Dietary nitrate reduces resting metabolic rate: a randomized, crossover study in humans[1–3]

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ABSTRACT

Background: Nitrate, which is an inorganic anion abundant in vegetables, increases the efficiency of isolated human mitochondria. Such an effect might be reflected in changes in the resting metabolic rate (RMR) and formation of reactive oxygen species. The bioactivation of nitrate involves its active accumulation in saliva followed by a sequential reduction to nitrite, nitric oxide, and other reactive nitrogen species.

Objective: We studied effects of inorganic nitrate, in amounts that represented a diet rich in vegetables, on the RMR in healthy volunteers.

Design: In a randomized, double-blind, crossover study, we measured the RMR by using indirect calorimetry in 13 healthy volunteers after a 3-d dietary intervention with sodium nitrate ($NaNO_3$) or a placebo (NaCl). The nitrate dose (0.1 mmol · kg^{-1} · d^{-1}) corresponded to the amount in 200–300 g spinach, beetroot, lettuce, or other vegetable that was rich in nitrate. Effects of direct nitrite exposure on cell respiration were studied in cultured human primary myotubes.

Results: The RMR was 4.2% lower after nitrate compared with placebo administration, and the change correlated strongly to the degree of nitrate accumulation in saliva (r^2 = 0.71). The thyroid hormone status, insulin sensitivity, glucose uptake, plasma concentration of isoprostanes, and total antioxidant capacity were unaffected by nitrate. The administration of nitrite to human primary myotubes acutely inhibited respiration.

Conclusions: Dietary inorganic nitrate reduces the RMR. This effect may have implications for the regulation of metabolic function in health and disease. *Am J Clin Nutr* 2014;99:843–50.

INTRODUCTION

The resting metabolic rate (RMR)[4] is the minimum energy required to sustain vital body functions in a resting state during fasting conditions. In humans, the daily energy output from the RMR exceeds the energy requirement of normal physical activities by >2-fold, even in active individuals (1). Thus, the RMR is the major component of the total energy expenditure, which may have implications for body weight control. The RMR is highly variable between individuals (2–4) even when fat-free mass is considered (5). Although a number of pharmaceuticals and nutrients have been shown to increase the RMR, very few compounds have the opposite effect. These exceptions include β-receptor antagonists (6) and the dietary antioxidant resveratrol (7).

Nitric oxide (NO) serves as a signaling molecule in numerous physiologic processes including the regulation of mitochondrial respiration by its reversible binding and inhibition of cytochrome *c* oxidase (8). NO generation is catalyzed by specific NO synthases but is also generated by serial reductions of the inorganic anions nitrate (NO_3^-) and nitrite (NO_2^-) (9). Nitrate is abundant in our everyday diet, particularly in green leafy vegetables that are naturally rich in nitrate. Ingested nitrate is actively taken up from blood by the salivary glands and accumulates in saliva. In the oral cavity, nitrate is reduced to the more-reactive nitrite anion by commensal bacteria, and swallowed nitrite is absorbed in the gut and rapidly distributed throughout tissues. Therefore, number of biochemical pathways in blood and tissues contribute to the additional metabolism of nitrite to NO and other molecules with NO-like activity (10, 11). In recent years, a number of studies have shown a biological activity of this nitrate-nitrite-NO pathway including protection against ischemia-reperfusion injury (12), a reduction in blood pressure (12, 13), and the modulation of mitochondrial function (14, 15).

We have previously shown that inorganic nitrate, in amounts readily achievable via the diet, improves mitochondrial respiratory efficiency in human skeletal muscle (15). This effect is coupled to a reduced proton leak across the inner mitochondrial membrane and a reduction in the expression of adenine nucleotide translocase (ANT), which is a protein involved in proton conductance (16).

These effects could theoretically be reflected by changes in the RMR and formation of reactive oxygen species (ROS) in vivo. We performed a short-term, randomized, placebo-controlled, crossover study in healthy volunteers with the intention to study if inorganic nitrate, in amounts that resembled those shown in a diet rich in vegetables, would affect the RMR and associated variables of normal metabolic function.

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[4] Abbreviations used: CR, caloric restriction; NO, nitric oxide; RMR, resting metabolic rate; ROS, reactive oxygen species; TOC, total antioxidant capacity.

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SUBJECTS AND METHODS

See supplemental experimental procedures under "Supplemental data" in the online issue for more detailed information including measurements of oxidative stress.

Subjects and ethical approval

This study was approved by the local ethics committee in Stockholm, Sweden. Subjects were informed and gave their written consent before participating in the study. All studies were performed according to the declaration of Helsinki. All subjects were healthy volunteers, between 18–49 y old. In total, 55 subjects (17 women) were recruited in this study. The number of subjects who took part in each substudy is indicated in each corresponding section. Average BMI (in kg/m^2) was 23.8 (range: 20.3–25.9). Regular smokers, vegetarians, and subjects who were taking chronic medications were not recruited.

