including GIR during hyperinsulinemic euglycemic clamps and plasma adiponectin and fasting plasma insulin concentrations, and that this effect occurs irrespective of previous training status.

Molecular Promotion of Insulin Sensitivity Following Physical Exercise Is Abrogated by Antioxidants.

Several molecular regulators of insulin sensitivity have been proposed in the past, including peroxisome-proliferator-activated receptor gamma (PPAR γ) and its 2 coactivators, PGC1 α and PGC1 β , all of which coordinate insulin-sensitizing gene expression within the nucleus of the cell. Using quantitative PCR (qPCR) to compare relative RNA expression levels for these regulators, we observed a strong induction of PGC1 α , PGC1 β , and PPAR γ expression in skeletal muscle following 4 weeks of exercise training in previously untrained, antioxidant naïve individuals (Fig 2 A, C, and E, *Left pair of bars*, all $P < 0.01$ to $P < 0.001$). By contrast, individuals treated with antioxidants showed a markedly reduced exercise-related induction (Fig 2 A, C, and E, *Right pair of bars*). Similarly, we observed induction of PGC1 α , PGC1 β , and PPAR γ expression by physical exercise in pretrained individuals in the absence of antioxidants (Fig 2 B, D, and F, *Left pair of bars*, $P < 0.05$ to $P < 0.001$), and antioxidant treatment prevented this induction (Fig 2 B, D, and F, *Right pair of bars*). Likewise, when comparing the relative expression of PGC1 α , PGC1 β , and PPAR γ in the postinterventional state in the presence and absence of antioxidants, exercise increased expression to a much lesser extent in antioxidant-supplemented individuals in all cases (Fig 2 A–E, *shaded bars*, $P < 0.05$ to $P < 0.001$). As with parameters of insulin sensitivity, this effect of antioxidant supplementation on postintervention gene expression was observed for the entire sample (Figs. S4 A–C, $n = 39$, $P < 0.001$ for ANOVA), and previous training status had no impact on the effects of exercise intervention and antioxidant supplementation on expression of these genes ($P = 0.21$ to 0.89 for interaction of vitamins by pretraining-status by ANOVA).

Taken together, these findings indicate that physical exercise induces several molecular regulators of insulin sensitivity irrespective of previous training status and that this induction is widely inhibited by antioxidant supplementation.

Molecular Promotion of Muscle Antioxidant Defense Following Physical Exercise Is Abrogated by Antioxidants.

The transcriptional coactivators PGC1 α and PGC1 β have not only been linked to increased insulin sensitivity but have also been shown to induce expression of several enzymes known to be involved into detoxification of reactive oxygen species (ROS), including superoxide dismutase 2 (SOD2), glutathione peroxidase 1 (GPx1) and possibly other enzymes of similar biochemical function (20). Accordingly, in the present study, physical exercise resulted in a strongly increased expression of *SOD1* (fig 2 G and H, Left pair of bars, $P < 0.05$ to $P < 0.001$), *SOD2* (fig 2 I and J, Left pair of bars, $P < 0.05$ to $P < 0.001$), and *GPx1* (fig 2 K and L, Left pair of bars, $P < 0.001$) in previously untrained and previously trained, antioxidant naïve individuals, whereas pretreatment with antioxidants prevented this induction (Fig 2 G, I, and K, Right pair of bars, $P = 0.92$, $P = 0.06$, and $P = 0.10$, respectively). Similar while less pronounced effects were observed for catalase (*CAT*) ($P = 0.045$ and $P = 0.13$, not depicted). When comparing the relative expression of these enzyme-encoding mRNAs in the postinterventional state in the presence and absence of antioxidants, exercise increased expression to a much lesser, if any, extent in antioxidant-supplemented individuals whether previously untrained or previously trained (Fig 2 G-L, shaded bars, $P < 0.05$ to $P < 0.001$). As observed for *PPAR γ* , *PGC1 α* , and *PGC1 β* , we found a strong effect of antioxidant supplementation to block exercise training-induced expression of antioxidant enzyme mRNAs for the entire sample of previously trained and untrained individuals ($n = 39$) (Fig. S4 D–F) (*SOD1*: $P < 0.001$; *SOD2*: $P = 0.01$; *GPx1*: $P < 0.001$; and *CAT*: $P = 0.81$, all for ANOVA). In some of these cases, previous training status had an impact on the effects of exercise intervention and antioxidant supplementation on 2 of the expression levels (*SOD1*: $P = 0.003$; *SOD2*: $P = 0.32$; *GPx1*: $P = 0.046$; and *CAT*: $P = 0.83$, all for interaction of vitamins by pretraining-status by ANOVA). However, this effect was restricted to *SOD1* and *GPx1*, while, notably, the mitochondrially active *SOD2* appeared to be unaffected by previous training status.

Taken together, physical exercise induces numerous molecular regulators of insulin sensitivity and antioxidant defense, most of which are almost completely inhibited by antioxidant pretreatment in healthy young men (Fig 3).

