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Mitochondrial coupling and capacity of oxidative phosphorylation in skeletal muscle of Inuit and Caucasians in the arctic winter

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During evolution, mitochondrial DNA haplogroups of arctic populations may have been selected for lower coupling of mitochondrial respiration to ATP production in favor of higher heat production. We show that mitochondrial coupling in skeletal muscle of traditional and westernized Inuit habituating northern Greenland is identical to Danes of western Europe haplogroups. Biochemical coupling efficiency was preserved across variations in diet, muscle fiber type, and uncoupling protein-3 content. Mitochondrial phenotype displayed plasticity in relation to lifestyle and environment. Untrained Inuit and Danes had identical capacities to oxidize fat substrate in arm muscle, which increased in Danes during the 42 days of acclimation to exercise, approaching the higher level of the Inuit hunters. A common pattern emerges of mitochondrial acclimatization and evolutionary adaptation in humans at high latitude and high altitude where economy of locomotion may be optimized by preservation of biochemical coupling efficiency at modest mitochondrial density, when submaximum performance is uncoupled from VO_{2max} and maximum capacities of oxidative phosphorylation.

During evolution, mutations have accumulated sequentially along maternal mtDNA lineages forming groups of mtDNA genotypes and haplogroups, which tend to be regionally and ethnically specific (Wallace et al., 1999). The haplogroup defining variants encompass transitions and transversions in both protein- and RNA-coding genes and have been proposed to induce variation in ATP and ROS production and/or proton leakage (Ruiz-Pesini et al., 2000; Gomez-Duran et al., 2010). Mitochondrial DNA haplogroup specificity linked to geographic and climate lineage is thought to allow for relatively rapid adaptation to environmental change related to climate, food availability, and energy demand, while nuclear encoded mitochondrial genes constitute distantly preserved structural and regulatory components of mitochondria (Wallace, 2010). It has been surmised that climatic pressures led to positive evolutionary selection for specific mitochondrial gene polymorphisms that lower oxidative phosphorylation (OXPHOS) coupling efficiency in favor of greater heat production, conferring survival advantage for populations habituating cold climates (Mishmar et al., 2003; Ruiz-Pesini et al., 2004). Previous studies have examined the selection hypothesis using the dN/dS (K_a/K_s) ratio (Mishmar et al., 2003). This ratio defines the number of non-synonymous substitutions per non-synonymous site (K_a) to the number of synonymous substitutions per synonymous site (K_s). This index proposes that climate has contributed to the selection of mtDNA haplotypes (Mishmar et al., 2003; Ruiz-Pesini et al., 2004). However, other studies do not support this

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hypothesis (Moilanen et al., 2003; Elson et al., 2004; Kivisild et al., 2006; Sun et al., 2007).

The bioenergetic steps in OXPHOS consist of electron flow that produces a proton motive force (Δp) across the inner mitochondrial membrane (substrate translocases, dehydrogenases, and components of the membrane-bound electron transfer system), reactions that couple the conservation of Δp in ATP formation, ADP/ATP exchange, and import of inorganic phosphate (ATP synthase, adenine nucleotide translocase, and P_i transporter), and reactions that dissipate Δp without formation of ATP (proton leak and slip) while stimulating oxygen consumption (non-phosphorylating LEAK respiration) (Amo & Brand, 2007; Gnaiger, 2014). LEAK respiration results in heat production as it occurs in brown adipose tissue mitochondria which exhibit very low coupling upon activation (Matthias et al., 2000). High LEAK respiration has been associated with weight loss, whereas low levels of LEAK are associated with weight gain (Thrush et al., 2015). The proposed link between the arctic haplotype and a less coupled mitochondrial phenotype has been investigated in isolated mitochondria from cybrid cell lines incorporating Arctic (A, C, D) and tropical (L) haplotypes (Amo & Brand, 2007), but the uncoupling hypothesis was not supported. However, the results in cybrid cells may depend on differences in nuclear background (Amo & Brand, 2007; Elson et al., 2007). With this consideration, no differences in mitochondrial bioenergetics were found in human lung carcinoma cell cultures between Europeans with haplogroups H and T (Amo et al., 2008).

Biochemical coupling *efficiency* can be operationally defined as the percentage of mitochondrial respiration linked to ATP synthesis at a given membrane potential (Gnaiger, 2014). Experimentally, uncoupling can be quantified as an increase of LEAK respiration at high membrane potential induced by substrate oxidation when ADP is depleted or phosphorylation to ATP is inhibited, relative to OXPHOS respiration stimulated by saturating ADP levels (Pesta & Gnaiger, 2012).