Dietary instructions and nitrate administration

Subjects were instructed to follow a diet that avoiding high-nitrate foods and avoid exercise for 36 h before each test. Nitrate was administered in a crossover, randomized, double-blinded fashion. During 2 separate 3-d periods, which were separated by ≥7 d, subjects received a daily dose of either 0.1 mmol $NaNO_3$/kg body weight or an equimolar dose of sodium chloride (placebo). This amount resembled the nitrate content in 200–300 g green leafy vegetable. Daily doses were ingested on 3 occasions, and the last dose was taken in the morning 1 h before measurements started.

Nitrite infusion

Nitrite ($NaNO_2$) for use in humans was obtained from Hope Pharmaceuticals (300 mg in 10 mL H_2O) and diluted in 0.9% saline solution. Nitrite was infused at 3 different rates (1, 10, and 30 nmol · kg^{-1} · min^{-1}) for 10 min/rate. Thus, the total amount of nitrite infused was <1 μmol.

Indirect calorimetry

The RMR was measured with an indirect calorimetric system connected to a ventilated hood (Jaeger Oxycon Pro). The RMR was recorded during 30 min, and the lowest steady oxygen uptake recorded for 10 min was used as measure of the RMR.

Euglycemic clamp

Insulin (Human Actrapid; Novo Nordisk) together with 20% dextrose (Fresenius Kabi) was infused at a constant rate (20 mU · m^{-2} · min^{-1}) into the left antecubital vein. The glucose infusion rate was adjusted to maintain a constant concentration of 5 mmol/L (17).

Oral-glucose-tolerance test

The oral-glucose-tolerance test was performed after ingestion of a glucose solution (75 g) dissolved in water. Venous blood samples were drawn periodically every 15 min for 120 min, and blood glucose was analyzed by using a portable blood glucose analyzer (HemoCue; HemoCue AB).

Muscle biopsies and isolation of myogenic stem cells

Muscle tissue (~100 mg) was obtained from the subjects' vastus lateralis by using a Blakesley's choncotom. The isolation of myogenic stem cells was based on the protocol according to Blau and Webster (18).

Cell culture procedures

Cells were cultured in T75 S cell$^+$ growth surface flasks (Sarstedt) and maintained at 37°C in a humidified atmosphere of 5% CO_2. DMEM (low glucose; Gibco) and F12 (Gibco) at 1:1 containing 20% fetal calf serum, 50 U penicillin/mL, 50 U streptomycin/mL, and 1.25 μg amphotericin/mL was used as growth medium. Before experiments, cells were differentiated by changing to medium that contained 2% fetal calf serum during 5 d.

Respirometric analysis

Cells were suspended in cell medium that contained 0.5 mmol EGTA/L, 3 mmol $MgCl_2$/L, 60 mmol K-lactobionate/L, 20 mmol taurine/L, 10 mmol KH_2PO_4/L. 20 mmol HEPES/L, 110 mmol sucrose/L, 1g bovine serum albumin/L, and 0.3 mmol dithiothreitol/L. A respirometric analysis was performed by using high-resolution respirometry (O2-K; Oroboros).

Blood and saliva sampling

Blood samples were drawn from an antecubital vein immediately after the calorimetric test and transferred into a tube that contained a nitrite-free EDTA solution (250 mmol/L). Samples were centrifuged at 700 × *g* for 10 min (2°C), and plasma aliquots were stored at −80°C. Saliva samples were collected in 1.5-mL Eppendorf tubes and immediately stored at −80°C. *See* supplemental experimental procedures under "Supplemental data" in the online issue for information on the analysis of nitrate and nitrite, thyroid hormones, isoprostanes, and the total antioxidant capacity (TOC).

Statistics

Data are presented as means ± SEMs. The statistical analysis was performed with Prism 5 software (GraphPad). Student's paired *t* test was used when differences between the placebo and dietary nitrate were compared. One-factor ANOVA was used when effects over several time points were analyzed. The normality distribution was determined by using the D'Agostino-Pearson omnibus normality test.

RESULTS

Nitrate and nitrite concentrations in blood and saliva increase after nitrate administration

After the 3-d supplementation period, nitrate concentrations were higher in both blood (placebo: 28 ± 12 μmol/L; nitrate: 168 ± 72 μmol/L; $P \leq 0.0001$; **Figure 1**A) and saliva (placebo: 118 ± 32 μmol/L; nitrate: 2293 ± 319 μmol/L; $P \leq 0.0001$, Figure 1B). The bacterial conversion of nitrate to nitrite was evident by a 10-fold increase in salivary nitrite (placebo: 117 ± 22; nitrate: 1059 ± 232 μmol/L; $P \leq 0.001$; Figure 1C) and a doubling of nitrite concentrations in plasma (placebo: 231 ±50 nmol/L; nitrate: 485 ±195 nmol/L; $P \leq 0.05$; Figure 1D).