DISCUSSION

Based on the evidence derived from the current study, we here propose an essential role for exercise-induced ROS formation in promoting insulin sensitivity in humans. This induction appears to involve the ROS-dependent transcriptional coactivators PGC1 α and PGC1 β , and the transcription factor *PPAR γ* and their targets *SOD1*, *SOD2*, *GPx1*, and, to a reduced extent, *CAT*. Most importantly, these changes in gene expression and the increase in insulin sensitivity following physical exercise are almost completely abrogated by daily ingestion of the commonly used antioxidants vitamin C and vitamin E. Thus, antioxidant supplementation blocks many of the beneficial effects of exercise on metabolism.

This direct molecular link between exercise-dependent formation of ROS, activation of PGC1 α , PGC1 β and *PPAR γ* on the one hand and increased insulin sensitivity on the other hand, strongly suggest that oxidative stress can be instrumental in preventing type 2-diabetes. The transcriptional coactivator PGC1 α has been previously linked to type 2 diabetes in humans (21, 22). This protein has also been shown to be inducible by various oxidative stressors (20), as well as physical exercise in rodents, notably in a vitamin C sensitive manner (17). Given its synergistic potency in coactivating the transcription factor *PPAR γ* to promote insulin sensitivity, the previously established role of PGC1 α as a ROS sensor in neurons that in turn induces ROS defense (20), as well as in rodent muscle (17), suggests that activation of PGC1 α and possibly PGC1 β may be important factors in promoting insulin sensitivity by both exercise and ROS in skeletal muscle.

Moreover, in addition to the increase in insulin sensitivity following exercise-induced ROS formation, we also observe an induction of all relevant ROS defense enzyme expression levels, namely *SOD1* and *SOD2*, *GPx1*, and, to a reduced extent, *CAT*. Notably, some of these enzymes have been previously linked to transcriptional promotion by PGC1 α (20), and, at least for *SOD2*, exercise has been shown to induce its expression in rodents (23).

Nevertheless, the published evidence is ambiguous with a number of studies suggesting that exposure to ROS may promote insulin resistance (24, 25) whereas others find the opposite (14). In the present study, we find that increased ROS formation efficiently counteracts insulin resistance. Previously published findings in nonprimate models also support this interpretation (13–17). One possible explanation for the apparent conflict between the different studies may be that those studies suggesting an inverse relation between ROS and insulin sensitivity were obtained in models of *continuous* exposure to increased levels of ROS (24, 25), whereas our current findings and those of other studies (23, 26) may reflect *transient* increases in ROS during limited periods of physical exercise only.

This notion is further supported by the fact that most negative effects of antioxidant supplements observed in the current study occur irrespective of previous training status. While most effects appear quantitatively more pronounced within the previously untrained study group (i.e., *Left arms* of fig 1, 2, and S1), the data do not support the assumption that antioxidant supplement intake is less detrimental in previously trained subjects. ANOVA, which considered covariables as stated in *Methods*, indicates that most effects of antioxidants are similar in both pretrained and untrained individuals (Fig. S3 and Fig. S4) and that there was no significant interaction of vitamins by pretraining status with the exception of *SOD1* and *GPx1* expression, where antioxidants had more pronounced effects in previously untrained subjects than in trained subjects following the 4 week exercise intervention. Hence, the negative effects of antioxidants on exercise training with regard to insulin sensitivity are similar in untrained and pretrained individuals.

If transient increases in oxidative stress are capable of counteracting insulin resistance in humans, it is possible that preventing the formation of ROS by, for example, antioxidants might actually increase, rather than decrease, the risk of type 2 diabetes. While this remains to be determined, one metaanalysis of previously published studies (27) suggests that high dietary intake of fruits and vegetables, a source of antioxidants but also of numerous other bio-active compounds, may actually decrease the risk for type 2 diabetes. Nevertheless, and as stated by Hamer and Chida (27), all larger intervention trials evaluating the diabetes-preventive potential of defined antioxidant supplements have been unable to find any positive effects of supplementation (28–30). Moreover, antioxidant use in type 2 diabetics has been linked to increased prevalence of hypertension (31) and use of antioxidant supplements has recently been proposed to increase overall mortality in the general population (32). Taken together, these previously published findings tentatively suggest that fruits and vegetables may exert health-promoting effects *despite* their antioxidant content and possibly due to other bio-active compounds. However, it should be noted that the current study applied comparably high doses of oral antioxidants, which have been tested in healthy young men only.