We studied skeletal muscle mitochondrial function in traditional and westernized Inuit of northern Greenland belonging to haplogroup A2 and D3 and descended from Thule and Dorset groups (Helgason et al., 2006) in whom the climate of extreme cold is considered to constitute a selective pressure on the mtDNA. Caucasian Danes from across Denmark belonging to haplogroup (H, J, T, U, K, I, W) were chosen as representing a population from a temperate climate much less likely to be selected for low coupling efficiency of mitochondrial respiration. As skeletal muscle has a large energy turnover capacity and represents a large fraction of body mass, we reasoned that if differences in mitochondrial coupling in muscle tissue existed between haplogroups, the physiological impact on whole body heat production would be significant and thus support the arctic haplotype hypothesis. Furthermore, as arms and legs are involved in locomotion (e.g., skiing) and demonstrate high plasticity in response to physical work, these muscles groups would be relevant for extending the analysis of mitochondrial coupling control further under conditions of markedly enhanced energy demand accompanying increased muscle loading in the extreme cold of the arctic winter. We tested the hypothesis that the Inuit of the arctic haplogroups would have a lower coupling of mitochondrial respiration in skeletal muscle compared to Caucasian Danes.

Methods

Study groups

Sixteen healthy Danes volunteered for the study (see also Boushel et al., 2015). The volunteers were not involved in formalized exercise training or competitive sports, but were classified as physically "active" through participation in recreational sport activity and regular use of a bicycle for transportation. Baseline measurements were undertaken in Copenhagen in February prior to their sojourn to northern Greenland to latitude of 80° at the Thule Air Base. They embarked upon a 42-day ski expedition across the polar ice. Each subject carried their own food in their sledge which was re-supplied by helicopter on the journey. The food was prepared daily en route from pre-mixed packaged meals consisting of 12–15% protein, 30% fat, and 55–58% CHO. The estimated daily use of energy was 15,000–24,000 kJ depending upon body size and load of the sledge. At the same time, eight traditional Inuit residing in remote northern villages of Greenland and 11 westernized Inuit who worked at the Thule Air Base volunteered to participate in the study after approval from the Commission for Scientific Research in Greenland (J.nr:505-89) and with the assistance of an interpreter. The traditional Inuit participants (*"Hunters"*) retained a lifestyle of hunting (with dog-sledge) and fishing year round (Munch-Andersen et al., 2012), performing daily physical work and subsisting primarily on a marine and arctic diet (seal, polar bear, reindeer, halibut, fowl and berries). They all had grandparents from northern Greenland, lived aside from western civilization for many years, and had high trichina antibody concentrations as evidence of long term intake of marine and arctic meat (Munch-Andersen et al., 2012). The westernized Inuit resided in permanent housing facilities on the Base, held a variety of maintenance jobs requiring some physical work, but were categorized as untrained, and they consumed a westernized cafeteria diet served at the Thule Air Base (Fig. 1). Anthropometric measures were taken and an exercise test was performed to determine maximal oxygen consumption on a cycle ergometer. The Inuit were older, shorter in stature, and heavier than Danes (Table 1). Their high body mass index, however, cannot be compared directly with Caucasians as a measure of overweight (Andersen, 2013).

Systemic oxygen consumption

Expired minute ventilation (V_E), O_2 fraction, and pulmonary oxygen uptake (VO_2) were measured continuously using an

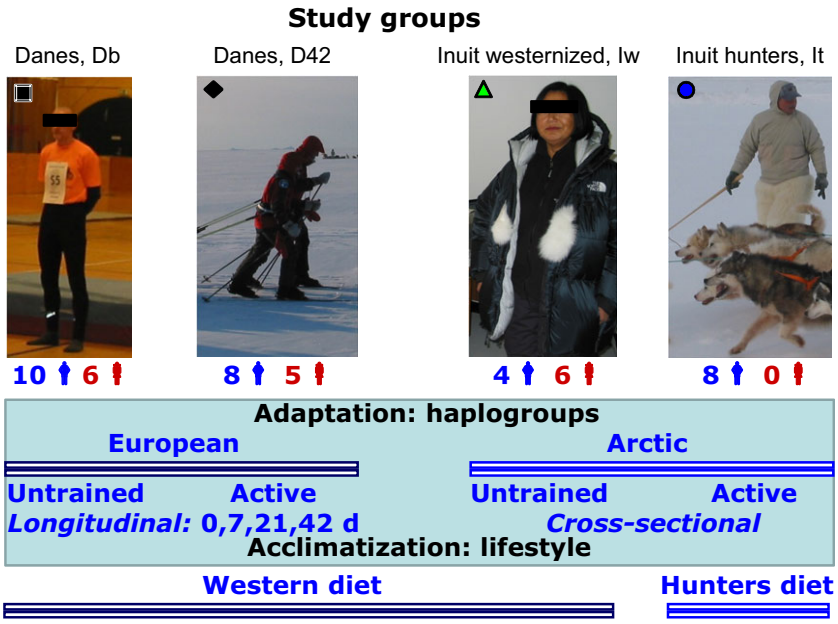

Fig. 1. Study groups: Haplogroups, lifestyle, and activity levels.

Table 1. Anthropomorphic and functional parameters of Inuit and Caucasian Danes

	Inuit Hunters ($N = 8$)	Inuit Westernized ($N = 11$)	Danes Baseline ($N = 15$)	Danes 42 days ($N = 13$)
Age [yrs]	40 ± 3	36 ± 4	31 ± 2*	
Height [cm]	169 ± 2	165 ± 3	175 ± 2*	
Weight [kg]	81 ± 3	78 ± 3	72 ± 3*	70 ± 3
BMI [kg/m^2]	28 ± 2	29 ± 2	23.6 ± 0.8*	22.9 ± 0.5
Fat %	19 ± 1	22 ± 2	19 ± 2*	18 ± 2
VO_{2max} ml·min^{-1}·kg^{-1}	41 ± 2	38 ± 3	48 ± 2*	49 ± 1*

Values are mean ± SEM.