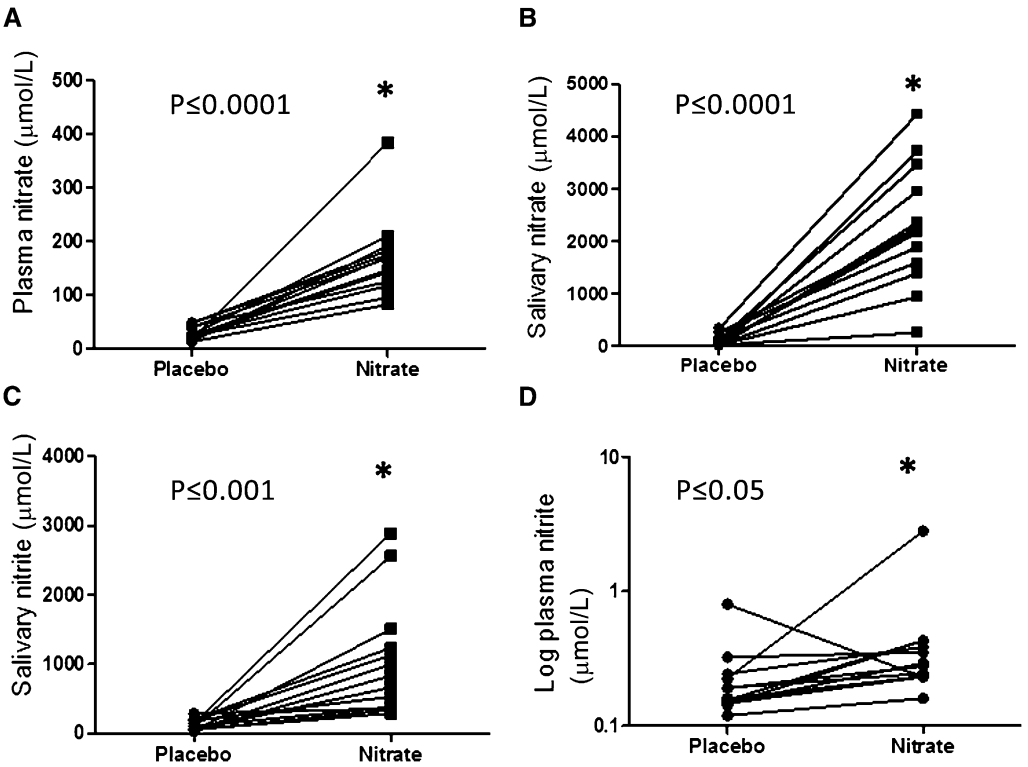

FIGURE 1. Dietary nitrate increases mean (±SEM) nitrate (A and B) and nitrite (C and D) concentrations in saliva and plasma. Concentrations in plasma and saliva were measured in healthy subjects after 3 d of dietary sodium nitrate supplementation (0.1 mmol · kg^{-1} · d^{-1}) or a placebo (sodium chloride) (n = 13). Plasma concentrations of nitrite (D) were not normally distributed and accordingly log transformed. *$P < 0.05$.

Nitrate administration reduces RMR in healthy humans

The RMR was measured by indirect calorimetry after an overnight fast. Oxygen uptake at rest was 266 ± 35 mL/min after the placebo intervention compared with 253 ±39 mL/min after the nitrate intervention (**Figure 2**A). As a consequence, the RMR was reduced by 4.2% from 1862 ± 232 kcal/24 h after the placebo intervention compared with 1780 ± 269 kcal/24 h after the nitrate intervention (Figure 2, B and C). Our observed value of