Free radicals causing oxidative stress are an inevitable by-product of mitochondrial metabolism and have been proposed to exert repetitive damage to individual cells of the body promoting increased disease prevalence and aging (33). However, and in specific regard to exercise, antioxidants were incapable of further extending exercise-induced lifespan extension in rats (26). Repeated exposure to sublethal stress has been proposed to cumulate in enhanced stress resistance and ultimately increased survival rates due to a process named hormesis. By analogy, for sublethal ROS-dependent processes emanating from the mitochondria, the term “mitohormesis” was recently proposed on a hypothetical basis (34). Evidence for this novel concept has been provided in model organisms such as nematodes (15) and rats (17), and the current study would extend the concept of mitohormesis to the amelioration of insulin resistance in humans, suggesting that potential harmful ROS may exert health promoting effects via defined molecular intermediates (Fig 3). In humans, this mitohormetic induction of

GIR is paralleled by, and may in part be due to, ROS-related induction of PPAR γ , PGC1 α , and PGC1 β , which then secondarily increase expression of ROS-detoxifying enzymes, including *SOD1*, *SOD2*, and *GPx1*. In this way exercise-induced ROS itself could increase endogenous ROS defense capacity in skeletal muscle, producing a mitohormetic state (Fig 3) as observed in nematodes (15) and rats (17).

Taken together, we find that antioxidant supplements prevent the induction of molecular regulators of insulin sensitivity and endogenous antioxidant defense by physical exercise. Consistent with the concept of mitohormesis, we propose that transiently increased levels of oxidative stress reflect a potentially health-promoting process at least in regards to prevention of insulin resistance and type 2 diabetes mellitus.

METHODS

The present study was approved by the ethics committee of the University of Leipzig, Leipzig, Germany. All study participants gave written informed consent before initiation of the study. The study design was registered at ClinicalTrials.gov registration number NCT00638560. Forty healthy males participated in a prospective randomized 4 week intensive training intervention study. The study design is depicted in Fig. S1. Subjects were selected from a computer-based volunteer database based on sex, age, body-mass index, physical training status, availability, and additional criteria as listed at the end of this paragraph. Sex was prespecified to be male, age was prespecified to be 25 to 35 years; BMI was prespecified to be below 27 kg/m², and the training status used is defined below. All subjects included in the study also needed to fulfill the following inclusion criteria: (i) absence of any acute or chronic inflammatory disease, (ii) absence of any metabolic disease including diabetes mellitus of any type, (iii) no medical history of hypertension and systolic blood pressure <140 mmHg and diastolic blood pressure <85 mmHg, (iv) no clinical evidence of cardiovascular or peripheral artery disease, (v) no thyroid dysfunction, (vi) no concomitant medication intake, (vii) no alcohol, nicotine, or drug abuse.

The study consisted of 2 parts (Fig. S1). The first part was performed as an open-label study and included 16 individuals; all of these terminated the study (no withdrawals). Based on the effects observed during this first part, the second part was initiated and subsequently performed as a double blind placebo-controlled study and included 24 individuals. Twenty-three participants completed the second part of study (one subject withdrew after baseline clamp for personal reasons, Fig. S1). None of the subjects participating in study part 2 had participated in study part 1.

Twenty of the 40 subjects enrolled were previously untrained, defined as performing less than 2 hours of exercise (including daily life activities) per week before the study was initiated. The remaining 20 subjects were considered pretrained, i.e., performed more than 6 hours of exercise per week. The different pretraining status was further verified by a significantly higher fitness level (measured as highest oxygen uptake per minute reached during a standardized graded bicycle test) in the pretrained compared to the untrained group.

Subjects of these 2 differentially pretrained groups were assigned by the randomization function of the statistical software package into an antioxidant treatment ($n = 10$ per subgroup) and a control group ($n = 10$ per subgroup). Participants in the antioxidant treatment groups ($n = 20$ each, out of which $n = 10$ were untrained and $n = 10$ were pretrained) received 500 mg vitamin C (ascorbic acid, Jenapharm) twice a day and 400 IU vitamin E (RRR-/D- α -tocopherol, Jenapharm) once a day orally. Optically matched placebo pills were provided by the Clinical Pharmacy Department of the University of Leipzig hospital.

All 40 subjects were subjected to supervised physical training, which consisted of training sessions on 5 consecutive days of the week for 4 weeks, i.e., 20 sessions in total. Each session included 20 min of biking or running, 45 min of circuit training, and 20 min periods for warming up and cooling down. All subjects completed a graded bicycle test to volitional exhaustion and had maximal oxygen uptake measured with an automated open circuit gas analysis system at baseline. The highest oxygen uptake per minute reached was defined as the maximal oxygen

uptake (VO_2 maximum), and subjects subsequently trained at their individual submaximal heart rate using heart rate monitors. Basal percentage fat-free mass was measured by dual x-ray absorptiometry.

At baseline and after 4 weeks of training, but 7 days after the last training session, blood samples were obtained in the fasting state. All baseline and postintervention blood samples and skeletal muscle samples were collected between 8–10 a.m. after an overnight fast. Plasma glucose concentrations were determined by the hexokinase method using an Immulite automated analyzer (Diagnostic Products Corporation). Plasma insulin concentrations were determined by an enzyme immunometric assay on the same instrument. Plasma adiponectin concentrations were determined by RIA (Linco Research) as previously described (35). Serum and skeletal muscle TBARS concentrations were determined fluorometrically according to standard procedures. Hyperinsulinemic euglycemic clamps were performed as previously described (18).