*Difference between Inuit and Danes.

automated metabolic cart (Quark b^2, Cosmed Srl., Rome, Italy). Volunteers performed exercise on a Monark cycle ergometer. Subjects performed leg exercise with incremental loads to determine leg VO_{2max}. The exercise protocol started with a 10-min warm up followed by incremental exercise beginning with unloaded pedaling which was increased by 40–60 watts every 2 min until exhaustion.

Genotyping/mtDNA haplotyping

In a subset of Inuit and Danes, mitochondrial haplogroups were determined by direct sequencing to verify that the expected haplogroup distribution among the participating individuals and previously reported frequencies in Denmark and Inuit of Greenland matched (Helgason et al., 2006; Benn et al., 2008) (Table 2). DNA was extracted from serum using QIAamp DNA Blood Midi Kit (QIAGEN, Hilden, Germany) and amplified using REPLI-g Mitochondrial DNA kit (QIAGEN). An annealing temperature of 60 °C was used in all PCR. The PCR products were sequenced using BigDye Terminator v1.1 Cycle Resequencing (ABI), and analyzed on an ABI3730 DNA Analyzer. Primer sequences are available on request. The resulting sequences were compared to the Revised Cambridge sequence (rCRS, GenBank ID: NC_012920) using Sequencher 4.8 software (Gene Codes, Ann Arbor, Michigan, USA). Haplogroup markers were chosen based on and classified according to PhyloTree mtDNA tree Build 11 (7 February 2011).

Skeletal muscle tissue

After local anesthesia (1% lidocaine) of the skin and superficial muscle fascia, biopsies were obtained from the vastus lateralis and posterior deltoid muscles using the Bergstrom technique. A portion of the obtained muscle tissue was frozen immediately in liquid nitrogen and stored at −80 °C for later analyses. A small portion of the biopsied muscle was placed in relaxing medium (10 mM Ca-EGTA buffer, 0.1 μM free calcium, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.56 mM MgCl2, 5.77 mM ATP, 15 mM phosphocreatine, pH 7.1) at 2–4 °C. Individual fiber bundles were then separated with two pairs of sharp forceps achieving a high degree of fiber separation. The fiber bundles were then permeabilized for 30 min in 3 ml of ice-cold relaxing medium with saponin (50 μg/ml). After chemical permeabilization, the tissue was rinsed twice for 10 minutes in chilled mitochondrial respiration medium MiR05, consisting of EGTA (0.5 mM), $MgCl_2.6H_2O$ (3 mM), K-lactobionate (60 mM), taurine (20 mM), KH_2P0_4 (10 mM), HEPES (20 mM), sucrose (110 mM), BSA (1 g/l), at pH 7.1 (Gnaiger et al., 2000). The muscle bundles were then blotted and measured for wet weight in a balance controlled for constant relative humidity providing stability of weighting measures in all biopsies. The muscle bundles were then transferred immediately into the respirometer (Oxygraph-2k; Oroboros Instruments, Innsbruck, Austria).

Respirometry

Biopsy samples of 2–5 mg were run in duplicate in the two-chamber system calibrated for chemical back-diffusion of

Table 2. Mitochondrial DNA haplogroup (%)

Ethnic group								
Danes	This study (N = 13)	H (46)	T (8)	I (16)	W (8)	U (8)	K (8)	V (8)
	Benn et al. (2008)	H (45.9)	T (9.9)	W/I (3.8)	W/I (3.8)	U (15.9)	K (6.2)	V (4.5)
Inuit hunters	This study (N = 9)	A2 (88)	D3 (12)					
	Helgason et al. (2006)	A2 (95.7)	D3 (4.3)					

oxygen into the chamber, oxygen leak to the exterior, and sensor O_2 consumption (Gnaiger, 2001). Respirometry was performed at a chamber temperature of 30 °C with chamber [O_2] maintained at (250–400 μM) to avoid O_2 diffusion limitation (Pesta & Gnaiger, 2012). All respiratory fluxes were adjusted to a standard temperature of 37 °C by multiplication with a factor of 1.62 (assuming a Q_{10} of 2; Gnaiger, 2009). Applying a substrate-uncoupler-inhibitor titration (SUIT) protocol (Fig. 2a and b; Gnaiger, 2014), resting (LEAK) respiration was assessed by addition of malate (2 mM) and octanoylcarnitine (1 mM) in the absence of adenylates. OXPHOS capacity of fatty acid oxidation (FAO_P) with octanoylcarnitine was achieved by addition of ADP (5 mM). Fatty acid oxidation requires the metabolism of CoA; hence the addition of malate to allow β-oxidation to proceed. Further stimulation of Complex I-linked respiration (CI_P) was achieved by addition of glutamate (10 mM). OXPHOS capacity with convergence of physiological electron supply to the Q-junction through Complexes I and II was stimulated by addition of succinate (10 mM; $CI\&II_P$). Inhibition of CI by addition of rotenone (1 μM) led to CII-linked OXPHOS (CII_P). Oligomycin (1 μM) was added to inhibit ATP synthase, demonstrating oligomycin-induced LEAK respiration (CII_L). Complex II-linked electron transfer capacity was assessed by uncoupler titrations using carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP; 0.7 μM per addition up to 2.5 μM; CII_E). The integrity of the outer mitochondrial membrane was assessed with the addition of cytochrome *c* (10 μM). Cytochrome *c* had no stimulatory effect on respiration confirming mitochondrial membrane intactness throughout all skeletal muscle samples included in the analysis. Antimycin A (2.5 μM) was added to terminate respiration by inhibiting Complex III to observe the decline toward residual oxygen consumption (ROX), but a correction for ROX was not applied (Pesta & Gnaiger, 2012).

