

Cigarette Smoking Impairs Sperm Bioenergetics

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ABSTRACT

Objective: The growing consensus on the negative impact of cigarette smoking on fertility prompted us to compare the rate of sperm respiration in smokers and non-smokers.

Materials and Methods: Semen samples from 20 smokers and 58 non-smokers consulting at the andrology laboratory for fertility evaluation were used. Smoking was defined as consumption of at least a half a pack per day. A phosphorescence analyzer that measures O₂ concentration in sperm suspensions as function of time was used to determine the rate of respiration. In a sealed vial, the rate of sperm respiration (k) was defined as $-d[O_2]/dt$; where [O₂] was obtained from the phosphorescence decay rate of a palladium phosphor. [O₂] in solutions containing sperm and glucose declined linearly with time, showing the kinetics of O₂ consumption was zero-order. Inhibition of O₂ consumption by cyanide confirmed the oxidations that occurred in the sperm mitochondrial respiratory chain.

Results: There were no differences ($p > 0.28$) between smokers and non-smokers for ejaculate volume, motility, concentration, normal morphology, viability and hypo-osmotic swelling test. The rate (mean \pm SD, in $\mu\text{M O}_2/\text{min}/10^8$ sperm) of sperm mitochondrial O₂ consumption in the smokers was 0.96 ± 0.58 and in the non-smokers 1.39 ± 0.67 ($p = 0.004$).

Conclusions: The rate of sperm respiration was significantly lower in smokers. This negative impact of cigarette smoking on sperm aerobic metabolism may, in part, explain the lower rate of fertility in smokers.

Key words: male infertility; smoking; sperm; respiration; human

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INTRODUCTION

The detection of nicotine, and its major metabolite cotinine, in the seminal plasma of smokers showed that the tobacco compounds cross the blood-testis barrier and create a toxic environment for the spermatozoa (1,2). Toxic components in the cigarette smoke can disrupt the testicular microcirculation and cause DNA or chromosomal damage in germinal cells (3,4). The combustion also reduces semen volume, sperm concentration, motility and normal morphology

(5-12). Cigarette smoking also decreases fertilizing capacity of the sperm (13,14). Moreover, oligozoospermia has been linked to prenatal tobacco exposure in a dose-dependent manner (15). Smokers have more oxidative DNA damage and aneuploidy in their sperm than non-smokers (16-19). These observations confirm the deleterious effects of cigarette smoking on human fertility.

Cigarette smoking is still a major health dilemma, especially in "reproductive age" men and women. In a recent survey in the United States, 30-

35% of these individuals smoke cigarettes (20). The growing consensus on the negative impact of cigarette smoking on fertility prompted us to compare the rate of sperm respiration in smokers and non-smokers. For this purpose, we used a phosphorescence analyzer that accurately measures O_2 concentration as function of time in sperm suspensions (21,22). In this study, we evaluated the effect of cigarette smoking on sperm bioenergetics.

MATERIALS AND METHODS

Chemicals and Solutions

Pd (II) complex of meso-tetra-(4-sulfonato-phenyl)-tetrabenzoporphyrin (Pd phosphor sodium salt) was purchased from Porphyrin Products (Logan, UT). Modified Human Tubal Fluid (mHTF), containing 97.8 mM NaCl, 4.69 mM KCl, 0.2 mM $MgSO_4$, 0.37 mM KH_2PO_4 , 2.04 mM $CaCl_2$, 4.0 mM $NaHCO_3$, 21 mM HEPES, 2.78 mM glucose, 0.33 mM Na pyruvate, 21.4 mM Na lactate, 10 $\mu g/mL$ gentamicin sulfate, 5 mg/L phenol red and 0.5% human serum albumin was purchased from Irvine Scientific (Santa Ana, CA). The remaining reagents were obtained from Sigma-Aldrich (Saint Louis, MO). Pd phosphor solution (2 mM) was made by dissolving the powder at 2.5 mg/mL in dH_2O and stored at $-20^\circ C$ in small aliquots. NaCN (1.0 M) was made fresh in dH_2O , and the pH was adjusted to ~ 7 with 12N HCl.

Study Population

Seventy-eight (20 smokers and 58 non-smokers) patients attending the Andrology Laboratory for fertility testing were included in this study. The study was approved by the Institutional Review Board for protection of human subjects and informed consent was obtained from each patient. The average for number of cigarettes smoked per day was 11.6 ± 6.1 (mean \pm SD). A single semen sample with a sexual abstinence period between 2-7 days was collected for each patient. The samples were evaluated according to World Health Organization 1999 criteria. Semen

samples were allowed to liquefy at $37^\circ C$ for 30 minutes and only samples with ≤ 5 amorphous cells per hpf were selected. Additionally, peroxidase staining was performed on each sample to detect leukocytes. Only samples with negligible or without any leukocytes were used. Samples with similar semen parameters for smokers and non-smokers were selected for O_2 measurement to rule out the contribution of other variables. To measure respiration, an aliquot was diluted (2-fold) in mHTF and centrifuged at $25^\circ C$ (300 xg) for 10 min. within one hour after collection. The pellets were suspended at $\sim 10^8$ sperm per mL in mHTF supplemented with 2 μM Pd phosphor. The sample was immediately transferred to 1-mL glass vial, sealed and placed in the instrument for O_2 measurement.

