

RESEARCH ARTICLE

A nutrient cocktail prevents lipid metabolism alterations induced by 20 days of daily steps reduction and fructose overfeeding: result from a randomized study

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(U-¹³C) fructose to assess liver de novo lipogenesis. Histological changes and related cellular markers were assessed from muscle biopsies collected on days 0 and 20. While the cocktail did not prevent the decrease in insulin sensitivity and its muscular correlates induced by the intervention, it fully prevented the hypertriglyceridemia, the drop in fasting HDL and total fat oxidation, and the increase in de novo lipogenesis. The cocktail further prevented the decrease in the type-IIa muscle fiber cross-sectional area and was associated with lower protein ubiquitination content. The circulating antioxidant capacity was improved by the cocktail following the OGTT. In conclusion, a cocktail of nutrient compounds from dietary origin protects against the alterations in lipid metabolism induced by physical inactivity and fructose overfeeding.

NEW & NOTEWORTHY This is the first study to test the efficacy of a novel dietary nutrient cocktail on the metabolic and physiological changes occurring during 20 days of physical inactivity along with fructose overfeeding. The main findings of this study are that 1) reduction in daily steps leads to decreased insulin sensitivity and total fat oxidation, resulting in hyperlipemia and increased de novo lipogenesis and 2) a cocktail supplement prevents the alterations on lipid metabolism.

countermeasure; nutrition; OGTT; omega-3 fatty acids; polyphenols; physical inactivity; sedentary behaviors; selenium; vitamins

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INTRODUCTION

Over the past decade, physical inactivity has emerged as an important risk factor for a number of chronic metabolic and

cardiovascular diseases. In addition, sedentary behaviors have been associated with adverse health effects (35) even in subjects who meet the levels of current recommendations on physical exercise (14). This sedentary death syndrome (41) is responsible for an equivalent number of deaths to smoking (19) and has prompted numerous research studies to understand the role of physical inactivity and sedentary behaviors in disease etiology and to develop efficient preventive strategies.

Research on the physiology of physical inactivity in humans has been pioneered by researchers investigating physiological adaptations of astronauts to space environment. To do so, they used a ground-based model analog to microgravity, the bed rest model (28). Indeed, the hypokinesia and hypodynamia induced during prolonged bed rest are largely responsible for the described adaptations to space. Over the past 60 years, bed rest studies ranging from as little as 3 days to 120 days have demonstrated that physical inactivity leads to muscle atrophy and a shift from slow oxidative fibers toward fast glycolytic fibers (59). These structural adaptations are strongly associated with the development of metabolic inflexibility, low-grade inflammation, and oxidative stress (56, 66, 70). There is an increase in fasting and postprandial glucose oxidation concomitant to an increased spillover of dietary lipids along with a reduced capacity to burn fat (see Ref. 9 for review). The resultant hypertriglyceridemia leads to ectopic fat storage in the liver, muscle, and bone marrow (81) and contributes to the development of insulin resistance at the muscle level (9). The role of physical inactivity and sedentary behaviors in the onset and progression of metabolic diseases has attracted increasing attention from the scientific biomedical community over the past decade. Some investigators have proposed to reduce the number of daily steps of physically active individuals to study the direct metabolic effects of physical inactivity (38, 39, 62). While these studies confirmed the findings observed during the bed rest studies at levels of physical inactivity closer to what is observed in the general population, they did not examine the underlying mechanisms. The physiology of physical inactivity therefore needs to be further delineated to develop strategies to prevent its deleterious effects.

Recent studies conducted to develop preventive strategies suggest that bioactive nutrients, such as polyphenols, vitamins, and essential fatty acids, may mitigate some metabolic features of physical inactivity. However, so far, most studies have been tested in rodent analogs of microgravity to induce muscle disuse atrophy and metabolic disorders. A large body of data exists in humans, rodents, and primates demonstrating the effect of polyphenols, such as quercetin, resveratrol, cinnamon, grape, or green tea extracts on insulin sensitivity, lipid metabolism, inflammation, and oxidative stress (see Refs. 2, 24, 33, 44, 55, 77, 78, 85 for recent reviews). In addition, polyphenols supplemented as pure molecules, such as 8-phenylnaringenin [flavanones (53)], quercetin, [flavonols (54)], resveratrol [oligostilbens (51)], epigallocatechin 3 [catechins (46)], or as extracts from dietary sources (green tea, apple extracts, or grape seed extracts), prevent several aspects of rodent muscle atrophy and metabolic disorders induced by disuse, whereas some promote a fatigue-resistant muscle fiber phenotype (3, 40, 46, 50, 63).

Vitamin E is also known to have antioxidant capabilities by acting as a reactive oxygen species (ROS) scavenger and anti-inflammatory properties through inhibition of the NF- κ B

pathway, which is well described in disuse atrophy models to activate proteolytic pathways (32, 37). Vitamin E supplementation mitigates disuse atrophy in rats (71), reduces adipose tissue fibrosis, inflammation, oxidative stress, and blood lipids in humans with obesity (1). Vitamin E intake is also related to muscle strength in the elderly (12, 16). Selenium is often cosupplemented with vitamin E as it scavenges ROS and boosts the intracellular effects of vitamin E. The combination of these two micronutrients was also shown to be beneficial on specific dystrophy (65). Furthermore, selenium has independent effects on insulin sensitivity and reduces insulin secretion (79). Although these effects are still debated, there are clear antioxidant and anti-inflammatory effects of selenium (22), but not on lipid metabolism (79).

The role of omega 3 fatty acids (ω -3) has been studied for decades. Studies in healthy and unhealthy humans and animals showed that they improve hepatic insulin sensitivity, lower very low-density lipoprotein (VLDL) production and de novo lipogenesis by the liver, and reduce inflammation and oxidative stress (15, 29, 60). All of these are metabolic features observed in the inactive/sedentary states (9). These observations were also reported in the presence of fructose overfeeding (21). Surprisingly, besides the protective effects of ω -3 supplementation during bed rest and spaceflight on bone markers (88), no studies have investigated their impact on muscle function during disuse. However, some studies suggest they could have beneficial effects to prevent against muscle atrophy. In rodent cancer-induced cachexia, ω -3 supplementation prevented muscle atrophy (74), and in the elderly, ω -3 increased activation of the protein synthesis mammalian target of rapamycin (mTOR) pathway in response to insulin stimulation (72, 73).

While the available literature suggests that some bioactive molecules, taken individually or as food extracts, improve several aspects of muscle and whole-body metabolic control, it is unlikely that any single micronutrient will be sufficiently powerful to reverse the wide range of deleterious effects induced by physical inactivity. Recently, the notion of nutrient cocktails to trigger additive and/or synergistic effects between bioactive compounds has been proposed (6). Several studies have shown that cosupplementing with ω -3 and green tea extracts (49), ω -3 and vitamin E (20, 83), vitamin E and selenium (25), fish oil and selenium (84), and epigallocatechin and resveratrol (52) improve, to some extent, insulin sensitivity, body composition, fat oxidation, inflammation, and oxidative stress in aging and in diseases associated with muscle atrophy, including obesity and type 2 diabetes.

While these cocktails have the potential to reduce the risk of metabolic disease, there are no studies in the literature examining their impact on physical inactivity-induced metabolic alterations in humans. The purpose of this study was to determine the preventive effect of a nutrient cocktail composed of polyphenols, ω -3, vitamin E, and selenium on the metabolic and physiological changes that occur during 20 days of reduced daily steps and exercise in trained men. To further trigger metabolic deterioration, dietary fructose supplementation was provided during the last 10 days of the trial. We hypothesized that the cocktail supplementation would reduce and/or prevent the deterioration in glucose and lipid metabolism, insulin sensitivity, and muscle atrophy that are associated with reduced physical activity and fructose overfeeding.