Skeletal muscle biopsies were obtained under local anesthesia from the right *vastus lateralis* muscle and immediately snap-frozen in liquid nitrogen. Biopsies before ("pre", Fig. S1) and after intervention ("post", Fig. S1) were obtained from all 39 study subjects; biopsies for the "early" time-point (Fig. S1) were obtained from 4 placebo-taking and 5 vitamin-treated individuals, while the remaining 3 individuals refused to undergo this additional biopsy. Human *PGC-1 α* , *PGC-1 β* , *PPAR γ* , *SOD1*, *SOD2*, *GPx1*, and *CAT* gene expression was measured by quantitative real-time (RT)-PCR in a fluorescent temperature cycler using the TaqMan assay, and fluorescence was detected on an ABI PRISM 7000 sequence detector (Applied Biosystems). Total RNA was isolated from skeletal muscle samples using TRIzol (Life Technologies), and 1 μ g RNA was reversely transcribed with standard reagents (Life Technologies) employing standard procedures. From each RT-PCR, 2 μ l were amplified in a 26 μ l PCR using the Brilliant SYBR Green QPCR Core Reagent Kit from Stratagene according to the manufacturer's instructions. Samples were incubated in the ABI PRISM 7000 sequence detector for an initial denaturation at 95 °C for 10 min, followed by 40 PCR cycles, each cycle consisting of 95 °C for 15 s, 60 °C for 1 min, and 72 °C for 1 min. The following primers were used: human *PGC-1 α* : 5'TGCCCTGGATTGTTGACATGA (sense) and 5'TTTGTCAGGCTGGGGGTAGG (antisense); human *PGC 1 β* : 5'TTGAGGAGTGCAGAGGTGCTG (sense) and 5'ATCTGGGCCAGCAGAAGTGC (antisense), human *PPAR γ* : 5'AGGCGAGGGCGATCTTGACAG (sense) and 5'GATGCGGATGGCCACCTCTT (antisense); human superoxide dismutase 1 (*SOD1*): 5'GGTGTGGCCGATGTGTCTATT (sense) and 5'CTGCTTTTTCATCGACCACCA (antisense); human *SOD2*: 5'TGCTGCTTGCCAAATCAGG (sense) and 5'CACACATCAATCCCCAGCAGT (antisense); human *GPx1*: 5'GCGGCGGCCAGTCGGTGTA (sense) and 5'GAGCTTGGGGTCGGTCATAA (antisense); human catalase: 5'TCCGGGATCTTTTTAACGCCATTG (sense) and 5'TCGAGCACGGTAGGGACAGTTCAC (antisense); human *18S* rRNA: 5'TGCCATGTCTAAGTACGCACG (sense); 5'TTGATAGGGCAGACGTTCTGA (antisense). SYBR Green I fluorescence emissions were monitored after each cycle. Expression of *PGC-1 α* , *PGC-1 β* , *PPAR γ* , *SOD1*, *SOD2*, *GPx1*, *CAT*, and *18S* rRNA were quantified by using the second derivative maximum method of the TaqMan Software (Applied Biosystems) determining the crossing points of individual samples by an algorithm which identifies the first turning point of the fluorescence curve. Amplification of specific transcripts was confirmed by melting curve profiles (cooling the sample to 68 °C and reheating slowly up to 95 °C with parallel measurements of fluorescence) at the end of each PCR. The specificity of the PCR was further verified by subjecting the amplification products to agarose gel electrophoresis.

Statistical Analysis.

All data collection processes and subsequent statistical analyses were performed with SPSS, Version 15.0. Variables were tested for normal distribution using the Kolmogorov-Smirnov test. Nonnormally distributed variables were log transformed to approximate a normal distribution before applying *t* test or general linear modeling statistics. P-values of less than 0.05 were considered significantly different.

Group comparisons ([Table 1](#)) were made using a 2-sided unpaired Student's *t* tests. Within previously untrained ($n = 20$) or pretrained ($n = 20$) subgroups, interindividual effects of antioxidant treatment between treatment groups at baseline, as well as after intervention, were compared with two-sided unpaired Student's *t* tests. For comparing intra-individual effects of exercise within treatment groups (pre- vs. postexercise), 2-sided paired Student's *t* tests were used ([Table 1](#) and Fig 1 and 2).

To determine putatively differential effects of initial training status (previously untrained vs. pretrained), multivariate analyses were performed using a general linear model approach on the delta values (postvalues minus prevalues) of all 39 individuals. The ANOVA statistics for effects of vitamin supplements as dependent variable are given after adjustment for the covariates "open" versus "blinded", and "initial training status" (previously untrained vs. pretrained). A significant P value would indicate that vitamin supplementation has an effect on outcome measurements irrespective of initial training status. This specific test has been denominated "ANOVA" throughout the *Results* and *Discussion* sections.