Cellular Respiration

$[O_2]$ in the sperm suspensions was determined as function of time, using the Pd phosphor. The phosphorescence decay ($1/\tau$) of the probe was exponential, with τ being linear in $[O_2]$, according to $\tau^\circ/\tau = 1 + \tau^\circ k_q [O_2]$; τ , lifetime in the presence of O_2 ; τ° , lifetime in the absence of O_2 ; and k_q , second-order O_2 quenching constant. Samples were exposed to light flashes (10 per sec) from a pulsed light-emitting diode array with peak output at 625 nm. Emitted light was detected by a Hamamatsu photomultiplier tube after passing through a wide-band interference filter centered at 800 nm. The amplified phosphorescence decay was digitized at a rate of 1 MHz by an A/D converter. The values of τ were determined in a series of ascorbate plus ascorbate oxidase solutions, simultaneously with electrochemical measurements of $[O_2]$. A plot of $1/\tau$ vs. $[O_2]$ was linear; the value of the quenching constant k_q (the slope) was $96.1 \pm 1.2 \mu M^{-1} s^{-1}$ and $1/\tau^\circ$ (the intercept) $10087 \pm 156 s^{-1}$ (21).

For each run, 1.0 mL of the sperm suspension was placed in 1-mL sealed glass vial. The changes in $[O_2]$ with time were measured at $37^\circ C$ (21,22). Mixing was accomplished with parylene-coated stirring bar. Rates of respiration were the negative of the slopes of $[O_2]$ vs. t (zero-order rate constant, k , in $\mu M O_2 \text{ min}^{-1}$ per 10^8 sperm). Representative runs are shown in Figure-1.

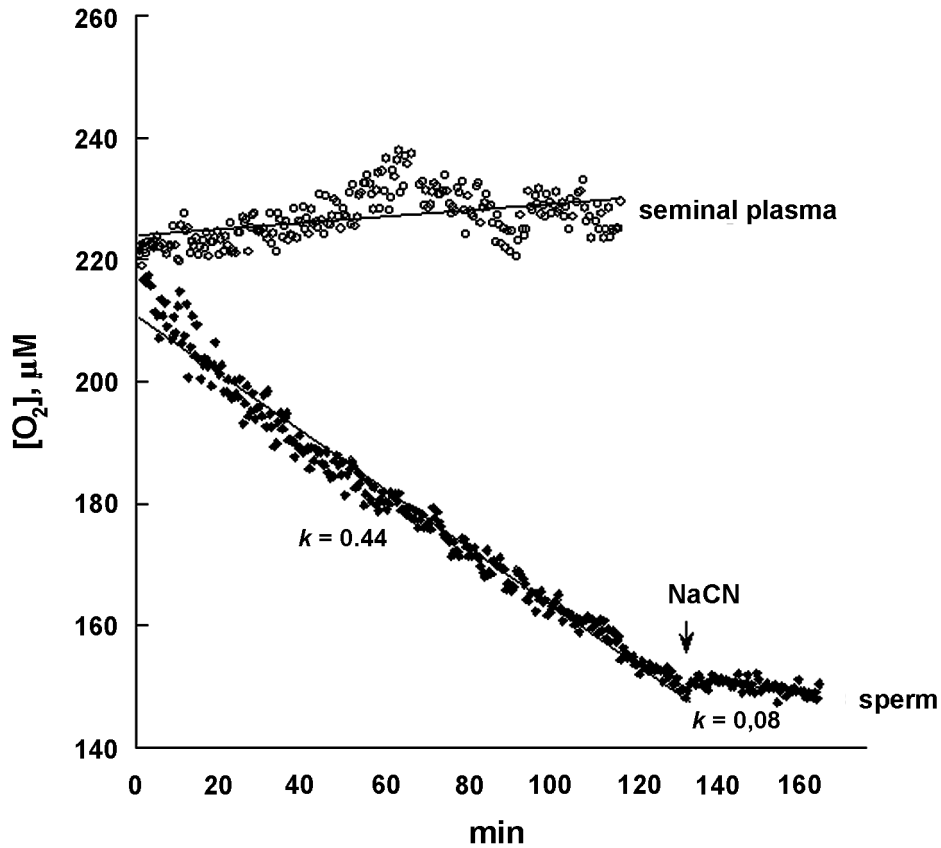


Figure 1 – Representative plot of O_2 consumption as function of time in sperm and seminal plasma from a smoker. O_2 consumption was not observed in the seminal plasma without sperm (open circles). $[O_2]$ declined linearly with time in the sperm suspension (closed diamonds). The decline in $[O_2]$ was inhibited by 87% after the addition of NaCN, confirming the oxidations occurred in the sperm mitochondrial respiratory chain. Best-fit curves ($r^2 = 0.978$) and values of k ($\mu M O_2 \text{ min}^{-1} \text{ per } 10^8 \text{ sperm}$) are shown.