METHODS

Subjects

Twenty healthy, trained young men were recruited from the local community. Characteristics are presented in Table 1. Habitual physical activity was assessed using hip-worn triaxial accelerometry (Actigraph GT3x+, Actigraph, Pensacola, FL) for 7 days. Subjects were included if they walk >10,000 steps/day, participated in at least 2 leisure sport sessions per week, and were free of any known diseases. Subjects were excluded if not able to drastically reduce professional, leisure, and transport-related physical activities. This study was approved by the local ethical committee, and all subjects provided written informed consent (NCT03313869, ID-RCB number: 2015-A00665-14).

Experimental Design

The experiment outflow is presented in Fig. 1. Participants were randomly assigned to control ($n = 10$) or daily nutrient cocktail supplemented ($n = 10$) groups. Random tables were generated by the study statistician. The participants, nurses, and physicians were not blinded as no placebo pills were given to the control group; however, the statistician was blinded. During the 20-day study in free-living conditions, volunteers from both groups were asked to stop exercising and drastically reduce their daily physical activity. Subjects were instructed to walk a maximum of 2,500–4,000 steps/day. This was controlled on a daily basis by the subject and the investigators through a wrist-worn Fitbit pedometer (Fitbit). Accurate data were assessed using an Actigraph GT3x+ (hip-worn) throughout the 20 days of the experiment. Metabolic stress induced by physical inactivity was boosted during the last 10 days of the protocol by ingesting 3 g·kg⁻¹·day⁻¹ of fructose (Vivis fructose) mixed with 0.5 g·kg⁻¹·day⁻¹ glucose in water to alleviate intestinal problems (61, 80), on top of their regular diet. Fasting blood collection, glucose tolerance, substrate oxidation, and de novo lipogenesis were measured at baseline and after 10 and 20 days of reduced activity. Muscle biopsies and body composition measurements were completed at baseline and after 20 days (Fig. 1). Diet was not controlled during the intervention; subjects were instructed to eat according to their appetite.

Cocktail Composition and Doses

The supplemented group received a polyphenol nutrient cocktail derived from food sources that consist of Liliaceae, Vernenaceae, Lamiaceae, Vitaceae, Rubiaceae, Theaceae, and Rutaceae genres consisting of *Allium cepa*, *Lippia citriodora*, *Ajuga reptans*, *Vitis vinifera*, *Coffea robusta*, *Camellia sinensis*, and *Citrus aurantium*. The cocktail is referred to as XXS-2A and was designed by Spiral Company (Dijon, France). The daily dose was achieved by the ingestion of 3 pills (one at breakfast, lunch, and dinner) to reach a total dose of 529.5 mg/day of polyphenols that was composed of 120 mg/day flavonols (including 50 mg quercetin), 75 mg/day oligostilbens (including 20 mg resveratrol), 91.5 mg/day hydroxycinnamic acids (including 40 mg chlorogenic acid), 135 mg/day flavanols (including 60 mg epigallocatechin gallate), and 108 mg/day flavanones (including 30 mg naringin). As there is no dietary references intake (DRI) available for polyphenols, the ~500 mg/day dose was based on several reviews on the bioavailability and bioefficacy of polyphenols in humans and other studies that tested the effects of polyphenols on exercise performance and oxidative stress (43, 57, 75). The 3-g daily dose of ω -3 (Omacor, Pierre Fabre Laboratories, Toulouse France) was based on French pharmacopeia recommendations for hypolipemic effects (2–4 g/day) and was provided as one pill per meal, which is within the daily dose used in most clinical studies (29, 60). This daily dose thus corresponded to 1.1 g of eicosapentaenoic acid and 1 g of docosahexaenoic acid. Vitamin E and selenium

Table 1. Anthropometry and physical activity data

Variables	Baseline		Day 10		Day 20		Mixed Model	
	Control	Supplemented	Control	Supplemented	Control	Supplemented	Supplementation	Intervention
	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 10$	$n = 10$		Interaction
Weight, kg	77.5 ± 2.3	76.5 ± 2.2	77.6 ± 2.2	76.7 ± 2.3	77.8 ± 2.1	76.9 ± 2.4	0.89	0.28
Fat mass, kg	16.9 ± 1.4	17.8 ± 1.8	17.2 ± 1.4	18.1 ± 1.7	17.4 ± 1.3	18.3 ± 1.7	0.44	<0.001
Fat mass, %	22.0 ± 1.4	23.4 ± 2.0	22.3 ± 1.4	23.8 ± 2.0	22.6 ± 1.3	24.1 ± 2.0	0.58	<0.001
Lean body mass, kg	56.7 ± 1.5	54.7 ± 1.7	56.5 ± 1.4	54.5 ± 1.8	56.4 ± 1.4	54.4 ± 1.9	0.87	0.32
Daily steps	13,032.6 ± 875.1	14,951.7 ± 1,720.1	2,511.8 ± 235.3	2,663.8 ± 359.7	2,644.9 ± 330.7	3,008.9 ± 297.8	0.69	<0.001
Time spent sitting during waking time, min/day	445.9 ± 37.7	461.7 ± 29.4	477.7 ± 43.3	528.2 ± 39.7	552.0 ± 42.2	527.6 ± 41.6	0.98	<0.01
Time spent in light intensity activity, min/day	213.8 ± 22.5	206.0 ± 21.9	114.5 ± 20.2	119.2 ± 23.4	125.8 ± 26.7	130.9 ± 23.8	0.58	<0.05
Time spent in moderate to vigorous intensity activity, min/day	104.5 ± 10.5	134.3 ± 17.7	18.2 ± 4.8	21.7 ± 7.7	17.6 ± 3.2	29.1 ± 9.2	0.67	<0.001
$n =$ no. of participants. Means are ± SE. Mixed models are adjusted on baseline.								0.69

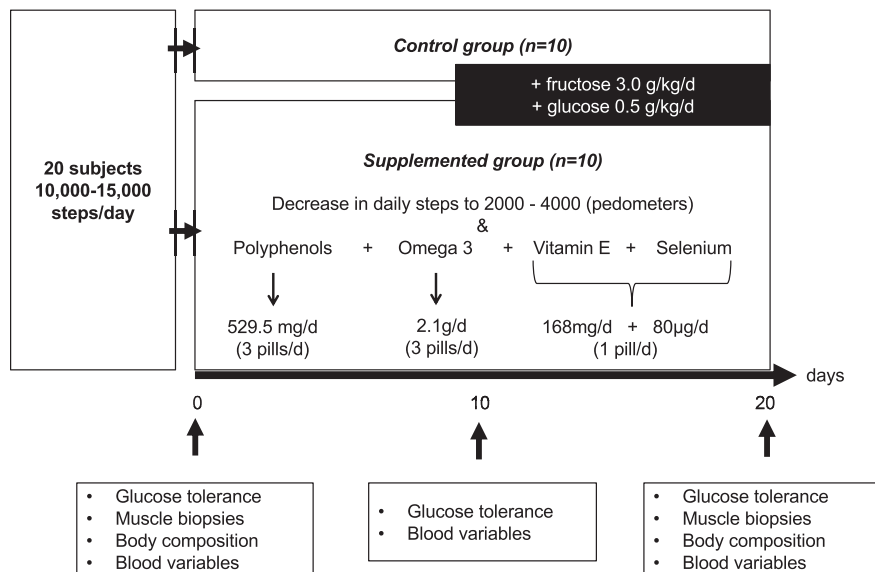


Fig. 1. Design of the study.

were given as a single daily pill, providing 168 mg of vitamin E associated with 80 µg of selenium (Solgar, Marne la Vallée, France). The DRI for Vitamin E is set at 15 mg/day. The tolerable upper limit (UL) for intake is set at 1 g in adults; therefore, the dose from the commercially available pill was 6 times lower than the UL but 11 times higher than DRI. To capitalize on the cocktail effect, the dose of vitamin E was twofold lower the doses that have provided positive effects on metabolism and muscle (25, 65). Regarding selenium, the intake reported in most countries presents a large variability. DRI and UL are respectively set at 55 and 400 µg/day. The selected dose provided a daily supplement that set the daily intake at 135 µg/day, which is 3 times lower than the UL and lower than the dose reported in previous studies (25, 65) to capitalize on cocktail effects. The control group did not receive any supplementation or placebo.