Coupling efficiency

Efficiency is rigorously defined in thermodynamics based on the 2nd law as the ratio of output/input power, with a maximum value of 1 (Gnaiger, 1993a,b). Metabolic power (per unit tissue mass; μW/g) is the product of flux [nmol·s^{-1}·g^{-1}] and conjugated force [kJ/mol or μJ/nmol]. For the process of OXPHOS, the mass-specific input power is oxygen flux [pmol·s^{-1}·mg^{-1}] times the molar Gibbs energy of oxygen consumption (close to −450 kJ/mol O_2; Gnaiger, 1993b). The output power is a function of the effective ~P/O_2 ratio and the molar Gibbs energy of phosphorylation (55 to 62 kJ/mol ADP phosphorylated to ATP, ~P; Gnaiger, 1993b). Uncoupling diminishes the effective ~P/O_2 ratio and shifts OXPHOS from efficient ATP production to the generation of heat. In the resting state of LEAK respiration, however, the output flux of phosphorylation and efficiency are always zero. Under these conditions, the resting oxygen flux (LEAK respiration) and the corresponding heat generation depend on mitochondrial content and the "biochemical coupling efficiency", defined as $\Delta j_{E\text{-}L} = (E\text{-}L)/E = 1\text{-}E/L$ within the theoretical boundaries of 0.0 to 1.0. When the phosphorylation system capacity is not limiting OXPHOS ($P = E$), then the biochemical coupling efficiency is simply related to the respiratory acceptor control ratio, RCR = P/L and $\Delta j_{E\text{-}L}$ = (RCR-1)/RCR = (P-L)/P (Gnaiger, 2014). This relation applies in human skeletal muscle under the condition of low FAO and CII-linked electron transfer capacity (E), when the phosphorylation system does not limit OXPHOS capacity (P) as shown by the equivalence of CII_P and CII_E (Fig. 2; in contrast to the CI&II substrate state supporting high ETS capacity, Amo et al., 2008; Rasmussen & Rasmussen, 2000; Gnaiger, 2009; Boushel et al., 2011). Our SUIT protocol was designed to validate the controlled state of resting respiration in the absence of adenylates (with octanoylcarnitine and malate; FAO_L) and by a second LEAK state after inhibition of ATP synthetase by oligomycin (with succinate and rotenone, CII_L). Consistent biochemical coupling efficiencies were obtained in both substrate states, that did not differ between Inuit traditional hunters (0.55 ± 0.02 for FAO; 0.57 ± 0.02 for CII) and Danes measured at baseline or after the 42-day sojourn in Northern Greenland (Db and D42: 0.59 and 0.58 ± 0.02 for FAO; 0.59 and 0.52 ± 0.02 for CII; Fig. 2c and d).

Muscle fiber type

Serial sections of frozen biopsies were cut in a cryostat at −20 °C, and ATPase histochemical analysis was performed after pre-incubation at pH 4.37, 4.6, and 10.3. The stained sections were visualized and analyzed for fiber type percentage. Five fiber subtypes were determined and then pooled into the three subtypes Type I, IIa, and IIx (Table 3).

UCP3 protein expression

Muscle samples for UCP3 protein content were homogenized in ice-cold phosphate-buffered saline (PBS), containing 1 mM EDTA and 0.4 mM PMSF and subsequently sonicated for 3 min 10 s. After sonication, two volumes of each skeletal muscle homogenate and one volume of SDS sample buffer were boiled for 4 min. Thereafter, samples were centrifuged for 5 min at 10,000 *g* and equal amounts of protein were loaded for gel electrophoration and Western blotting. For detection of UCP3, blots were incubated, respectively, with an affinity purified rabbit polyclonal antibody against UCP3 (code 1331; provided by LJ Slieker, Eli Lilly). After washing with 0.05% Tween20 in PBS, blots were incubated with horseradish peroxidase-conjugated swine anti-rabbit Ig (SWARPO, DAKO, Glostrup, Denmark) for 60 min at room temperature, and subsequently washed for 90 min with 0.05% Tween20 in PBS, and 10 min in PBS. Chemiluminescence was performed using a Super Signal West Dura Extended kit (Pierce, Rockford, Illinois, USA). Finally, the reaction product of each blot was analyzed by densitometry using Imagemaster (Pharmacia Biotech, Roosendaal, The Netherlands) and a representative blot is provided (Fig. 3).