We also performed an additional ANOVA including an interaction term (training status by supplement) again using delta values (postvalues minus prevalues) and adjusting for the above covariates. A significant P value would indicate that the effect of vitamin supplementation is dependent on initial training status. This test has been denominated "interaction of vitamins by pretraining-status by ANOVA" throughout the *Results* and *Discussion* sections.

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FOOTNOTES

The authors declare no conflict of interest.

Data deposition: The study design described in this paper has been deposited at ClinicalTrials.gov (registration no. NCT00638560).

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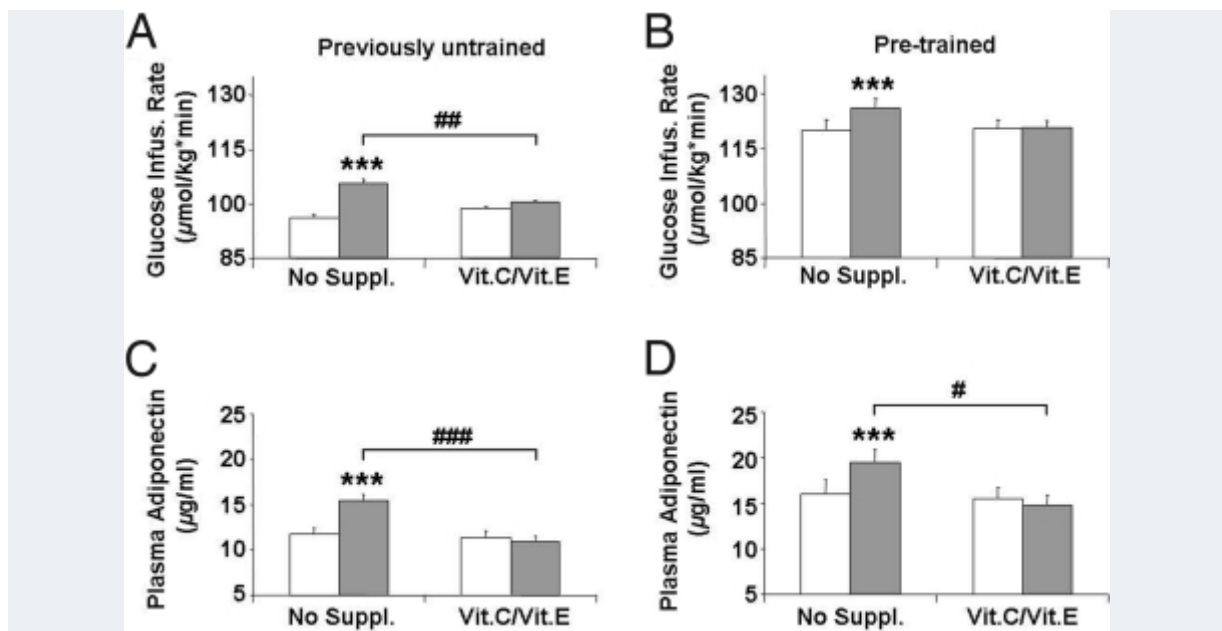


Fig. 1.

Antioxidants prevent exercise-dependent induction of insulin sensitivity. (A) Glucose infusion rates (GIR) during euglycemic hyperinsulinemic clamps in previously untrained individuals before (white bars) and after (shaded bars) physical exercise over 4 weeks. (Left pair of bars) Individuals not taking any medication or placebo; (Right pair of bars) individuals taking both vitamin C (1000 mg/day) as well as vitamin E (400 IU/day). Bars depict means, error bars show standard error means (applies to all subsequent panels and figures). Significances (applies to all subsequent panels and fig 3* indicates $0.01 < P < 0.05$ comparing data before and after 4 weeks of exercise, # indicates $0.01 < P < 0.05$ comparing "no suppl." with "Vit.C/Vit.E" groups after intervention, ** indicates $0.001 \leq P \leq 0.01$ comparing data before and after 4 weeks of exercise, ## indicates $0.001 \leq P \leq 0.01$ comparing "no suppl." with "Vit.C/Vit.E" groups after intervention, *** indicates $P < 0.001$ comparing data before and after 4 weeks of exercise, ### indicates $P < 0.001$ comparing "no suppl." with "Vit.C/Vit.E" groups after intervention. (B) The same set of data derived from a physically pretrained group of individuals. (C) Plasma adiponectin levels in the previously untrained and previously trained (D) state.