Body Composition

Fat mass (FM) and lean body mass (LBM) were assessed by using a dual-energy X-ray absorptiometer (DXA, HOLOGIC QDR 4500W) at baseline and on day 20 of the experiment.

Physical Activity Patterns

Time spent sitting and being active was determined using a triaxial accelerometer (ActiGraph GT3X+; ActiGraph). Participants were instructed to wear the accelerometer at their right hip at all times, except for bathing, during 1 wk before the inclusion and during the experimental protocol. At each visit, both raw accelerometry and activity counts per minute were downloaded using manufacturer software (Actilife 6.13). An automatic activity-recognition algorithm (7) that identifies sitting time and an activity-specific energy expenditure model (23), both developed by our group, were used to determine time spent in a sitting position and in different activity intensities; cut-points of 1.5–3 METs and >3METs were used for light intensity activity and moderate-to-very vigorous activity, respectively.

Glucose Tolerance and Substrate Use

Subjects reported to the clinic the evening before the test. A standard dinner was given, containing 47.7% carbohydrates, 32.5% lipids, and 17.8% protein for a total of 918 Kcal intake. An oral glucose tolerance test (OGTT) was performed after an overnight fast using a mix of 1 g/kg of glucose and 0.5 g/kg of fructose diluted in 300 ml of water; 1% of fructose was labeled with U-¹³C-fructose

(Eurisotop, Paris). Following baseline collection and glucose/fructose ingestion, blood samples were collected every 15 min for the first 3 h and every 30 min for the fourth hour. Carbohydrate and fat oxidation rates were determined every hour using canopy dilution respirometry (Quark, Cosmed, Italy) and the classical equations of indirect calorimetry corrected for urinary nitrogen excretion. Insulin sensitivity was estimated using the Matsuda index (45). Metabolic flexibility, defined as the ability to adjust nutrient oxidation to nutrient availability and demand, was assessed during the OGTT by examining the relationship between the variance of plasma insulin and the variance of the nonprotein respiratory quotient (NPRQ). As explained previously (9), the variance-derived indexes assume a metabolically flexible state when the variance in insulin is low and the variance in NPRQ is high, in other words, when the body has a high capacity to switch from fat to carbohydrate oxidation in association with small changes in insulin concentration in response to the OGTT. A transition toward a metabolically inflexible state is assumed when the variance in insulin increases and/or the variance in NPRQ decreases.

Exogenous Fructose Oxidation

Exhaled breath samples were collected at the same time as blood samples. Breath ¹³C/¹²C isotopic ratio was measured in triplicate on a GasBench system (Thermo Scientific, Germany) connected to a continuous-flow isotope ratio mass spectrometer (IRMS, DELTA V, Thermo Scientific). U-¹³C-fructose oxidation was calculated as the cumulated percentage dose recovery of ¹³C in expired CO₂/h over the OGTT, as previously described (42).

Liver de Novo Lipogenesis

De novo lipogenesis was measured as the apparition of 1-¹³C-palmitate from U-¹³C-fructose in VLDL. In brief, as previously described (42), VLDLs were separated by sequential ultracentrifugation, and total lipids were extracted from VLDL by a modified Folch technique. Triglycerides (TGs) were further separated by solid phase extraction and derivatized into methyl esters. The absolute concentration of both unlabeled and labeled palmitate was measured by gas chromatography/mass spectrometry (Agilent 5975, Inert XL) through a dual acquisition program in single ion monitoring mass-to-charge ratios (*m/z*) of 270 and 271 and calculated by reference to internal standards added to the plasma. The concentration of 1-¹³C-palmitate was calculated by multiplying its molar percent enrichment by the concentration of total palmitate.

Blood Sample Analyses

Plasma insulin was assessed by radioimmunoassay, and glucose, nonesterified fatty acids, and TGs were measured by colorimetric assays and enzymatic methods, as previously described (42). Reduced and oxidized glutathione concentrations were simultaneously measured by reverse-phase high-performance liquid chromatography as previously described (87). Total plasma malondialdehyde was determined by reverse-phase high-performance liquid chromatography (82). The antioxidant defenses were examined using a test based on an *in vitro* free radical-induced blood hemolysis KRL test (Kirial International/Spiral, Couternon, France) as previously described (68).

Muscle Biopsies and Parameters

Muscle biopsies were performed on the mid vastus lateralis (VL) under aseptic conditions and after anesthesia (2% wt/vol lidocaine HCl) using a Bergström skeletal muscle biopsy needle (Lauran Médical, KBM 5/10). One piece was mounted in tragacanthum gum (OCT Compound) for histological analysis, cooled to the temperature of liquid nitrogen in isopentane, and the remainder was snap-frozen in liquid nitrogen and stored at -80°C until further analysis.

Immunohistochemical classification of muscle fibers and cross-sectional area measurements. Transverse serial cross sections (10 μm thick) of VL muscle samples were obtained using a cryostat at -25°C (HM-560, Microm H) and mounted onto glass microscope slides. Before labeling, sections were dried and fixed for 10 min in acetone. Sections were then washed in PBS, blocked, and permeabilized with 0.1% Triton-X100 and 20% horse serum. For muscle fiber-typing and cross-sectional area (CSA) determination, sections were incubated with anti-myosin heavy chain (MyHC) primary antibodies [anti-slow (I) MyHC, (BA-D5, Developmental Studies Hybridoma Bank, 1:10), anti-fast (II) MyHC (M4276, Sigma-Aldrich, 1:200), and anti-fast (IIA) MyHC (SC-71, Developmental Studies Hybridoma Bank, 1:10)] for 1 h at 37°C , followed by washes in PBS and incubation with the secondary antibodies [ALEXA 488 (A11029, Invitrogen, 1:800) and ALEXA 568 (A11031, Invitrogen, 1:800)] for 1 h. Fiber typing was manually determined, and the fiber sizes were analyzed with Image J software (1.46r version). A total of ~ 300 myofibers per sample were thus measured in double-immunostained pre- and post-intervention VL cryosections.

Protein expression. Muscle samples were homogenized in 10 volumes of lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 100 mM NaF, 5 mM Na_3VO_4 , 1% Triton X-100, 1% SDS, 40 mM β -glycerophosphate, and protease inhibitor mixture (P8340; Sigma-Aldrich)] and centrifuged at 10,000 g for 10 min (4°C). Protein extract (60 μg) was loaded into Stain-Free 4%–20% precast gels (4568095; Bio-Rad) before electrophoretic transfer onto nitrocellulose membranes (Bio-Rad; Trans-Blot Turbo Blotting System). After transfer, the membranes were blocked with 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween 20 containing 5% skimmed milk or bovine serum albumin and incubated overnight at 4°C with primary antibodies. The membranes were then incubated for 1 h with a peroxidase-conjugated secondary antibody. The immunoblots were revealed using a Pierce ECL kit (32106; Thermo Scientific), and proteins were visualized by enhanced chemiluminescence using the Chemi-Doc Touch Imaging System and quantified with Image Laboratory Touch Software (version 5.2.1). Stain-Free technology or red Ponceau or GAPDH was used as loading control.

Citrate synthase activity. Citrate synthase activity of muscle samples was measured using a commercially available kit, as reported before (36).

Data and Statistical Analysis

The effects of the intervention and the cocktail supplementation on the outcomes were assessed by using mixed linear models with group,

intervention, group-by-intervention interaction, and baseline values as fixed effect; taking repeated measurements among subjects into account by including the subjects' ID as random effect. Additional adjustment for changes in FM and LBM was performed for substrate oxidation. Between-group and within-group differences were assessed by using post hoc tests. Data obtained during OGTTs are presented as area under the curve (AUC). Significance was set at 0.05 for main effects and 0.10 for interaction effects. Values are mean $\pm$ SE, unless otherwise stated. Statistical analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Subject characteristics and changes in time spent physically active and sitting. Subject characteristics and changes in activities are presented in Table 1. Participants' compliance was very good; over the 20-day intervention, daily steps went down from $14,952 \pm 1,720$ steps to $3,009 \pm 298$ steps in the supplemented group and from $13,032 \pm 875$ steps to $2,645 \pm 331$ steps in the control group. We observed in both groups a reduction of both light intensity activity and moderate-to-very vigorous intensity activity, along with a significant increase in time spent sitting during waking hours. Although the intervention did not modify body and lean mass, we observed a significant increase in FM of less than 1 kg, independent of the cocktail supplementation.