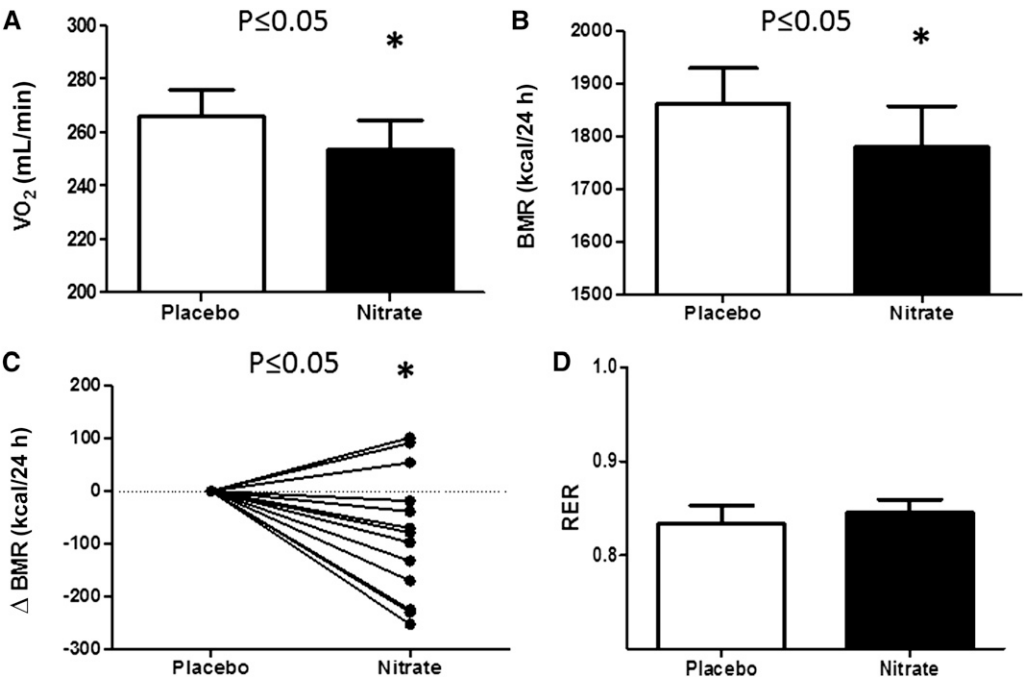

FIGURE 2. Mean (±SEM) effects of dietary nitrate on oxygen uptake, the resting metabolic rate, and substrate partitioning. A: Indirect calorimetry was used to measure the resting oxygen uptake (VO_2) in 13 healthy subjects after 3 d dietary sodium nitrate supplementation (0.1 mmol · kg^{-1} · d^{-1}) or a placebo (sodium chloride). The calculated resting metabolic rate (B and C) and substrate partitioning RER (D) are shown. *$P < 0.05$. BMR, basal metabolic rate; RER, respiratory exchange ratio; VO_2, volume of consumed oxygen.

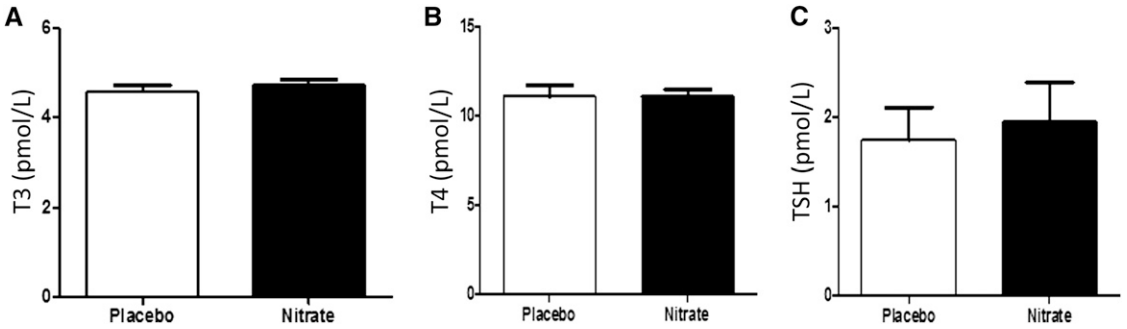

FIGURE 3. There were no mean (±SEM) changes in thyroid hormone concentrations after dietary nitrate supplementation. Plasma concentrations of thyroid hormones T3 (A), T4 (B), and TSH (C) were measured after 3 d dietary supplementation with nitrate or a placebo (n = 12). TSH, thyroid stimulating hormone; T3, thriiodothyronine; T4, thyroxine.

1862 ± 232 kcal/24 h was close to that predicted from the Harris-Benedict equation (1887 kcal/24 h) on the basis of subject characteristics (n = 13 men; height: 185 cm; weight: 80 kg; age: 27 y).

The respiratory-exchange ratio (volume of exhaled carbon dioxide:volume of consumed oxygen) was unaffected at 0.834 ± 0.06 after placebo administration and 0.845 ± 0.05 after nitrate administration (Figure 2D), which indicated that there was no change in the partitioning of glucose and fat oxidation.

Reduction in RMR is independent of changes in thyroid hormone concentrations

The thyroid gland is in direct control of energy metabolism, and iodine is an obligate precursor in thyroid hormone synthesis. It is known that the uptake of iodine at the sodium iodide symporter in the thyroid gland occurs in competition with nitrate (19). This competition may result in reduced thyroid hormone synthesis and a lower RMR. We measured plasma concentrations of triiodothyronine, thyroxine, and thyroid stimulating hormone but showed that they were unchanged after 3 d nitrate administration (**Figure 3**, A–C; n = 13).

Increase in salivary nitrate correlates with reduction in RMR

The active uptake of nitrate from blood by the salivary glands is believed to be central in the bioactivation of this anion (10). Therefore, we ran correlation analyses between changes in salivary and plasma nitrate and nitrite concentrations and changes in the RMR. We showed no correlation between plasma or salivary nitrite concentrations and the response in the RMR (data not shown), but there was a strong negative correlation between the increase in salivary nitrate and decrease in the RMR ($R = -0.84$, $P = 0.0003$; **Figure 4**). These results indicated that the uptake of nitrate by salivary glands and the concentration of nitrate in saliva are central for the overall response to nitrate administration.