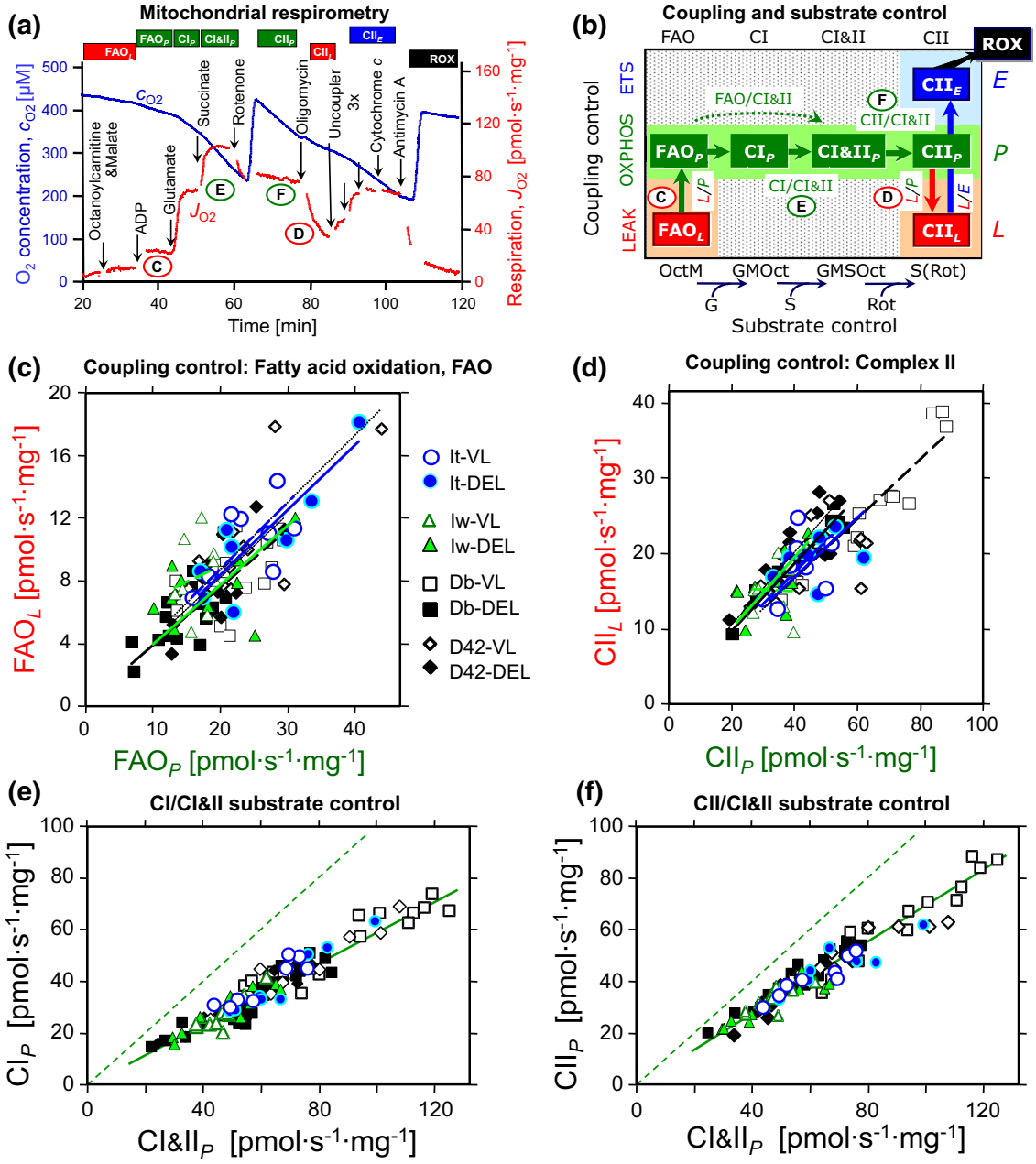

Fig. 2. Coupling and substrate control of mitochondrial respiration in permeabilized muscle fibers. (a) Record of substrate-uncoupler-inhibitor titration protocol showing oxygen concentration, c_{O2} (blue line; µM), and respiration per mg tissue wet weight, J_{O2} (red line; pmol·s^{-1}·mg^{-1}) of vastus lateralis measured by high-resolution respirometry. (b) Coupling/substrate control diagram representing the protocol in Fig. 2a. Non-phosphorylating respiration is low in the absence of adenylates at a high chemiosmotic gradient, when oxygen flux is controlled by proton leak, slip, and cation cycling (LEAK, *L*). Active respiration at saturating ADP concentration represents OXPHOS capacity (*P*). Further stimulation of OXPHOS by glutamate (G) providing NADH for Complex I (CI). Convergent electron supply through Complex I and II (CI&II) to the Q-junction after addition of succinate (S) to achieve maximal physiological OXPHOS capacity. CII-linked OXPHOS capacity after inhibition of CI by rotenone. Second LEAK state for CII-linked respiration in the presence of adenylates after inhibition of ATP synthase by oligomycin, followed by evaluation of CII-linked electron system capacity (ETS, *E*) after uncoupler titration (FCCP). Residual oxygen consumption (ROX) determined after inhibition of Complex III with antimycin A. (c and d) Preserved coupling control based on fatty acid oxidation and CII-linked respiration as shown by the proportional dependence of LEAK respiration on OXPHOS capacity in Inuit and Danes across life styles and training (It and Iw, traditional hunters and westernized Inuit; Db and D42, Danes at baseline and after the 42-day sojourn in Northern Greenland, in leg (vastus lateralis, VL) and arm muscle (deltoid, DEL). Symbols represent the average of two measurements for each biopsy. Mean *L*/*P* coupling control ratios were 0.43 ± 0.01 (N = 91) and 0.46 ± 0.01 (mean ± SEM; N = 69) for ETF and CII, respectively, corresponding to RCR (*P*/*L*) of 2.6 ± 0.08 and 2.3 ± 0.07. (e and f) Preserved Complex I- and II-linked substrate control shown by the proportional relation to convergent CI&II respiration over the entire range of OXPHOS capacities. The CI/CI&II and CII/CI&II flux control ratios (slope of the full lines) are tightly maintained at 0.60 ± 0.01 and 0.70 ± 0.01 (mean ± SEM; N = 95), respectively; dashed line, theoretical line of correspondence with slope of 1.0.

Table 3. Muscle composition

	Inuit hunters		Inuit westernized		Danes baseline		Danes 42 days	
	DEL	VL	DEL	VL	DEL	VL	DEL	VL
Fiber type (%)								
Type I	69 ± 3	57 ± 4*	54 ± 4*,†	43 ± 3*,†	56 ± 3*,‡	64 ± 3*,‡	64 ± 2‡,§	57 ± 2*,‡,§
Type IIa	23 ± 4	28 ± 3*	31 ± 4*,†	34 ± 2*,†	26 ± 2	29 ± 2‡	24 ± 2‡	30 ± 2‡
Type IIx	8 ± 2	15 ± 4*	14 ± 2*,†	22 ± 3*,†	18 ± 3‡	7 ± 2*	12 ± 2*,‡	13 ± 2*,‡,§
UCP3 (AU)	40 ± 11	60 ± 12*	50 ± 15	75 ± 12*	41 ± 10	41 ± 6‡		

Data are means ± SEM.
*Difference, arm vs leg (It vs Iw).
†Difference between Inuit (Iw).
‡Different from Inuit (Iw).
§Difference baseline–42 days (Db vs D42).

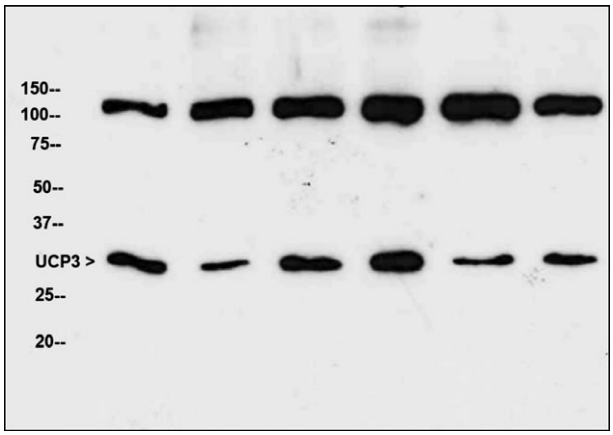

Fig. 3. A representative Western blot of UCP-3 expression.

Data analysis

All values are given as means ± SE for all experiments. A two-tailed Wilcoxon signed-rank sum test was used to compare mitochondrial parameters, VO_{2max} and muscle characteristics between groups and between muscles (SigmaStat version 3.11). A student paired *t*-test was applied to test for differences pre to post 42-day skiing in Danes . For all evaluations, a *P*-value <0.05 was considered significant.

Results and discussion

Our primary findings were that coupling control of OXPHOS was not different between haplogroups in either the leg or arm muscles exemplified by LEAK respiration plotted as a function of OXPHOS capacity (Fig. 2c and d). Biochemical coupling efficiencies measured with FAO-linked and CII-linked substrates were identical in Inuit and Europeans, arm and leg, and before and after the 42-day acclimatization to life in the cold.