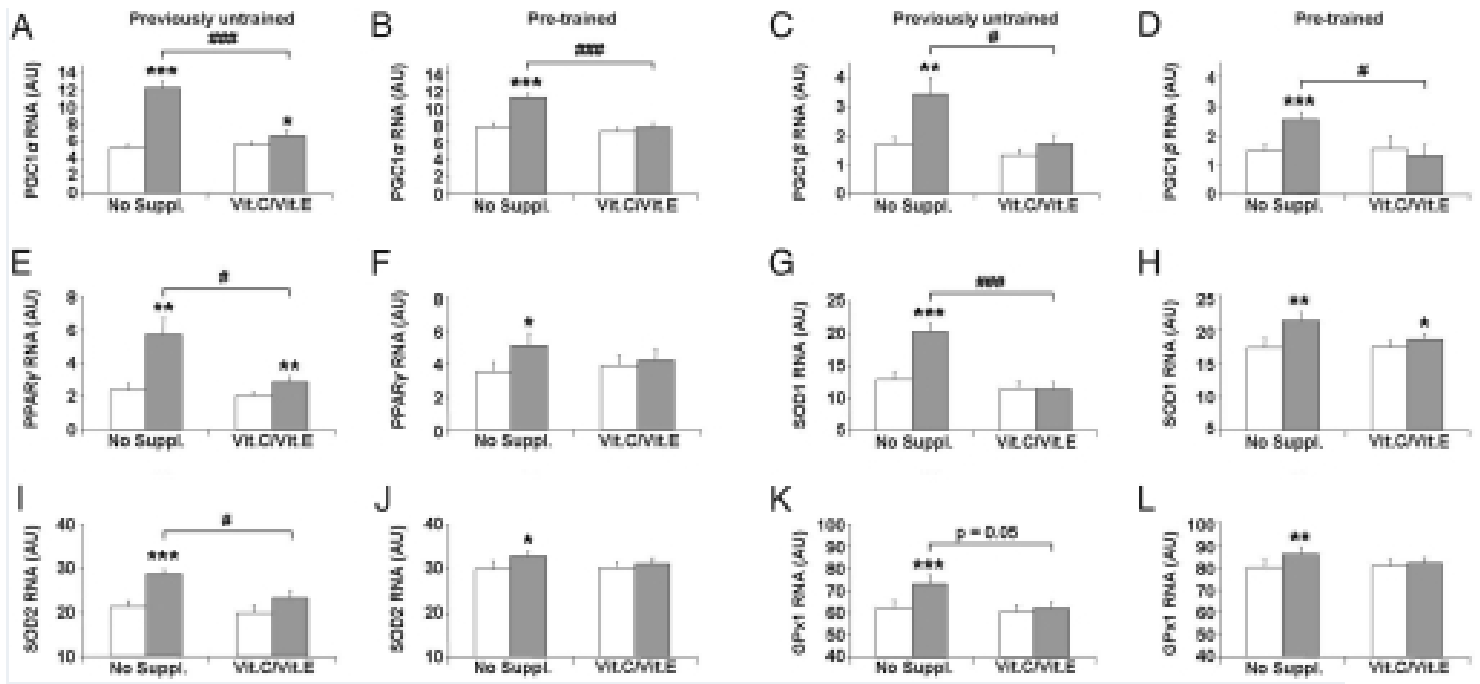


Fig. 2.

Antioxidants prevent induction of molecular mediators of insulin sensitivity and antioxidant defense in exercised skeletal muscle. (A) depicts expression levels of *PGC1 α* RNA transcripts in skeletal muscle biopsies derived from previously untrained individuals before (white bars) and after (shaded bars) physical exercise over 4 weeks as described in the *Methods* section. (*Left pair of bars*) Individuals not taking any medication or placebo; (*Right pair of bars*) individuals taking both vitamin C (1000 mg/day) as well as vitamin E (400 IU/day). Bars depict means, error bars show standard error means, "AU" abbreviates normalized arbitrary units. (B) depicts expression levels of *PGC1 α* RNA transcripts in skeletal muscle biopsies derived from pre-trained individuals before (white bars) and after (shaded bars) physical exercise over 4 weeks. (C and D) expression levels of *PGC1 β* RNA transcripts in a similar fashion; (E and F) expression levels of *PPAR γ* RNA; (G and H) levels of superoxide dismutase 1 (*SOD1*) RNA expression; (I and J) RNA levels of superoxide dismutase 2 (*SOD2*); (K and L) glutathione peroxidase 1 (*GPx1*) RNA expression levels.