Nitrate administration does not alter blood markers of oxidative stress

Mitochondrial respiration is associated with the generation of ROS. Our finding of a lower RMR after nitrate administration could theoretically lead to changes in the amount of ROS generated. The rate of living theory predicts a reduced ROS production because of the lower flux in the electron transport system (20), whereas the uncoupled to survive theory would, in contrast, predict a higher ROS production considering the elevated membrane potential in mitochondria that respire with a lower electron flux (21).

To investigate whether the reduced RMR had any impact on ROS concentrations in this study, we analyzed the TOC and concentrations of 3 isoprostane species in plasma (5-iPF2α-VI, 8,12-iPF2α-VI, and 8-isoPGF2α). Despite the reduction in the RMR, the TOC and all 3 isoprostanes were unchanged after nitrate compared with placebo interventions (**Figure 5**, A–D; n = 13). However, we did observe a significant correlation between increases in salivary nitrite concentrations and the decrease in 2 markers of isoprostanes after nitrate administration (Figure 5, E and F).

Reduction in RMR is independent of changes in insulin sensitivity or glucose uptake at rest

The metabolic rate directly controls glucose uptake and insulin sensitivity in mammals (22). Therefore, we wanted to study the effects of nitrate administration on these variables. In separate sets of experiments in healthy volunteers, we performed an oral-glucose-tolerance test and a euglycemic hyperinsulinic clamp after 3 d nitrate administration.

We showed no evidence of changes in glucose uptake during the euglycemic clamp (7.8 ± 0.9 mg · kg^{-1} · min^{-1} after placebo administration and 7.8 ± 1.0 mg · kg^{-1} · min^{-1} after nitrite administration; **Figure 6**A; n = 12) or the AUC for glucose or insulin during the oral-glucose-tolerance test; Figure 6, B and C; n = 16).

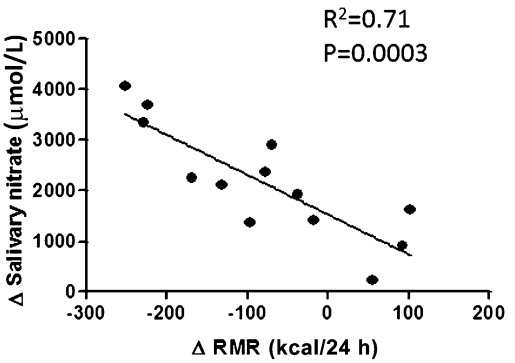

FIGURE 4. Mean (±SEM) changes in salivary nitrate correlated with changes in energy expenditure. A linear regression analysis of the increase in salivary nitrate after dietary nitrate supplementation and associated changes in energy expenditure (n = 13). RMR, resting metabolic rate.