The relationship between Complex I- and Complex II-linked OXPHOS capacity was preserved across haplogroup and training status. If only CI-linked or CII-linked electron entry was provided to fuel the electron transfer system, electron gaiting reduced OXPHOS capacity to 0.6 or 0.7 of maximal respiration with convergent electron flow through CI&II (Rasmussen & Rasmussen, 2000; Gnaiger, 2009; Boushel et al., 2011). These tight relationships provide the basis for using CI&II-linked OXPHOS capacity as a functional marker of mitochondrial density (Pesta et al., 2011), which varied between arm and leg, without effect on coupling (Fig. 2). Taken together, these results indicate that in muscle tissue, haplogroup populations of the circumpolar arctic are not parturient for a selection characteristic of higher heat production by mitochondria and a lower coupling efficiency as an adaptation to cold climates. Our findings do not exclude the possibility of lower coupling efficiency in other tissues such as adipose tissue. The physically active Inuit Hunters had a higher capacity to oxidize fatty acids than the westernized Inuit and Danes (Fig. 4c). For the Inuit, this was attributed entirely to the low mitochondrial content in the inactive westernized (Iw) group. In contrast, the OXPHOS capacity in the arm of the Danish group increased due to a reprogramming toward higher relative fatty acid oxidation capacity during the time of the 42-day skiing expedition. Qualitative changes are unequivocally demonstrated by substrate control ratios normalized for CI&II-linked OXPHOS (Fig. 4d) (Pesta et al., 2011). Accordingly, for a given CI&II-linked OXPHOS capacity, the physically active Inuit Hunters had a higher capacity to oxidize fatty acids. In Danes, 42 days of skiing for 6 h per day caused a shift to more oxidative muscle fibers (Type I) in the arms and enhancement or preservation of fatty acid oxidation capacity per muscle mass in the arm and leg, respectively, despite constant or decreased mitochondrial density as indicated by the CI&II OXPHOS capacity consistent with fiber type (Fig. 4b). We found no difference in mitochondrial fat oxidation capacity between Danish males and females in either the leg or arm muscles and the increase in capacity

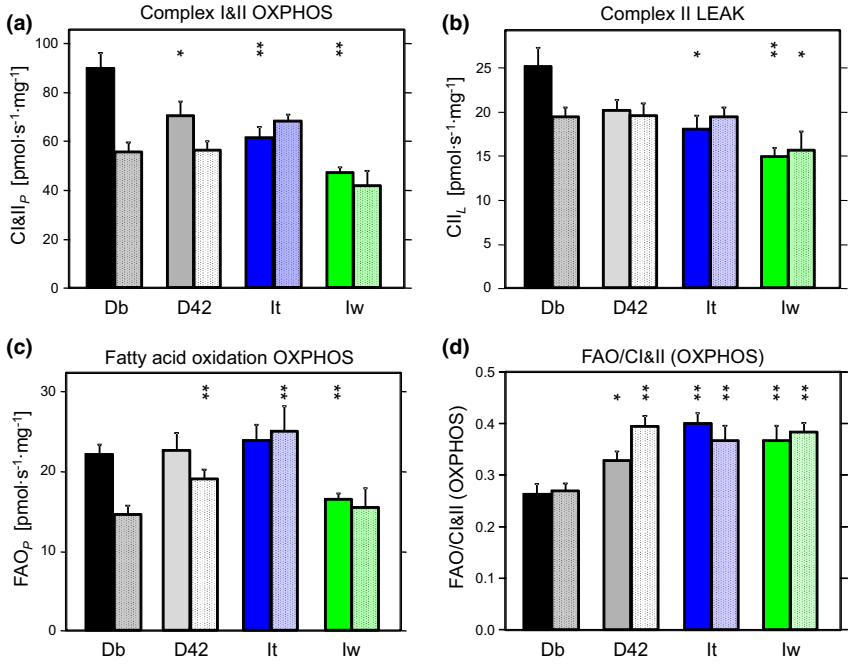

Fig. 4. Quantitative and qualitative changes of mitochondrial function in muscle of Danes after 42-day sojourn in Northern Greenland. Mitochondrial function in Danes at baseline (Db) and after 42 days of skiing (D42) compared to traditional and westernized Inuit (It and Iw), in the leg (VL, left bars) and arm (DEL, right bars). (a) OXPHOS capacity with a physiological substrate cocktail (convergent electron flow to the Q-junction from CI&II and FAO) as a functional marker of mitochondrial density (compare Fig. 2e and f). In Db, mitochondrial density in the arm was only half of the leg. Surprisingly, after 42-day expedition, the Danish volunteers lost mt-density in the leg but maintained constant mt-density in the arm, without decline in VO_{2max} (Table 1). There was no difference of mt-density in the legs and arms of the Inuit. Westernized Inuit had a low mt-density at only c. 60% with respect to the Inuit with an active traditional lifestyle. The Inuit hunters had the highest mt-density in the arm, but unexpectedly the mt-density in the leg was lower compared to the Danes. (b) Mitochondrial heat production in a tissue at rest is not only determined by coupling control, but increases with mt-density. As CI&II- and CII-linked OXPHOS capacity were equivalent functional markers of mt-density (Fig. 2f), CII-linked LEAK respiration per mg muscle mass integrates the qualitative effect of coupling and the quantitative effect of mt-density. Mass-specific LEAK respiration was highest in the leg of Db, declined after 42 days in the cold to the same level of the arm (Db) and of the traditional Inuit (It), and was lowest in Iw. (c and d) The decline in mt-density (Db to D42) was compensated by a qualitative shift of the mitochondria to a higher FAO/CI&II flux control ratio, expressing OXPHOS capacity with octanoylcarnitine and malate relative to the physiological substrate cocktail. Therefore, fatty acid oxidation capacity remained constant in the leg for Db and D42. This qualitative acclimatization was even more pronounced in the arm (Db to D42). In conclusion, the transition from Db to D42 induced a mitochondrial phenotype in the Danes that was nearly identical to the muscle mitochondria of the Inuit in arm and leg muscle. $*P < 0.05$; $**P < 0.01$ significant difference to matched muscle in the Danish baseline group.