Glucose tolerance and insulin sensitivity. Fasting plasma glucose and insulin were neither affected by the intervention nor by the cocktail supplementation (Table 2). Concentrations of glucose, TGs, and insulin during the OGTT are presented in Fig. 2, A–C, respectively. To increase readability of the results, data are also presented as AUC in Fig. 3. During the OGTT, glucose concentration did not change between control and supplemented groups (Fig. 3B); insulin increased after 10 days of reduction in daily steps (Fig. 3A) but did not further change after 10 more days of inactivity combined with fructose overfeeding. Reduction in daily steps decreased insulin sensitivity in both groups, as indicated by the decrease in the Matsuda index (Fig. 3C). Fasting high molecular weight adiponectin increased during the intervention in both groups, but the increase was greater in the supplemented group compared with the control group after 20 days of intervention (Table 2). Total carbohydrate oxidation (Fig. 3D) and exogenous fructose oxidation (Fig. 3E) during the OGTT increased similarly in both groups after both 10 and 20 days of intervention. While a group-by-intervention interaction almost reached significance for an increase in insulin variance, significant decrease in NPRQ variance was observed during the OGTT in the control group after both 10 and 20 days of intervention, which was fully prevented by the supplementation (Fig. 3F). This indicates a development of metabolic inflexibility in the control group but not in the supplemented group, which was likely due to decreased oxidative capacities in association with whole-body metabolic adaptations. Of note, most metabolic changes were observed after 10 days of inactivity only; the addition of 10 more days of reduced daily steps combined with fructose overfeeding did not lead to further modifications in metabolic flexibility.

Lipid metabolism. Fasting TGs and HDL, respectively, increased and decreased in the control group after both 10 and 20 days of intervention (Table 2). Similar responses were ob-

Table 2. Fasting metabolites, hormones and substrate oxidation

Variables	Baseline			Day 10			Day 20			Mixed Model	
	Control	Supplemented		Control	Supplemented		Control	Supplemented		Supplementation	Intervention
	n = 10	n = 10		n = 10	n = 10		n = 10	n = 10			Interaction
Metabolites											
Glucose, mM	5.58 ± 0.16	5.29 ± 0.12		5.39 ± 0.09	5.22 ± 0.12		5.39 ± 0.11	5.45 ± 0.10	0.83	0.28	0.18
HOMA index	0.76 ± 0.13	0.80 ± 0.13		1.00 ± 0.07	0.84 ± 0.12		1.30 ± 0.21	1.16 ± 0.21	0.15	<0.001	0.54
TG, mmol/l	0.95 ± 0.06	1.01 ± 0.09		1.05 ± 0.07	0.86 ± 0.07##		1.26 ± 0.14##	0.93 ± 0.09***	0.01	0.04	0.01
HDL, mmol/l	1.31 ± 0.04	1.23 ± 0.06		1.18 ± 0.04	1.29 ± 0.08***		1.20 ± 0.06	1.30 ± 0.07***	<0.001	0.25	<0.001
LDL, mmol/l	2.90 ± 0.22	2.69 ± 0.19		2.88 ± 0.21	2.94 ± 0.18		3.08 ± 0.26	3.05 ± 0.25	0.18	<0.01	0.18
Hormones											
Insulin, mU/l	3.05 ± 0.49	3.40 ± 0.52		4.16 ± 0.29	3.57 ± 0.49		5.33 ± 0.77	4.74 ± 0.85	0.07	<0.001	0.35
Total adiponectin, µg/ml	5.19 ± 0.68	6.99 ± 1.54		5.36 ± 0.94	7.27 ± 1.04		6.73 ± 1.05	9.22 ± 1.33	0.29	<0.001	0.75
HMW adiponectin, µg/ml	2.93 ± 0.43	4.18 ± 0.74		3.08 ± 0.41	4.60 ± 0.66		3.99 ± 0.64##	6.27 ± 0.85*##	0.15	<0.0001	0.05
Substrate oxidation											
	n = 10	n = 8		n = 10	n = 8		n = 10	n = 8			
NPRQ	0.93 ± 0.03	0.94 ± 0.03		1.04 ± 0.03	1.02 ± 0.03		1.07 ± 0.03	0.97 ± 0.03	0.20	<0.01	0.16
Lipid oxidation, mg/min	24.48 ± 8.47	15.24 ± 9.54		-8.47 ± 8.47	-6.64 ± 9.51		-19.77 ± 8.48	4.03 ± 9.50	0.53	<0.01	0.18
Glucose oxidation, mg/min	196.08 ± 15.08	203.71 ± 16.88		232.07 ± 15.05	220.82 ± 16.88		252.86 ± 15.04	254.50 ± 16.90	0.97	<0.01	0.81

n = no. of participants. Means are ± SE. Mixed models are adjusted on baseline. Post hoc tests. HOMA, Homeostasis model assessment of insulin resistance; HMW, high molecular weight; NPRQ, nonprotein respiratory quotient; TG, triglycerides. * $P < 0.05$, *** $P < 0.001$ vs. control. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$ vs. baseline.

served during the OGTT. TGs (Fig. 4A), VLDL-TGs (Fig. 4B), palmitate VLDL-TGs (Fig. 4C), and de novo lipogenesis from fructose (Fig. 4D) gradually and significantly increased after 10 and 20 days of intervention in the control group. The intervention decreased total lipid oxidation in association with an increase in net lipid synthesis both in the fasting states and following the OGTT (Table 2 and Fig. 4E), as indicated by the negative values of lipid oxidation, thus confirming the increase in de novo lipogenesis as measured by stable isotopes. The cocktail supplementation fully prevented the increase in lipemia (Table 2) in both the fasting state and during the OGTT (Fig. 4A), the decrease in total lipid oxidation (Table 2 and Fig. 4E), and the increase in de novo lipogenesis measured both by the tracer technique (Fig. 4D) and indirect calorimetry (Table 2) induced by the daily step reduction with or without fructose overfeeding.

Antioxidant capacity. Fasting oxidative stress markers remained unaffected by either the intervention or the supplementation (Table 3). However, blood antioxidant capacity following the OGTT decreased in the control group when the fructose overfeeding was added to the daily step reduction, whereas the supplemented group remained at significantly higher values of whole-blood antioxidant capacity (Fig. 3F).

Skeletal muscle analysis. Muscle parameters are presented in Table 4. While type I fibers' CSA was lower in both groups at the end of the intervention, the decrease in type IIa CSA observed in the control group was prevented by the cocktail supplementation. In line with this observation, ubiquitination content, a protein marker of proteolysis processes, increased only in the control group. At the mitochondrial level, both mitofusin-2 and peroxysome proliferator-activated receptor gamma coactivator 1 alpha contents dropped in response to the intervention. We failed to see a protective effect of the cocktail. Fatty acid transport protein 1, involved in fatty acid transportation at the mitochondrial level, tended to rise in the cocktail group only ($P = 0.07$), supporting the maintenance of lipid oxidation during the intervention in the supplemented group but not in the control group. No changes in citrate synthase activity, skeletal muscle intracellular signaling protein contents, and oxidative stress markers were observed.

DISCUSSION

The main findings of this randomized, interventional study in lean, healthy, trained young men are that 1) a reduction in daily light intensity activity and moderate-to-vigorous activity along with an increase in time spent sedentary leads to decreased insulin sensitivity, total fat oxidation, hyperlipemia, and increased de novo lipogenesis and 2) a nutrient cocktail supplement prevents the alterations on lipid metabolism but not insulin sensitivity. This is the first study to test the efficacy of a nutrient cocktail to counteract the negative effects of physical inactivity along with fructose overfeeding.