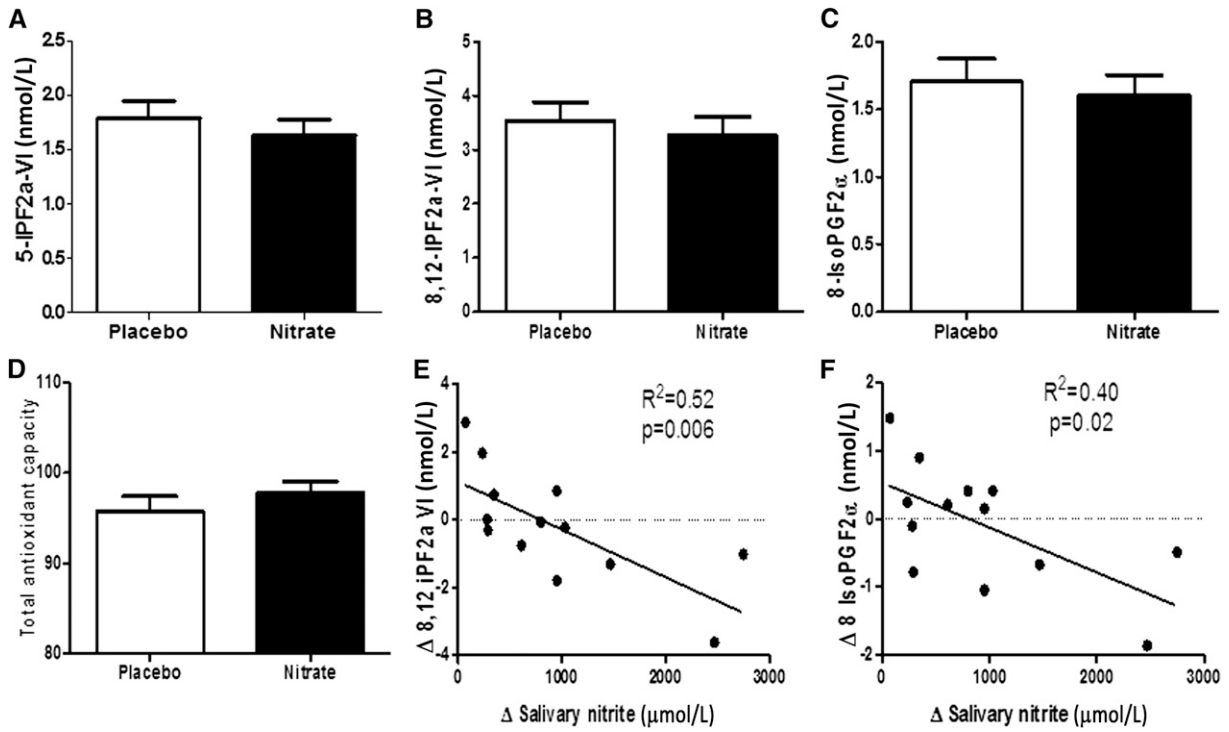

FIGURE 5. There were no mean (±SEM) changes in blood markers of oxidative stress after dietary nitrate supplementation. Isoprostanes (A–C) and the total antioxidant capacity (D) were measured in plasma after 3-d dietary intervention with inorganic nitrate or a placebo (n = 13). The change in salivary nitrite concentrations was correlated with the change in 8,12 IPF2a and 8-IsoPGF2α concentrations (E and F). Data are means ± SEMs.

Acute infusion of nitrite does not affect RMR

Previous findings suggested that nitrite is an intermediate in the bioactivation of nitrate (12, 23). In an attempt to investigate if systemic increases in nitrite would acutely affect the RMR, we infused increasing doses of sodium nitrite in healthy volunteers.

Despite increasing plasma concentrations of nitrite from 135 ± 23 to 1831 ±299 nmol/L at the end of the protocol (n = 2; data not shown), we did not observe any changes in the volume of consumed oxygen, respiratory exchange ratio, or RMR during any dose of infused nitrite (**Figure 7**, A–C; n = 8). This result suggested that the effect of a 3-d supplementation period with nitrate was not readily mirrored by an acute increase in plasma nitrite. This finding might have indicated that the mechanism might have involved structural changes that required a longer treatment protocol or, alternatively, that nitrate-derived bioactive nitrogen oxides other than nitrite were the final mediators of the observed effects.

Nitrite inhibits basal oxygen consumption of primary human skeletal myotubes

In a previous study, we have shown that the administration of nitrate for 3 d improved mitochondrial efficiency in healthy volunteers (15). An important finding from the study was that the effects seen after in vivo nitrate supplementation were not present with the addition of nitrite in vitro to isolated mitochondria. One possible explanation could be that components of the cellular cytosol are important in mediating the effects of nitrate supplementation.

To expand the mechanistic basis on how nitrate regulates mitochondrial energetics, we isolated myogenic stem cells from human skeletal muscle. As shown in **Figure 8**A, 25 μM nitrite reduced the basal myotube oxygen consumption to ~60% of untreated cells ($P < 0.05$). When cells were treated with the cellular permeabilizer digitonin to release cytosolic compartments into the medium and directly expose mitochondria to

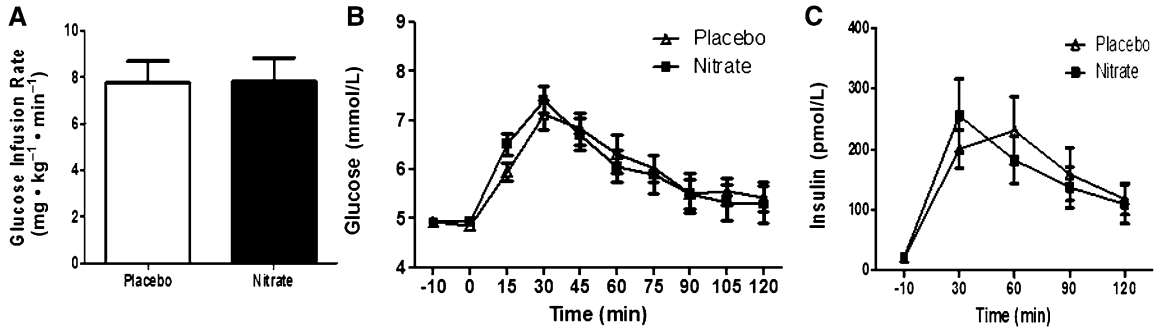

FIGURE 6. Dietary nitrate did not affect mean (±SEM) glucose uptake or insulin sensitivity. Insulin sensitivity (A) was measured during a euglycemic hyperinsulinic clamp after dietary supplementation with nitrate or placebo (n = 12). In separate experiments, the AUC for blood glucose (B) and insulin (C) during an oral-glucose-tolerance test was measured after nitrate or placebo supplementation (n = 17).