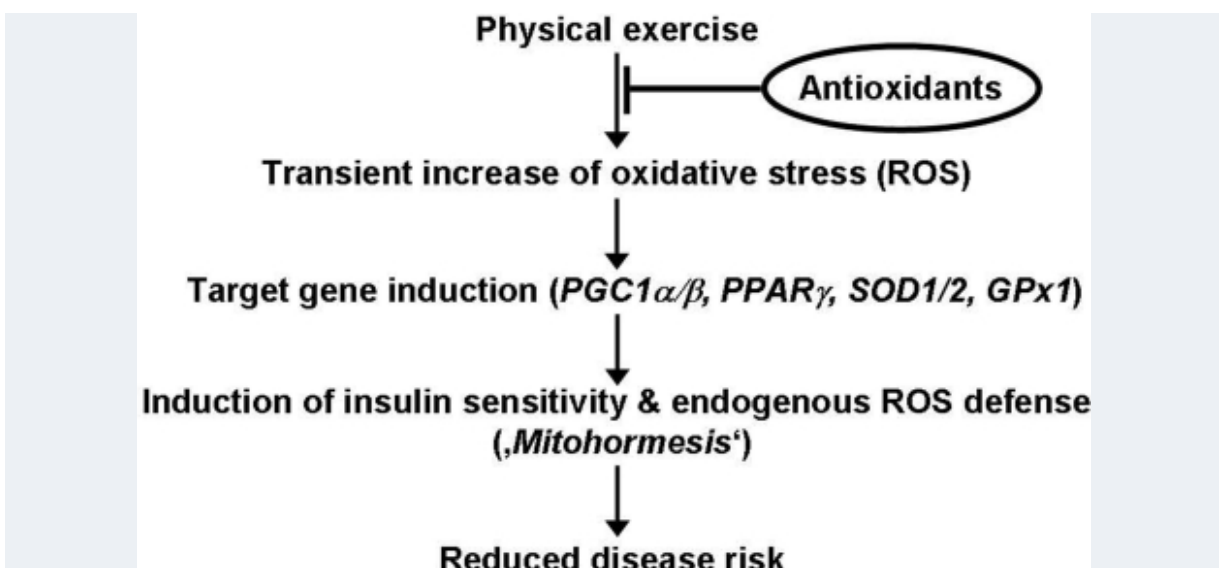


Fig. 3.

Mitohormesis links physical exercise and subsequent formation of reactive oxygen species to insulin sensitivity and antioxidant defense. Physical exercise exerts ameliorating effects on insulin resistance by increasing mitochondrial formation of reactive oxygen species in skeletal muscle to induce expression of *PGC1 α* , *PGC1 β* , and *PPAR γ* as inducers of insulin sensitivity, as well as superoxide dismutases 1 and 2 and glutathione peroxidase 1, key enzymes of ROS defense. Notably, by blocking exercise-dependent formation of reactive oxygen species due to ingestion of antioxidant supplements, health promoting effects of physical exercise are abolished, and physical exercise fails to promote insulin sensitivity and antioxidant defense in the presence of vitamin C and vitamin E.

Supporting Information

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Fig. S1. Study design. The study consisted of 2 parts including an open-label first part (*Upper*), and double-blinded placebo-controlled second part (*Lower*), including previously untrained individuals (*Left*) and previously trained individuals (*Right*). Both groups were randomly split into antioxidant-treatment or no/placebo-treatment and analyzed after exercise intervention and collection of samples as described in *Methods* section.

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Fig. S2. Antioxidants prevent exercise-induced formation of oxidative stress in skeletal muscle. TBARS concentrations in skeletal muscle following a 3 day exercise intervention in the presence (*Left pair of bars*) and absence (*Right pair of bars*) of antioxidants before (white bars) and after (shaded bars) physical exercise. Bars depict means, error bars show standard error means. # indicates $0.01 < P < 0.05$ comparing "no suppl." with "Vit.C/Vit.E" groups, ** indicates $0.001 < P < 0.01$ comparing data before and after 3 days of exercise.

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Fig. S3. Antioxidants prevent exercise-induced induction of insulin sensitivity (combined analysis). (A) depicts glucose infusion rates (GIR) during euglycemic hyperinsulinemic clamps in previously untrained individuals (open circles) and pretrained individuals (black triangles) before (pre, *Left*) and after (post, *Right*) physical exercise over 4 weeks. (*Left*) Individuals not taking any medication or placebo; (*Right*) individuals taking both vitamin C (1000 mg/day) and vitamin E (400 IU/day). Horizontal red lines depict means for untrained and trained individuals together ($n = 19$ and $n = 20$, respectively). Significances: *** indicates $P < 0.001$ comparing delta values before and after 4 weeks of exercise (ANOVA). B depicts plasma adiponectin levels in a similar manner as A.

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Fig. S4. Antioxidants prevent induction of molecular mediators of insulin sensitivity and antioxidant defense in exercised skeletal muscle (combined analysis). (A) Expression levels of *PGC1 α* RNA transcripts in skeletal muscle biopsies derived from previously untrained (white circles) and pretrained (black triangles) individuals before (pre, *Left*) and after (post, *Right*) physical exercise over 4 weeks as described in the *Methods* section. (*Left*) Individuals not taking any medication or placebo; (*Right*) individuals taking both vitamin C (1000 mg/day) and vitamin E (400 IU/day). Horizontal red lines depict means for untrained and trained individuals together ($n = 19$ and $n = 20$, respectively). (B) expression levels of *PGC1 α* RNA transcripts; (C) expression levels of *PPAR α* RNA; (D) levels of superoxide dismutase 1 (*SOD1*) transcripts; (E) RNA levels of superoxide dismutase 2 (*SOD2*); (F) glutathione peroxidase 1 (*GPx1*) RNA expression levels, all in a similar fashion as in (A). Significances: * indicates $0.01 < P < 0.05$ comparing delta values before and after 4 weeks of exercise (ANOVA), *** indicates $P < 0.001$ (ANOVA).