to oxidize fatty acids in the arms with skiing was similar in males and females. This training response was localized to the muscle and was not exhibited at the whole body level where maximal oxygen consumption (VO_{2max}) was unchanged after the 42 days of skiing. During this type of skiing activity at a low intensity of 60% of maximal heart rate, pulling a 60-kg sled, the legs function primarily to stabilize the torso, while the arms accomplish most of the pulling work. The 42-day ski journey in the cold induced a mitochondrial phenotype in the arms and legs of Danes that was nearly identical to the muscle mitochondria of the physically active Inuit Hunters. An environmental effect of cold on muscle OXPHOS was apparent in the Danes with a transient reduction in both arm (27%) and leg (40%) muscles after the first 7 days skiing in the extreme cold which was approximately −30 to −40 °C. OXPHOS capacity of the arms, the most active muscle group, recovered to baseline with acclimatization from day 21 to 42 and with warming temperatures in the range of −20 to −15 °C by the end of the expedition. Despite this, OXPHOS capacity of the leg remained 23% lower than baseline, at the same level as the Inuit Hunters. Although the causal mechanisms are different, the effect of a reduced OXPHOS in the legs in the cold despite maintained activity level appears similar to the reduction in muscle OXPHOS at high altitude (Jacobs et al., 2013). In both extreme cold and altitude environments, VO_{2max} is reduced (Quirion et al., 1989; Boushel et al., 2001) and economy of locomotion is optimized by preservation of biochemical coupling efficiency at modest mitochondrial density, when VO_{2max} and sustained submaximum exercise performance are not dependent on peripherally increased capacities of OXPHOS (Jacobs et al.,

2013; Boushel et al., 2014). However, with acclimatization to altitude, VO_{2max} remains reduced and muscle OXPHOS continues to decline (Jacobs et al., 2013), whereas with acclimatization to cold, both OXPHOS and VO_{2max} recover as seen in the arms (Boushel et al., 2014). We attribute the sustained lowering of OXPHOS in the legs of Danes to the training (lifestyle) stimulus (also seen in the reduction in type I fibers), where the muscles were engaged in 6 hours/day of stabilizing work while the arms performed the more demanding pulling work, similar to the training stimulus of the Inuit Hunters. We do not have detailed dietary comparisons between Danes and Inuit, which may have played a role in the differences in fat oxidation capacity between groups.

Muscle UCP3 protein content was higher in the legs compared to arms in both Inuit groups, while the Danes showed similar levels of UCP3 across limbs. We found no relationship between UCP3 protein content and fatty acid oxidation capacity or LEAK respiration. The high capacity of the Inuit Hunters to oxidize fat substrates and the improved fat oxidation capacity of the Danes with training indicate plasticity of mitochondrial phenotype in relation to lifestyle and environment, while the preserved coupling in muscle tissue suggests evolutionary selection for conservation of energy over heat production at the level of muscle mitochondria despite the extreme cold of the arctic winter. The results on skeletal muscle do not exclude the possibility that evolutionary cold adaptation may alter coupling and thus thermoregulatory mechanisms in other tissues (Huttenen et al., 1981; Cypess et al., 2009). These findings may lend support to a "tissue-specific" coupling hypothesis, suggesting that high ATP production is conserved over heat generation across adaptations to different climates in muscle tissue required to perform work. Accordingly, it would be of great interest to investigate whether climatic selection for haplogroup manifests functionally in other tissues such as adipocytes and the extent to which chronic cold exposure alters thermoregulation by distinct heat dissipation mechanisms, extending comparative functional studies of mitochondria to populations adapted to more diverse climates and lifestyles.

Key words: Mitochondrial haplogroup, proton leak, thermogenesis, Inuit, substrates.

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Notes: *E. Gnaiger designed the mitochondrial respiratory protocols, conducted experiments, analyzed data, generated the figures, and co-wrote the manuscript. *R. Boushel conducted experiments, analyzed data, and co-wrote the manuscript; H. Søndergaard was expedition manager, conducted exercise testing, supervised all subjects throughout the study, and provided comments on the manuscript; T. Munch-Andersen conducted exercise testing, data analysis, co-supervised all subjects throughout the study, and provided comments on the manuscript; C. Hagen, C Díez-Sánchez and M. Christiansen performed mitochondrial haplogroup analysis, and contributed to the manuscript; P. Schrauwen, M. Hesselink performed UCP3 analysis, and provided input to the manuscript; I. Ara undertook muscle fiber type analyses and provided comments on the manuscript; R. Damsgaard and J. Calbet were medical monitors of the study, took muscle biopsies and J Calbet also contributed to the manuscript; ¶J.W. Helge co-organized and planned the expedition and provided comments on the manuscript; ¶B. Saltin was leader of all aspects of the study, co-wrote the manuscript. E. Gnaiger is founder and CEO of OROBOROS INSTRUMENTS Corp, www.oroboros.at.

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