Physically Active Men Show Better Semen Parameters than Their Sedentary Counterparts

Paula C. Lalinde-Acevedo, B.Sc.1, B. Jose Manuel Mayorga-Torres, B.Sc.1, Ashok Agarwal, Ph.D.2, Stefan S. du Plessis, Ph.D.3,4, Gulam Ahmad, Ph.D.3,4, Ángela P. Cadavid, Ph.D.1, Walter D. Cardona Maya, Ph.D.5*

1. Reproduction Group, Department of Microbiology and Parasitology, Medical School, University of Antioquia, Antioquía, Colombia
2. Center for Reproductive Medicine, Cleveland Clinic, Cleveland, Ohio, USA
3. Division of Medical Physiology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa
4. Department of Physiology and Cell Biology, University of Health Sciences, Lahore, Pakistan

Abstract

Background: The quality of semen depends upon several factors such as environment, lifestyle, physical activity, age, and occupation. The aim of this study was to analyze and compare the conventional and functional semen parameters in men practicing vigorous physical activity to those of sedentary men.

Materials and Methods: In this descriptive cross-sectional study, semen samples of 17 physically active men and 15 sedentary men were collected for analysis. Semen analysis was performed according to the World Health Organization (WHO) guidelines, while functional parameters were evaluated by flow cytometry.

Results: Results showed that several semen parameters (semen volume, viability, progressive motility, total motility, normal morphology, and moribund cells) were superior in the physically active group in comparison with the sedentary group. Semen parameters such as viability, progressive motility and total motility, as well as the percentage of moribund spermatozoa were significantly different between both groups. However, sperm DNA damage, lipid peroxidation and mitochondrial potential were not significantly different among the groups.

Conclusion: Nevertheless, the physical activity shows better semen parameters than sedentary group. Taken together, our results demonstrate that regular physical activity has beneficial impact in sperm fertility parameters and such a lifestyle can enhance the fertility status of men.

Keywords: Sperm, Fertility, Physical Activity, Sedentary, Lifestyle

Citation: Lalinde-Acevedo PC, Mayorga-Torres BJM, Agarwal A, du Plessis SS, Ahmad G, Cadavid A’P, Cardona Maya WD. Physically active men show better semen parameters than their sedentary counterparts. Int J Fertil Steril. 2017; 11(3): 156-165. doi: 10.22074/ijfs.2017.4881.

Introduction

The conventional semen analysis involves the macroscopic (volume, pH, and colour) and microscopic (motility, concentration, viability, and morphology) examination (1). It reflects the secretory activity of the testes, epididymis and accessory sex glands indirectly (2). Although conventional semen analysis provides both quantitative and qualitative
information, it does not include evaluation of the functional properties of spermatozoa (3-7). Furthermore, oxidative stress which may directly contribute to the origin of male infertility, is not measured (8). Oxidative stress occurs due to the imbalance between the reactive oxygen species (ROS), reactive nitrogen species (RNS), and seminal antioxidant reserve in the male reproductive tract (9, 10). These ROS or RNS are produced during normal cellular metabolism and can be from either endogenous (normally produced by oxidative phosphorylation in mitochondria) or exogenous origin (e.g. produced by leukocytes) (10, 11).

Physiological levels of ROS exert a critical role in spermatozoa, triggering and mediating important signaling events to acquire essential functions such as hyperactivation, capacitation, and acrosome reaction (10-12). However, an excess in ROS levels is detrimental to cellular function and spermatozoa are highly susceptible to oxidative stress due to a lack in repair mechanisms (8, 13). This may result in damage to the structural components of the axoneme which may impact on the motility patterns (14, 15). It may also induce lipid peroxidation of cellular membranes (16), thereby disrupting the fluidity of mitochondrial and plasma membranes (12, 17) and furthermore lead to oxidative damage to proteins involved in the fusing of the spermatozoon with the oolemma (15). Additionally, ROS may cause DNA damage due to impaired histone remodeling during sperm maturation (12). Oxidative damage to spermatozoa has been related with recurrent pregnancy loss (13, 18, 19) and male infertility (5, 20).

It is well known that certain environmental factors including prolonged and continued exposure of the whole body, testes or scrotum to: i. Increased temperature, even at 37°C (21-23), ii. Environmental pollutants and endocrine disruptors (21, 24), iii. Electromagnetic radiation (21, 25), as well as lifestyle factors such as smoking, recreational drug use, alcohol consumption, obesity and sedentary occupation or lifestyle (25-29), may influence sperm quality and male fertility potential (21, 23, 25) mediated by induction of oxidative stress leading to cell apoptosis. Among several lifestyle factors, sedentarism have been found associated with several medical conditions and considered as one of the main causes of major public health issues at present (30). According to the definition of Bernstein et al. (31), individuals are considered sedentary when they spend less than 10% of their daily energy expenditure on performing moderate to vigorous-intensity activities. Also a sedentary person frequently spends much time sitting or lying down and performing activities usually associated with this low energy consumption state such as sleeping or watching television. Also it is commonly avoiding any form of exercise or sporting activities (32). Over the past five decades, changes in the occupational activities and leisure time have promoted the sedentary behavior and impacted on lifestyle (33, 34). As is true for other medical conditions (obesity and heart diseases), this phenomenon is equally deleterious for semen quality (35).