Statistical Analysis

The data for semen parameters between smokers and non-smokers was compared using Student t-test. The Mann-Whitney rank sum test was used to compare respiration rate in sperm.

RESULTS

Semen parameters and sperm respiration rates for smokers and non-smokers are summarized in Table-1. No differences between smokers and non-smokers were observed for the ejaculate volume, motility, concentration, normal morphology, viability

and hypo-osmotic swelling (HOS) test ($p > 0.28$). However, the rate of sperm respiration (mean \pm SD, k , in $\mu M O_2/\text{min}/10^8 \text{ sperm}$) in the non-smokers was 1.39 ± 0.67 and in the smokers 0.96 ± 0.58 ($p = 0.004$).

COMMENTS

Our results show that cigarette smoking lowers sperm mitochondrial O_2 consumption (sperm respiration), Table-1. The mechanism for this inhibitory effect in sperm remains unclear. Cigarette smoke yields many toxic compounds which have inhibitory or stimulatory effects on various cells and body functions, one of which is total inhibition of

Table 1 – Sperm parameters and rates of respiration in smokers and non-smokers.

	Smokers (N = 20)	Non-smokers (N = 58)
Ejaculate volume (mL)	3.2 ± 1.3	3.6 ± 1.4
Total motility (%)	62.3 ± 8.4	61.8 ± 9.1
Progressive motility (%)	50.2 ± 12.1	48.7 ± 10.2
Sperm concentration (10 ⁶ /mL)	125 ± 73	134 ± 83
Normal morphology (%)	21.1 ± 13.4	20.4 ± 10.0
Viability (%)	55.8 ± 10.4	55.9 ± 10.8
HOS (%)	71.1 ± 7.3	72.0 ± 8.9
Rate of respiration	0.96 ± 0.58	1.39 ± 0.67

The values are mean ± SD. The *p* value between smokers and non-smokers for the rate of respiration (in $\mu\text{M O}_2/\text{min}/10^8$ sperm) was 0.004. No statistical differences were observed for the remaining parameters. HOS = hypo-osmotic swelling.

ciliary movement at bronchial level (23). The decreased cellular respiration observed in smokers can occur due to toxic compounds in the cigarette smoke. These include nicotine, cadmium, carbon monoxide, hydrogen cyanide, ammonia volatile hydrocarbons, alcohol, aldehydes and ketones, etc.

No differences were observed for conventional semen parameters between smokers and non-smokers in the present study. Similar findings have been observed previously (24). Significant negative impact of cigarette smoking on semen quality; however, has also been reported (5-12). Lower sperm mitochondrial function (respiration) in smokers may be a consequence of sperm exposure to smoke-related toxins present in seminal plasma. The impact of these toxins on mitochondrial function seems non-reversible because washed sperm devoid of seminal plasma were used in this study. Irreversible degenerative damage to the architectural elements of the sperm tail or axoneme in smokers has been previously observed (25). This ultra structural damage includes the absence of one or more fiber doublets, central fibers, and coarse outer fibers. The mitochondria are a principal part of sperm tail therefore, such ultra structural damage in the tail region can also result in impaired mitochondrial function. Exposure of non-smoker's sperm to the seminal plasma of a smoker resulted in significantly reduced motility and membrane functional integrity (26). Oxidative stress produced in seminal plasma due to smoking can be another factor for this phenomenon (27).

In summary, our results show a significantly lower sperm mitochondrial O₂ consumption rate in smokers. Further studies are needed to identify those constituents of cigarette smoke that mediate this effect.

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CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

Smoking is a lifestyle hazard for both active and passive smokers (1). Although sperm concentrations, motility, and/or morphology are often reduced compared to results observed in non-smokers, they often remain within the normal range (2). In this article, no differences were found between smokers and non-smokers for these parameters. However, it does show a negative impact of smoking on sperm mitochondrial function. Although sperm mitochondrial O₂ consumption is not a routine test, as a functional test, it may represent a predictive value for smoking men with normal sperm count aiming pregnancy. In accordance with literature, spermatozoa from smokers have reduced fertilizing capacity and embryos display lower implantation rates (3).

As discussed in the article, the mechanism for the inhibitory effect of smoking in sperm mitochondrial O₂ respiration remains unclear. This decreased cellular respiration can occur due to toxic compounds (1), to oxidative stress (4) or to both. According to Gaur et al., direct exposure of spermatozoa to the toxins in cigarette smoke probably tilts the delicate

balance of reactive oxygen species that are produced by spermatozoa for their special functions like decapitation (1).

In conclusion, couples in reproductive age should be strongly discouraged to smoke (3).

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