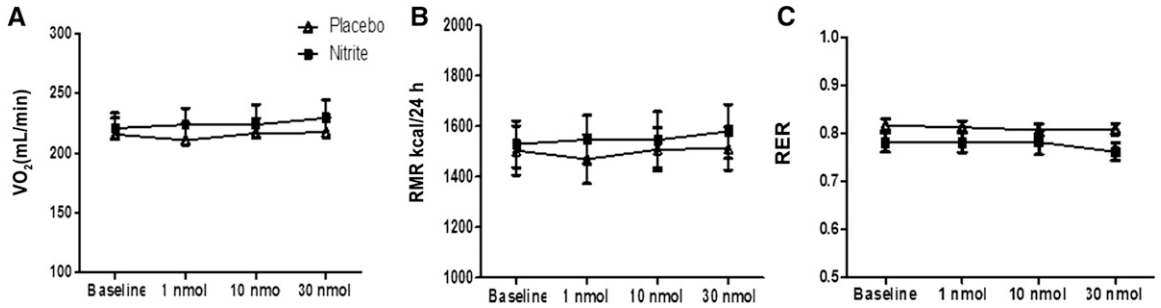

FIGURE 7. Acute intravenous infusion of nitrite did not decrease the RMR. Mean (±SEM) oxygen uptake (A), RMR (B), and RER (C) was measured in 8 healthy subjects during an intravenous infusion of sodium nitrite at a rate of 1, 10, or 100 nmol · kg^{-1} · min^{-1} (10 min/dose) (n = 8). RER, respiratory exchange ratio; RMR, resting metabolic rate; VO_2, volume of consumed oxygen.

respiratory substrates, no significant effects of nitrite were seen on leak respiration with mitochondrial substrates but without ADP and ADP-stimulated respiration (Figure 8, B and C). Even when reflecting basal respiration through steady state infusion of ADP, nitrite had no effect. This observation supports the theory that a cytosolic component, possibly in close contact with the mitochondria, mediates the inhibitory effects of nitrite on mitochondrial oxygen consumption, possibly by converting nitrite to another bioactive nitrogen oxide species.

DISCUSSION

In the current study, we showed that dietary supplementation with inorganic nitrate, which is a nutrient abundant in vegetables, reduced the RMR in humans by >>4%. We have recently shown that an identical dietary nitrate protocol increased the oxidative phosphorylation ratio (P:O ratio) and reduced oxygen consumption during state 4 and leak respirations in isolated human skeletal muscle mitochondria. These effects were accompanied by the downregulation of 2 mitochondrial proteins, ANT and uncoupling protein 3 (UCP-3), which has been suggested to be involved in proton leakage (15). Such a nitrate-induced reduction in proton leak is a likely mechanism for the effects on the RMR observed in the current study. It is well known that thyroid hormone concentrations affect energy expenditure (24). The concern that the intervention may have changed the thyroid hormone concentration as a result of the competitive inhibition by nitrate of iodide uptake at the sodium iodide symporter in the thyroid gland was ruled out by the nonsignificant changes in thyroid hormone concentrations. Effects of nitrate on sympathetic activity might also theoretically explain these results because NO, which is a nitrate-derived metabolite, has inhibitory effects on sympathetic nerve signaling (25).

We could not detect any effects by the dietary intervention on insulin sensitivity, glucose uptake, or plasma markers of oxidative stress. ROS are continuously generated during oxidative phosphorylation, and a lower respiratory rate has been coupled to higher ROS generation because of the higher mitochondrial membrane potential (21). In this study, we showed no evidence of higher oxidative stress although small changes in mitochondrial ROS formation might not have been reflected in the plasma markers used. A nonsignificant numeric decrease was seen in 2 of the measured oxidative markers, and this decrease correlated to changes in salivary nitrite. This result may indicate an interplay between nitrite formation and mitochondrial ROS production. Alternatively, it is possible that nitrite reduces ROS generation from nonmitochondrial sources including NADPH oxidases (26, 27) or xanthine oxidases.

The strong correlation between salivary nitrate uptake and its effects on the RMR was intriguing. Although nitrate itself is thought to be inactive, its accumulation in saliva allows for bioactivation in a process that involves oral bacteria, which effectively reduces nitrate to the more reactive nitrite anion (10). Indeed, in humans and rodents, the disruption of oral bacterial nitrate reduction by an antibacterial mouthwash or spitting abolishes the blood pressure–lowering effects of dietary nitrate (12, 28). Besides oral bacteria, gastric acid is also important for