During the intervention, subjects decreased, on average, their activity to <3,000 steps/day. Body weight and LBM remained stable during the study, but FM and the percentage of body fat increased. The effect on body weight is not surprising. It is likely to be influenced by 1) the known delayed compensatory adjustment in energy intake, 2) the duration of the intervention, given that some (27, 38), but not all, studies (5, 26) with 7–14 days of decreased activity reported weight gain,

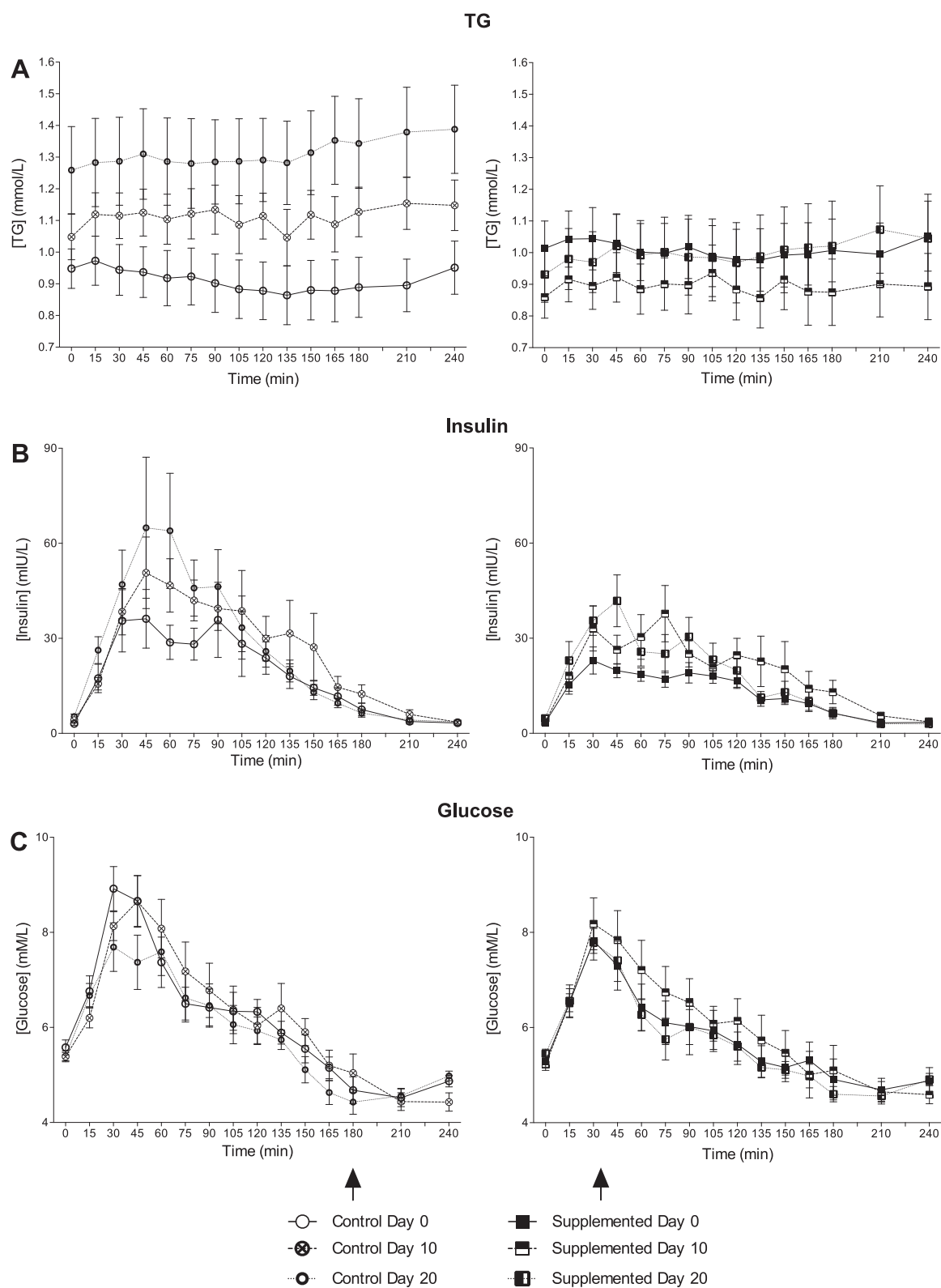


Fig. 2. Plasma triglycerides (TG), insulin, and glucose concentrations during the oral glucose tolerance test (OGTT). Evolution of plasma TG (A), insulin (B), and glucose (C) concentrations during an OGTT in the control (left; $n = 10$) and supplemented (right; $n = 10$) groups before the intervention, after 10 days of reduced physical activity, and after 10 more days of physical inactivity coupled with fructose/glucose overnutrition. Values are means $\pm$ SE.

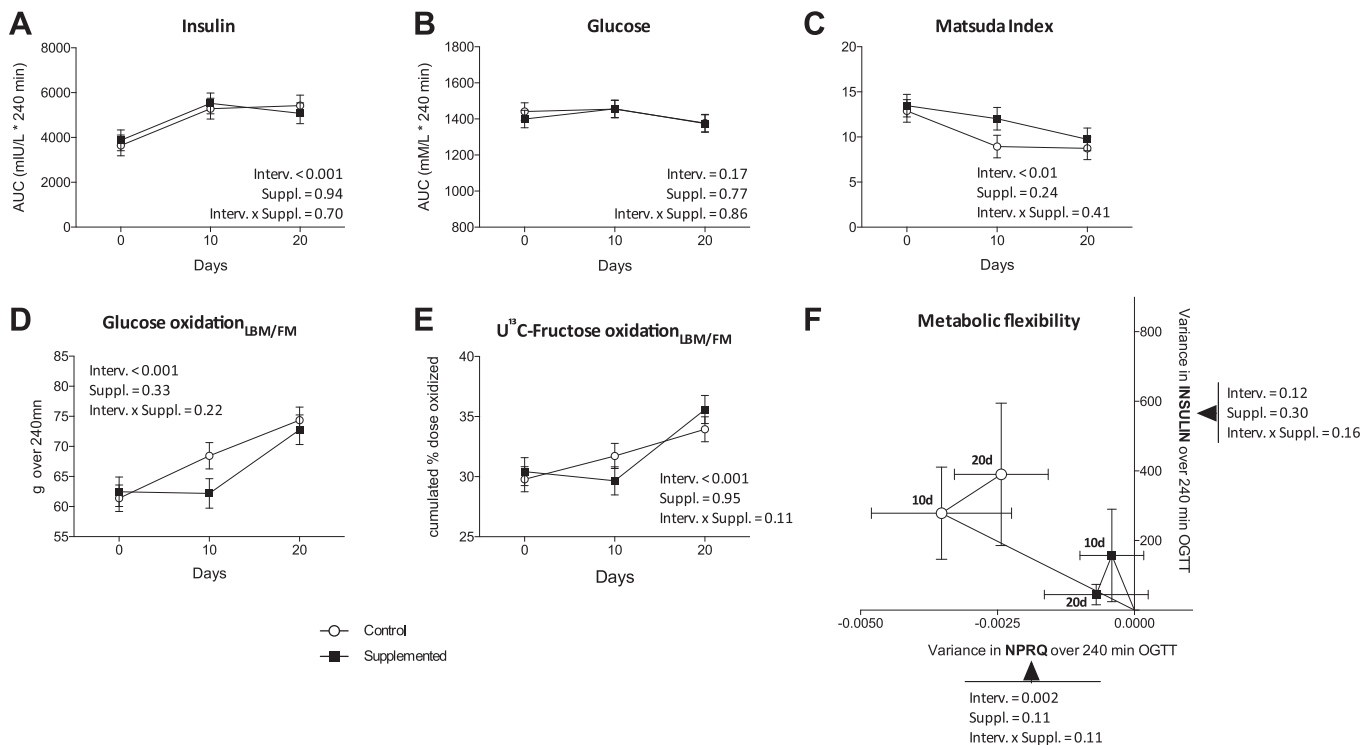


Fig. 3. Carbohydrate metabolism. Area under the curve calculated over each oral glucose tolerance test (OGTT) for plasmonic insulin (A), glucose concentration (B), Matsuda index (C), and $U^{13}C$ -fructose oxidation (E) over 20 days of reduced physical activity in control ($n = 10$) and supplemented ($n = 10$) groups. Area under the curve (AUC) calculated over each OGTT for glucose oxidation (D) and metabolic flexibility (F) over 20 days of reduced physical activity in control ($n = 10$) and supplemented ($n = 8$) groups. Reported data have been adjusted for baseline. Oxidation data are adjusted on fat mass (FM) and fat-free mass. Results of the linear mixed model analysis are displayed on the figure with P value for intervention (Interv.), cocktail supplementation (Suppl.), and the supplementation-by-intervention interaction (Interv. $\times$ Suppl.). Between-group differences were assessed by using post hoc tests at each time point. Values are means $\pm$ SE. LBM, lean body mass; NPRQ, nonprotein respiratory quotient.

and 3) the fructose overfeeding between 10 and 20 days, as others have also reported an increase in FM with overfeeding (38). The increase in FM is thus in line with the design of the study. Overall, LBM was preserved during the study, though there was a decrease in type II muscle fibers' CSA in the control group, suggesting that the cocktail supplementation may delay muscle disuse atrophy.