On the other hand; physical activity has beneficial effect on human health and is defined as any voluntary and repetitive body movements produced by skeletal muscle action that substantially increases energy expenditure above the basal state (34, 36). It may be included in the occupational activities or have diverse purposes like being aerobic training or training strength, flexibility and balance, therefore it encompasses exercise and sport (37, 38). Physical activity is classified according to the intensity with which it is practiced and may be quantified in terms of the energy expenditure as a multiple of the resting metabolic rate (39). Using the Metabolic Equivalent of the Task (MET, a physiological measure expressing the energy cost of physical activities), the moderate physical activity producing noticeable accelerated heart rate, ranges from 3.0 to 6.0 MET while the vigorous physical activity, demanding greater physical effort causing rapid breathing and a substantial increase in heart rate, are all the physical activities above 6.0 MET (38, 39). Some studies have reported a positive relationship (27, 33, 40-44), while others reported a negative one (45-47) between the practice of physical activity and the semen quality. Others report no impact of physical activity on sperm quality (48-51), therefore, the effect of a physically active lifestyle to improve semen quality is still controversial (52). The present study was conducted with the specific aim to evaluate and compare the semen parameters, conventional as well as functional, of men practicing vigorous physical activity to those having a sedentary life style.

Materials and Methods

In this descriptive cross-sectional study, thirty two men of reproductive age (physically active group 27.5 ± 6.0 and sedentary group 26.6 ± 5.3
years) from Medellin, Colombia were included. The inclusion criteria were: healthy men, without testicular disease, with a body mass index (BMI)<26 kg/m², and those who followed the same lifestyle pattern for the 12 months preceding the study, it is, either be physically active group (PAG, practice vigorous physical activities with a >6 MET for more than 2 hours per occasion at least 3 times per week; activities included are cycling, stationary cycling, calisthenetics, weightlifting, dancing, running, marital arts, football and swimming) or be sedentary group (SG, minimal physical activity ≤3 MET, do not practice sportive activities) (39, 53) (Table 1). Recreational drug and anabolic steroid users, smokers or medicated men were excluded from the study. Ethical approval was obtained from the Research Ethics Committee of the University of Antioquia and all patients gave informed consent. Semen samples were collected from volunteers between January and July 2014 by masturbation after a recommended ejaculation abstinence of 3-6 days. In addition to sample collection, certain anthropometric measurements (height and weight) necessary to calculate BMI were also measured (Table 1).

Also Participants had to complete a self-administered questionnaire by providing information regarding their reproductive history and whether or not they routinely practice any physical activity. If they did, they were asked to fill the description, type, frequency, intensity and duration of the physical activities practiced. This information was used to calculate the physical activities MET using the “Compendium of physical activities” as proposed by Ainswort et al. (39) which provided a measurement of their intensity level.

Conventional semen analysis

After complete liquefaction of the semen samples (30-60 minutes, at 37°C), a basic semen analysis was performed according to the World Health Organization (WHO) guidelines (1) while the sperm concentration was determined by using a Makler chamber (Sefi-Medical Instruments, Haifa, Israel) (54). Finally, sperm morphology was analyzed following the Tygerberg strict criteria (55), and semen samples with leukocytospermia (>1×10⁶ white blood cells/mL) were excluded.

Functional analysis

All flow cytometry analysis reported in this study were conducted on an Epics XL flow cytometer (Becton Dickinson, CA, USA) with a 488 nm excitation wavelength supplied by an argon laser. Forward scatter and side scatter measurements were used to gate spermatozoa and exclude debris and aggregates limiting undesired effects in the overall fluorescence. All data were acquired and analyzed using WinMDI 2.9 Software (Scripps Research Institute, La Jolla, CA) and a total of 10000 events were collected per sample.

Mitochondrial membrane potential

Mitochondrial membrane potential (ΔΨm) was measured by using 3, 3′-dihexyloxycarbocyanine iodide stain (DIOC6; Molecular Probes Inc., The Netherlands) (3) a cationic lipophilic dye selective for the mitochondria of living cells. Propidium iodide (PI, Molecular Probes Inc., The Netherlands) was used as counter stain to discriminate necrotic/dead cells. Briefly, 2×10⁶ spermatozoa were incubated in 300 μL of phosphate buffer saline (PBS, pH=7.4) containing DIOC6 (final concentration of 10 nM) and PI (final concentration of 12 μM) in the dark (30 minutes, at 25°C). Then, samples were washed in PBS (180 x g, 5 minutes), the pellet re-suspended in PBS and subjected to flow cytometry. Data were acquired as the percentage of living spermatozoa showing high (ΔΨm high) or low (ΔΨm low) green fluorescence and dead spermatozoa-red fluorescence.