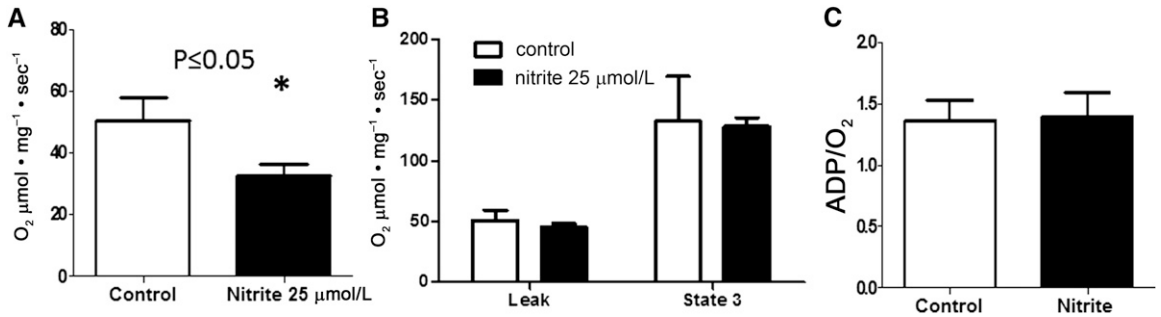

FIGURE 8. Nitrite inhibits mean (±SEM) basal oxygen consumption in primary myotubes. Human skeletal muscle myoblasts cultured from 6 individual donors were analyzed by using high-resolution respirometry. Respiratory variables were compared between nitrite-treated and untreated cells from the same subject. Nitrite-treated cells were exposed to sodium nitrite (25 μmol/L) while measuring oxygen consumption (A) followed by cellular membrane permeabilization with digitonin of nitrite-treated and control cells for oxygen-consumption measurements during leak respiration with mitochondrial substrates but without ADP and state 3 respiration (B). C: The ADP/O_2 ratio during the steady state infusion of a nonsaturating concentration of ADP was not affected. n = 8. *$P < 0.05$.

the activation of the nitrate-nitrite-NO pathway. A low pH facilitates the nonenzymatic conversion of nitrite to biologically active nitrogen oxides including NO, *S*-nitrosothiols, and nitro fatty acids which can be exported systemically (29). Thus, the accumulation of nitrate in saliva and its additional metabolism within the gastrointestinal tract to form bioactive nitrogen oxides seems central in the bioactivation of this anion. This effect may partly explain why we did not observe any effects of acute nitrite infusion on the RMR in this study, despite the fact that plasma nitrite rose to concentrations far greater than those achieved by dietary nitrate. In contrast, nitrite did repress respiration in vitro when applied directly onto primary myotubes, although the dose of nitrite was even higher in these experiments. Although a downregulation of mitochondrial uncoupling proteins (15) was the probable mechanism behind the nitrate-induced reduction in the RMR observed in the current study, it is unlikely that such protein modifications accounted for the acute effect of nitrite on myotube respiration because the incubation time with nitrite was <30 min. The effects on respiration seen in vitro may, instead, have been a result of the direct inhibition of cytochrome *c* oxidase by NO generated from nitrite.

The natural polyphenolic compound resveratrol, which is present in grapes and some other dietary components, was recently administered to obese human subjects and was shown to decrease the resting energy expenditure to a similar degree as nitrate did in the current study (7). However, a striking difference when we compared these nutrients is that the dose of resveratrol used by Timmers et al (7) to lower the RMR exceeded by far what would be achievable through a normal diet, likely limiting any role for this polyphenol in the nutritional or physiologic control of energy expenditure. In contrast, the current effect of nitrate on the RMR was seen already with a dietary dose that can be achieved by the ingestion of 200–300 g spinach or 2–3 beetroots (10)

A sustained reduction in the RMR of 4% might have implications for body-weight regulation. Indeed, the calculated weight gain over a 1-y period would amount to ∼4 kg if not compensated for by a lowered energy intake. However, on the basis of the established protective effects of green leafy vegetables against a number of major diseases, it is not advisable to reduce the intake of vegetables in an attempt to control weight. In this context, it is tempting to speculate that the lowering of the metabolic rate by nitrate in vegetables represents an evolutionary favorable metabolic response to preserve energy.

Another dietary intervention that has a similar effect on energy metabolism is caloric restriction (CR). Although different studies have yielded somewhat divergent results, CR in humans seems to be associated with a reduced RMR and favorable metabolic adaptations linked to reduced risk of cardiovascular disease (30–33). Dietary nitrate has also been shown to exert beneficial effects related to cardiovascular risk including the lowering of blood pressure, prevention of atherosclerosis, improvement in vascular endothelial function, and reversal of features of the metabolic syndrome in animal models (34–36). Moreover, green leafy vegetables, which are particularly high in nitrate, have been increasingly being identified as protective against cardiovascular disease in large epidemiologic studies (37–39). In this context, CR is the only dietary intervention model that consistently increases the life span in a wide range of animal models (40). To our knowledge, such studies have not been performed with dietary nitrate.

In conclusion, we showed that inorganic nitrate, in doses readily achievable through a normal diet, reduces the RMR in humans. These findings may have implications for the control of metabolic function in health and disease.

The authors' responsibilities were as follows—FJL: performed experiments, wrote the manuscript, and analyzed data; BE: performed experiments and wrote the manuscript; TAS, MPM, AC, TN, and CEW: performed experiments and analyzed data; and JOL and EW: wrote the manuscript. JOL and EW are codirectors of HeartBeet Ltd, which is a company that owns patent applications related to the therapeutic uses of inorganic nitrate and nitrite. FJL, TAS, BE, MPM, AC, CEW, and TN had no conflicts of interest.

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