There was a decrease in insulin sensitivity during the 20-day intervention, in agreement with other studies when activity was reduced for 3–14 days (5, 27, 31, 38, 48, 67). This was mainly due to increased insulin AUC during the OGTT, whereas we did not show a change in glucose, in agreement with some (2, 9), but not all, studies (5, 31). The additional fructose provided after day 10 did not affect insulin sensitivity, but there was a significant increase in carbohydrate oxidation and exogenous fructose oxidation. Knudsen et al. (38) reported similar glucose and insulin responses to an OGTT following 14 days of reduced activity and a 50% increase in energy intake. Whereas the Matsuda index in that study was significantly lower at day 7 but not day 14, clamp-derived insulin sensitivity had decreased by ~44%. In agreement with other studies of decreased activity (38) or bed rest (34), we reported an increase in fasting plasma adiponectin after 20 days of decreased activity. This is in contrast with studies where decreased adiponectin is associated with insulin resistance. It is possible that, in these healthy individuals, an increase in adiponectin may be a short-term compensatory mechanism to preserve insulin sensitivity. In support of this, we did not find a change in Akt and

glycogen synthase kinase 3 proteins following the intervention. This would need to be further investigated, but given the preservation of metabolic flexibility, the effects appear to be more pronounced on lipid metabolism.

By using bed rest ranging from 7 to 90 days in men and women, we have shown that a hallmark of physical inactivity physiology is a decrease in exogenous lipid clearance because of both a reduced uptake and oxidative capacity at the muscle level (see Ref. 9 for review). We further showed in the general population, through training/detraining studies, that activity energy expenditure is a major determinant of lipid trafficking (plasma pools, chylomicrons, and VLDL) and partitioning through uptake (lipoprotein lipase, fatty acid transport CD36, and fatty acid binding protein plasma membrane) between oxidation (long chain acyl co-A synthase and carnitine palmitoyl transferase 1) and storage (mitochondrial glycerol-3-phosphate acyltransferase) within the skeletal muscle (8). In the present study, we observed that physical inactivity by voluntary step reduction and physical inactivity plus fructose overfeeding gradually decreased fasting and postprandial fat oxidation to an extent where only net lipid synthesis was observed during the OGTT, similar to our observation during an OGTT following 7 days of bed rest in men and women (11). One of the striking results of the present study is the capacity of the cocktail to fully prevent the effects on lipid oxidation, thus likely preventing the hypertriglyceridemia of the control group.

Although no similar data exist, numerous recent studies investigating the impact of various bioactive compounds from

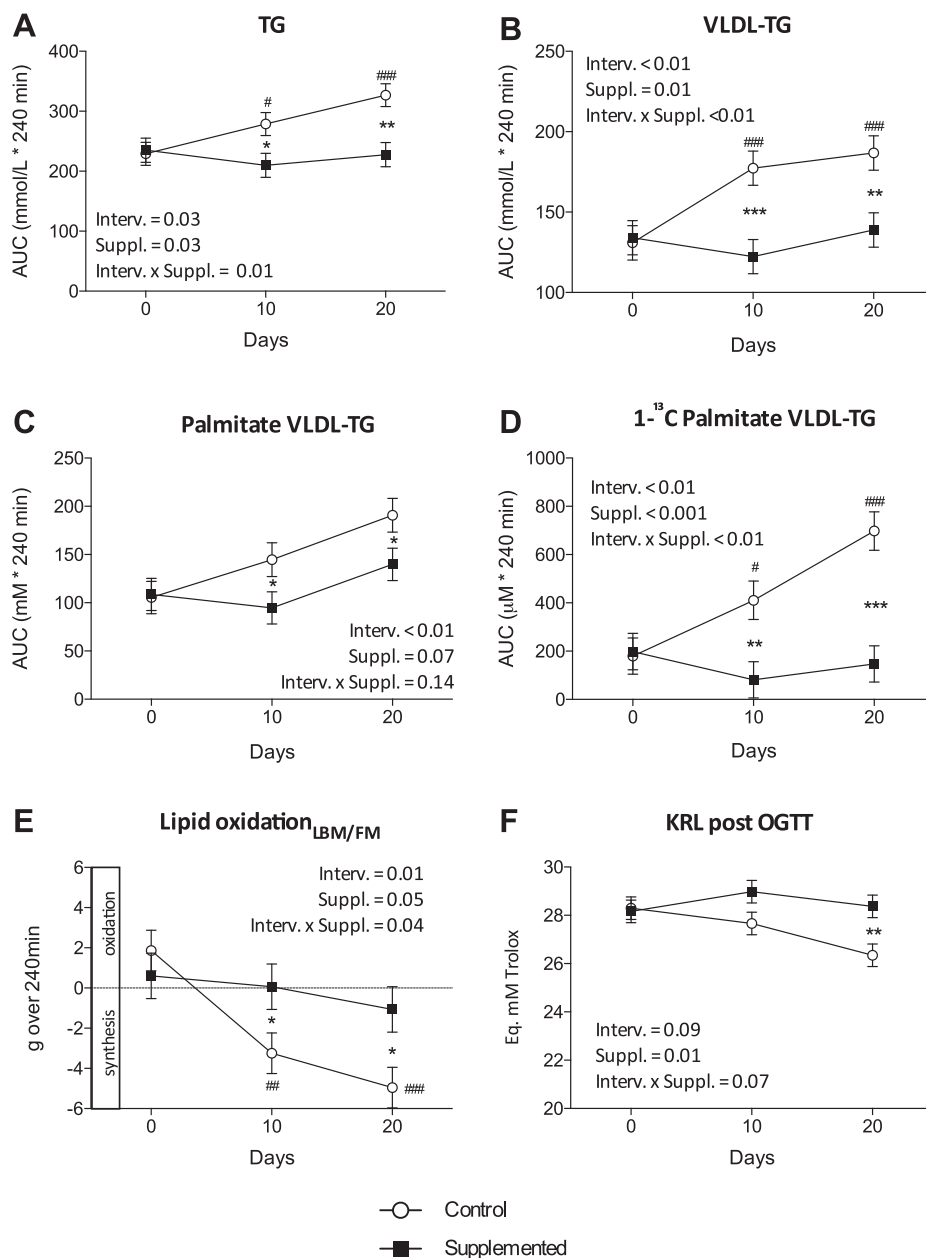


Fig. 4. Lipid metabolism and free radicals defense. Area under the curve (AUC) calculated over each oral glucose tolerance test (OGTT) for plasmatic triglycerides (TG) (A), very low-density lipoprotein (VLDL)-TG (B), palmitate VLDL-TG (C), 1-¹³C palmitate VLDL-TG (D), and KRL (F) over 20 days of reduced physical activity and fructose overfeeding in control ($n = 10$) and supplemented ($n = 10$) groups. AUC calculated over each OGTT for lipid oxidation (E) over 20 days of reduced physical activity and fructose overfeeding in control ($n = 10$) and supplemented ($n = 8$) groups. Reported data have been adjusted for baseline. Oxidation data are displayed on the figure with P value for intervention (Interv.), cocktail supplementation (Suppl.), and the supplementation-by-intervention interaction (Interv. $\times$ Suppl.). Between-group differences were assessed by using post hoc tests at each time point and are represented as follows: $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$; $\#P < 0.05$, $##P < 0.01$, and $###P < 0.001$ vs. baseline. Values are means $\pm$ SE. KRL, KRL, total antioxidant defense potential (in French, Kit Radicaux Libres); LBM, lean body mass.