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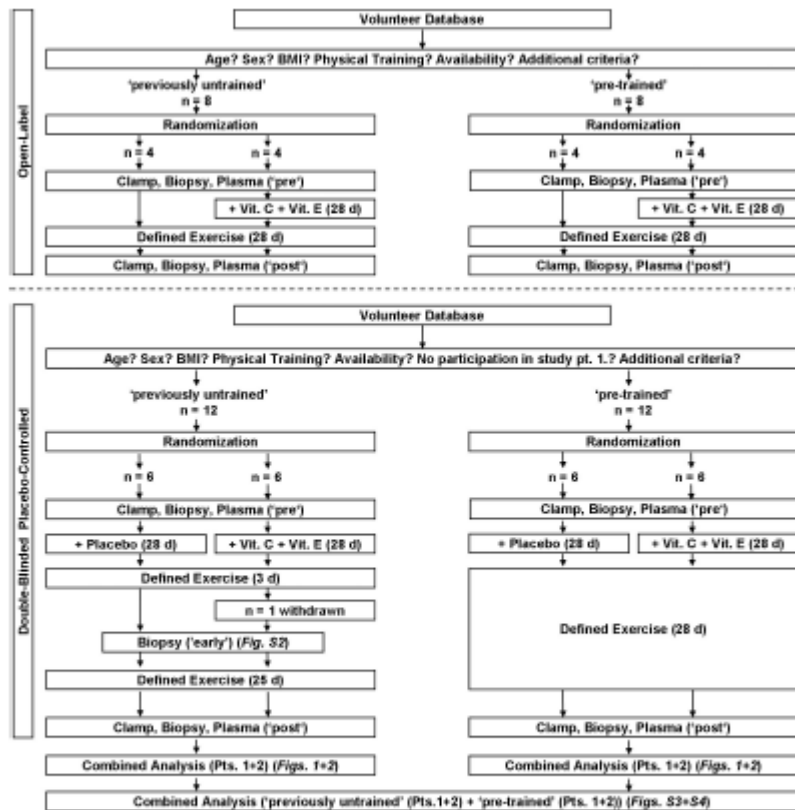


Fig. S1. Study design. The study consisted of 2 parts including an open-label first part (Upper), and double-blinded placebo-controlled second part (Lower), including previously untrained individuals (Left) and previously trained individuals (Right). Both groups were randomly split into antioxidant-treatment or a) placebo-treatment and analyzed after exercise intervention and collection of samples as described in Methods section.

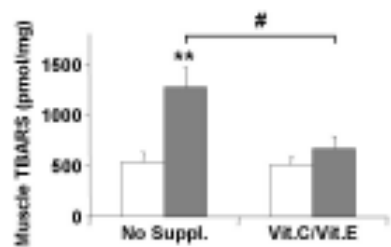


Fig. S2. Antioxidants prevent exercise-induced formation of oxidative stress in skeletal muscle. TBARS concentrations in skeletal muscle following a 3 day exercise intervention in the presence (Left pair of bars) and absence (Right pair of bars) of antioxidants before (white bars) and after (shaded bars) physical exercise. Bars depict means, error bars show standard error means. # indicates $0.01 < P < 0.05$ comparing "no suppl." with "Vit.C/Vit.E" groups, ** indicates $0.001 \leq P \leq 0.01$ comparing data before and after 3 days of exercise.

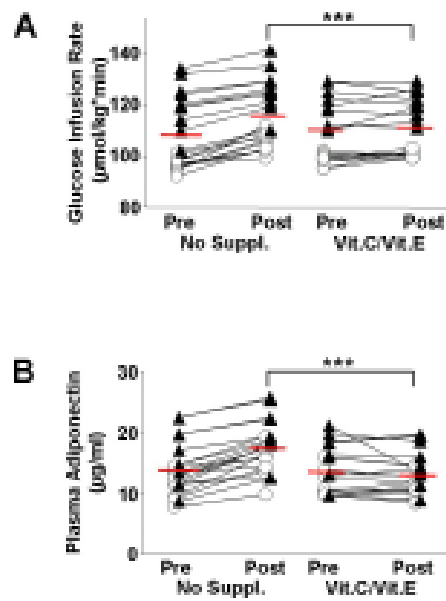


Fig. 2B. Antioxidants prevent exercise-induced induction of insulin sensitivity [combined analysis]. (A) depicts glucose infusion rates (GIR) during euglycemic hyperinsulinemic clamps in previously untrained individuals (open circles) and pretrained individuals (black triangles) before (pre, Left) and after (post, Right) physical exercise over 4 weeks. (Left) Individuals not taking any medication or placebo; (Right) individuals taking both vitamin C (1000 mg/day) and vitamin E (400 IU/day). Horizontal red lines depict means for untrained and trained individuals together ($n = 19$ and $n = 20$, respectively). Significance: *** indicates $P < 0.001$ comparing delta values before and after 4 weeks of exercise (ANOVA). B depicts plasma adiponectin levels in a similar manner as A.