the diet, taken as cocktails, in various physiopathological states and models support our results (4, 13, 29, 52). However, mechanistic evidence primarily comes from rodent models. Vitamin E plus ω -3 supplementation was hepatoprotective in naturally aging rats, with major antioxidant properties, especially in the brain (58). It was argued that vitamin E reduces the peroxidation of ω -3, thus allowing synergistic effects. In support of that, fish oils associated with grape polyphenols in rats fed a high-sucrose diet improved numerous blood biochemical parameters, such as HDL and TGs, as in our study, by significantly enhancing fatty acid beta oxidation (47). Interestingly, the same study showed a major suppression of lipogenic enzymes by their cocktail, in agreement with our human data. A recent review (69) reported evidence that polyphenols play key controlling roles in suppressing de novo lipogenesis in various models of nonalcoholic fatty liver diseases. Selenium

is often cosupplemented with vitamin E but is likely to have had limited impact in this study. Supplementation with selenium has independent effects on insulin sensitivity and insulin secretion but no effect on lipid profiles (79). In this study, the main changes were in lipid metabolism with no changes in glucose/insulin parameters.

The beneficial effects of fish oil ω -3 on lipemia by lowering TGs and rising HDL have been well known for decades (86). In subjects who are overweight/obese and supplemented with ω -3 and polyphenols, there is a substantial improvement in fasting plasma HDL and their subclasses (13) as well as postprandial TGs and TG-rich lipoproteins (4). Annuzzi et al. (4) showed reduced oxidative damage through a reduced excretion of 8-isoprostane. These data support the improved global antioxidant capacity we observed in our study during the OGTT. The impact on oxidative stress could have been aug-

Table 3. *Fasting oxidative stress and damages*

Variables	Baseline		Day 10		Day 20		Mixed Model		
	Control	Supplemented	Control	Supplemented	Control	Supplemented	Supplementation	Intervention	Interaction
	n = 10	n = 10	n = 10	n = 10	n = 10	n = 10			
Malondialdehyde, $\mu\text{g/ml}$	0.78 $\pm$ 0.05	0.70 $\pm$ 0.07	0.67 $\pm$ 0.05	0.81 $\pm$ 0.05	0.69 $\pm$ 0.08	0.74 $\pm$ 0.05	0.27	0.79	0.22
KRL, mEq trolox	24.16 $\pm$ 1.16	23.38 $\pm$ 0.89	23.09 $\pm$ 0.84	23.70 $\pm$ 0.81	22.72 $\pm$ 1.00	22.26 $\pm$ 0.64	0.54	0.13	0.48
Reseda 1, glucosides, mEq trolox	3.34 $\pm$ 0.18	3.52 $\pm$ 0.19	3.75 $\pm$ 0.23	4.02 $\pm$ 0.22	3.54 $\pm$ 0.21	3.32 $\pm$ 0.16	0.81	0.10	0.56
Reseda 2, sulfates, mEq. trolox	2.65 $\pm$ 0.16	2.65 $\pm$ 0.19	2.83 $\pm$ 1.13	3.10 $\pm$ 0.22	2.69 $\pm$ 0.12	2.78 $\pm$ 0.15	0.78	0.43	0.77
Reseda 3, glucuronides, mEq. trolox	4.95 $\pm$ 0.20	5.26 $\pm$ 0.25	5.95 $\pm$ 0.28	5.64 $\pm$ 0.30	5.00 $\pm$ 0.19	4.99 $\pm$ 0.21	0.13	<0.01	0.11
GSH	143.0 $\pm$ 19.7	227.5 $\pm$ 51.2	146.2 $\pm$ 15.0	229.9 $\pm$ 21.6	227.7 $\pm$ 71.9	292.2 $\pm$ 74.2	0.54	0.17	0.99
GSSG	334.9 $\pm$ 22.3	313.5 $\pm$ 28.9	329.0 $\pm$ 10.85	339.4 $\pm$ 25.6	331.4 $\pm$ 14.5	343.1 $\pm$ 26.9	0.21	0.79	0.27
GSH-GSSG ratio	0.42 $\pm$ 0.05	0.86 $\pm$ 0.28	0.45 $\pm$ 0.05	0.72 $\pm$ 0.10	0.64 $\pm$ 0.16	0.85 $\pm$ 0.18	0.52	0.27	0.77

n = no. of participants. Means are $\pm$ SE. Mixed models are adjusted on baseline. Post hoc tests. GSH, glutathione; GSSG, glutathione disulfide; KRL, total antiradical defense potential (in French, Kit Radicaux Libres); Reseda (in French, RESErves Défenses Antioxydantes) 1, 2 and 3, antiradical defense reserves unmasked by treatment of serum with respectively glucosides, sulfates or glucuronides.

mented by the cosupplementation of vitamin E and selenium, both recognized as ROS scavengers (32, 37, 65). Of note, the impact of our cocktail on various markers of antioxidant capacity or oxidative markers was quite modest. One may thus argue that, in our study, the principal effects were due to the ω -3, known to boost the whole-muscle machinery of lipid metabolism (18) and, to a lesser extent, to the polyphenols and

vitamin E/selenium. However, a recent study of a 12-wk supplementation with epigallocatechin-3-gallate and resveratrol showed a major impact on mitochondrial capacity and fat oxidation in men and women who are overweight/obese (52). In agreement with our data, they did not observe an improvement in insulin sensitivity. In addition, Annuzzi et al. (4) found that the polyphenols reduced circulating TGs and VLDL,

Table 4. *Vastus lateralis Western blots and enzyme activity*

Variables	Baseline		Day 20		Supplementation		Mixed Model	
	Control	Supplemented	Control	Supplemented	Supplementation		Intervention	Interaction
	n = 8	n = 10	n = 10	n = 10	n = 10	n = 9		
Muscle fiber type								
CSA MHC1, μm^2	5,330.6 $\pm$ 399.0	4,968.8 $\pm$ 314.3	3,951.5 $\pm$ 267.6	4,453.4 $\pm$ 349.2	0.27		<0.01	0.18
CSA MHC2A, μm^2	5,477.8 $\pm$ 478.3	5,184.0 $\pm$ 329.4	4,303.4 $\pm$ 230.3###	4,990.3 $\pm$ 261.7***	0.001		<0.01	<0.01
CSA MHC hybrid, μm^2	0.0 $\pm$ 0.0	582.3 $\pm$ 582.3	2763.8 $\pm$ 759.8	2,514.2 $\pm$ 756.6	0.35		<0.001	0.25
MHC1, %	67.8 $\pm$ 5.8	62.3 $\pm$ 4.2	52.7 $\pm$ 3.4	46.7 $\pm$ 4.2	0.38		0.001	0.85
MHC2A, %	32.2 $\pm$ 5.8	37.7 $\pm$ 4.2	46.0 $\pm$ 2.9	51.4 $\pm$ 4.1	0.37		<0.01	0.89
Hybrids, %	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	1.3 $\pm$ 0.7	1.9 $\pm$ 1.0	0.79		0.02	0.79
Insulin/glucose metabolism								
pAKT serine 473/AKTtotal	15.00 $\pm$ 8.04	15.03 $\pm$ 4.56	8.77 $\pm$ 2.69	17.71 $\pm$ 5.56	0.08		0.61	0.21
pGSK3 α ³	2.91 $\pm$ 1.28	2.36 $\pm$ 0.92	1.74 $\pm$ 0.50	2.88 $\pm$ 1.13	0.14		0.61	0.19
pGSK3 β	2.00 $\pm$ 1.09	1.44 $\pm$ 0.79	1.19 $\pm$ 0.48	1.13 $\pm$ 0.45	0.57		0.22	0.58
Protein synthesis/degradation								
atrogin-1	0.17 $\pm$ 0.08	0.20 $\pm$ 0.09	0.25 $\pm$ 0.10	0.25 $\pm$ 0.12	0.55		<0.05	0.62
MURF-1	0.017 $\pm$ 0.004	0.016 $\pm$ 0.004	0.016 $\pm$ 0.003	0.015 $\pm$ 0.002	0.94		0.71	0.87
Ubiquitination	0.64 $\pm$ 0.06	0.36 $\pm$ 0.04	0.83 $\pm$ 0.08	0.36 $\pm$ 0.05**	0.01		0.1	0.09
HSP90	4.59 $\pm$ 0.26	4.26 $\pm$ 0.41	4.26 $\pm$ 0.34	4.41 $\pm$ 0.70	0.35		0.77	0.40
Oxidative stress								
NRF2	6.52 $\pm$ 1.55	4.39 $\pm$ 0.62	4.99 $\pm$ 0.76	5.28 $\pm$ 0.63	0.68		0.50	0.45
Catalase	2.00 $\pm$ 0.84	1.20 $\pm$ 0.41	1.14 $\pm$ 0.28	1.43 $\pm$ 0.38	0.55		0.68	0.59
CBR1	4.13 $\pm$ 0.60	4.61 $\pm$ 0.78	4.55 $\pm$ 0.50	4.30 $\pm$ 0.79	0.52		0.97	0.41
AKR7A2	8.41 $\pm$ 0.59	8.51 $\pm$ 0.79	8.92 $\pm$ 0.63	8.17 $\pm$ 0.83	0.33		0.86	0.33
TNF α	3.55 $\pm$ 0.59	3.39 $\pm$ 0.61	2.97 $\pm$ 0.50	3.60 $\pm$ 0.76	0.21		0.52	0.19
Mitochondria markers								
Mitofusin	5.98 $\pm$ 0.77	6.14 $\pm$ 1.51	4.18 $\pm$ 0.96	4.36 $\pm$ 1.15	0.95		<0.01	0.99
PGC-1 α	0.37 $\pm$ 0.04	0.25 $\pm$ 0.02	0.38 $\pm$ 0.09	0.20 $\pm$ 0.03	0.21		0.65	0.64
Citrate synthase activity, $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{mg protein}^{-1}$	0.17 $\pm$ 0.03	0.14 $\pm$ 0.02	0.14 $\pm$ 0.03	0.15 $\pm$ 0.03	0.44		0.81	0.28
FATP1	3.50 $\pm$ 0.33	2.71 $\pm$ 0.39	3.34 $\pm$ 0.34	3.40 $\pm$ 0.70	0.07		0.45	0.07

n = no. of participants. Means are $\pm$ SE. Mixed models are adjusted on baseline. Post hoc tests. AKR7A2, aldo-keto reductase family 7 member A2; CBR1, carbonyl reductase 1; CSA, cross sectional area; FATP1, fatty acid transport protein 1; HSP90, heat shock protein 90; MHC, myosin heavy chain; MURF-1, muscle-ring-finger protein 1; NRF2, nuclear factor erythroid-derived 2 like 2; pAKT: phosphorylated AKT; PGC1 α , peroxisome proliferator-activated receptor gamma coactivator 1 α ; pGSK3, phosphorylated glycogen synthase kinase 3; TNF α , tumor necrosis factor α . ** P < 0.01, *** P < 0.001 vs. control; ### P < 0.001 vs. baseline.

whereas ω -3 reduced postprandial cholesterol and VLDL apolipoprotein B-48. While the cocktail may benefit physically inactive and unhealthy individuals, it could also optimize the effects of training on lipid metabolism. In this line, Ota et al. (64) showed that supplementation in a green tea-extract beverage rich in catechins for two months, concomitant to aerobic exercise training, increased fat oxidation rates during exercise. Altogether, these data support the positive effect on lipid metabolism associated with the cocktail supplementation in our study.

While there is strong evidence that supports our results on lipid metabolism following polyphenols and ω -3 supplementation, we also observed interesting effects on the muscle phenotype itself. Indeed, the muscle is known to be plastic in response to mechanical loading and unloading, and it was expected that a reduction in physical activity by $\sim$ 10,000 steps/day would have a detraining and deconditioning effect in healthy, trained subjects. There was a global decrease in muscle fiber CSA with significant reductions in type 1 and type 2 fibers. Such plasticity in response to exercise or chronic hypoactivity is well known (17). Interestingly, these adverse effects on type 2 muscle fibers were prevented by the cocktail and may be associated with the decrease in ubiquitination content, factors known to be associated to muscle deconditioning and fiber atrophy. We have previously shown that resveratrol prevented disuse muscle atrophy of hind-limb suspended rats (51), and a recent study reported that ω -3 delays muscle degradation in mice (76). It is possible that these components of the nutrient cocktail are responsible for preserving type 2 fiber CSA in this study, as there is no evidence for a role of vitamin E or selenium. However, there are no mechanistic studies examining the effects of nutrient cocktails, and further experiments are required.

Limitations must be acknowledged. The control group did not take a placebo supplement because of difficulties to find a neutral oil to encapsulate. This being said, the magnitude of the effects we observed suggests this would have a minor impact on the conclusion of the study. The molecular parameters we selected did not allow us to fully unravel the mechanisms at play, and further mechanistic studies are required. Finally, we acknowledge that subjects taking $>14,000$ step/day does not represent the general population, and the interpretation of the results are delimited to young active men. While recommending physical activity is a central component of health promotion policies, not enough people achieve the recommendations, and therefore nutritional strategies to offset some of the deleterious effects of physical inactivity are important. Therefore, further studies are needed on less active populations and on populations at-risk for developing metabolic diseases.

In conclusion, we have demonstrated that the negative effects of physical inactivity on lipid metabolism can be mitigated by micronutrient supplementation. We have established that the early decrements in lipid oxidation contribute to increased de novo lipogenesis but that a nutrient cocktail containing polyphenols, ω -3, and other compounds can effectively prevent these changes during 20 days of decreased physical activity. These data highlight the importance of regular physical activity to maintain carbohydrate and lipid metabolism. It is possible that this supplementation could be an effective prevention strategy for chronic diseases, such as

cardiovascular disease and type 2 diabetes, but long-term studies, especially in high risk groups, are now required.

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DISCLOSURES

M. Prost, director of Spiral Company, developed the cocktail XXS-2A to market it, if efficient.

AUTHOR CONTRIBUTIONS

A.D., R.D., J.N., I.C., A.Z., S.N., T.B., F.C., I.d.G., E.L., A.C., D.C.-C., M.P., P.S., G.P., S.B., and C.S. performed experiments; A.D., R.D., J.N., I.C., A.Z., T.B., F.C., E.L., A.C., J.D., D.C.-C., M.P., G.P., S.B., C.S., and D.J.O. analyzed data; A.D., R.D., J.N., I.C., A.Z., T.B., F.C., I.d.G., E.L., A.C., J.D., M.P., G.P., S.B., C.S., A.B., and D.J.O. interpreted results of experiments; A.D., R.D., J.N., I.C., A.Z., T.B., F.C., E.L., A.C., M.P., G.P., S.B., C.S., and D.J.O. prepared figures; A.D., R.D., J.N., I.C., A.Z., T.B., F.C., I.d.G., E.L., A.C., S.B., C.S., and D.J.O. drafted manuscript; A.D., R.D., J.N., I.C., A.Z., S.N., T.B., F.C., I.d.G., E.L., M.-P.B., A.C., J.D., D.C.-C., M.H., G.G.-K., M.P., P.S., G.P., S.B., C.S., A.B., and D.J.O. edited and revised manuscript; A.D., R.D., J.N., I.C., A.Z., S.N., T.B., F.C., I.d.G., E.L., M.-P.B., A.C., J.D., D.C.-C., M.H., G.G.-K., M.P., P.S., G.P., S.B., C.S., A.B., and D.J.O. approved final version of manuscript.

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