Intracellular reactive oxygen species production

The intracellular ROS and RNS (specifically H₂O₂, HO-, ROO- and ONOO−) levels were evaluated using 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA, Sigma-Aldrich, St Louis, MO, USA). Upon cleavage of the acetate groups by intracellular esterases, DCFH is selectively oxidized by the above mentioned ROS and RNS to the green fluorescent DCF. PI was used to exclude the necrotic/dead cells (5). DCFH-DA was diluted to a final concentration of 1 μM in 300 μL of PBS containing 2×10⁶ spermatozoa and PI (final concentration 12 μM). The cell suspensions were incubated in the dark for 5 minutes, at 25°C, washed three times with PBS (180 x g, 5 minutes) and the pellet re-suspended in PBS before being analyzed by flow cytometry. Results are expressed as the percentage of live spermatozoa exhibiting the green DCF fluorescent response (DCF positive spermatozoa), as well as the green media fluorescence intensity (MFI).
Plasma membrane integrity evaluation

The LIVE/DEAD® Sperm Viability Kit (Molecular Probes Inc., The Netherlands) which distinguishes three populations of sperm based on their staining patterns, was used to assess the integrity of the plasma membrane according to the manufacturer’s instructions. Briefly, $2 \times 10^6$ spermatozoa were incubated in 300 μL of PBS with Sybr-14 and PI (green and red fluorescence emission, final concentration of 1 μM and 12 μM, respectively) in the dark (30 minutes, 25°C), washed once and re-suspended in PBS prior to flow cytometry analysis. Data are expressed as the percentage of viable spermatozoa-intact plasma membrane cells (positive to SYBR-14 and negative to PI), necrotic/dead cells (positive for PI only) or moribund sperm (positive for both dyes).

Lipid peroxidation assay

Oxidative degradation of lipids was measured using the BODIPY (581/591) C11 (Molecular Probes Inc., The Netherlands) according to the method proposed by Aitken et al. (16). BODIPY (581/591) C11 once incorporated into sperm membranes, undergoes a fluorescent emission shift from orange to green upon peroxidation by ROS. Briefly, $2 \times 10^6$ spermatozoa suspended in 300 μL of PBS were incubated in the dark (30 minutes, at 25°C) with BODIPY C11 (final concentration 6.6 μM), washed and re-suspended in PBS before flow cytometry analysis. Results are expressed as the percentage of spermatozoa exhibiting the green fluorescence response.

Sperm Chromatin Structure Assay

The Sperm Chromatin Structure Assay (SCSA) was used to determine the sperm DNA fragmentation index by Evenson (56) as previously described, 400 μl of acridine orange (Sigma-Aldrich, St Louis, MO, USA) staining solution (final concentration of 6 μg/mL). The ratio of single stranded DNA (red) to single plus double stranded DNA (green) MFI was expressed as the DNA fragmentation index (DFI).

Statistical analysis

The distribution of the data was evaluated with the normality test of residuals. The t test was used to compare groups of data that assumed Gaussian distribution, while the Mann-Whitney test used to compare the variables that did not assume Gaussian distribution. Correlations between sperm variables were determined with the Pearson correlation coefficient. Data were analyzed by using Prism 5.0 (GraphPad Software, San Diego, CA) statistical software and a P<0.05 considered to be significant. Data following Gaussian distribution are expressed as the mean ± SD and those not assuming Gaussian distribution are expressed as median and range.

Results

According to the MET scores, men were stratified into a physically active group (PAG, 8-48 MET, n=17) and a sedentary group (SG, <3 MET, n=15). Both PAG and SG present similar characteristics with regards to abstinence (4.1 ± 0.69 vs. 3.7 ± 0.75 days), height (1.74 ± 0.06 vs. 1.72 ± 0.05 m) and BMI (23.7 ± 1.5 vs. 22.7 ± 1.8 kg/m²). The average weight was slightly higher in the PAG in comparison with SG (71.6 ± 7.3 vs. 67 ± 5.3 kg), because these men had increased body mass in the form of muscle not of fat (Table 1).

Table 1: Characteristics of the participants

| Characteristic              | Physically active group | Sedentary group | P value |
|-----------------------------|-------------------------|-----------------|---------|
| Age (Y)                     | 27.5 ± 6.0              | 26.6 ± 5.3      | 0.66*   |
| Sexual abstinence (days)    | 4.1 ± 0.69              | 3.7 ± 0.75      | 0.56*   |
| Weight (Kg)                 | 71.6 ± 7.3              | 67 ± 5.3        | 0.07*   |
| Height (m)                  | 1.74 ± 0.06             | 1.72 ± 0.05     | 0.3*    |
| BMI (Kg/m²)                 | 23.7 ± 1.5              | 22.7 ± 1.8      | 0.11*   |
| Metabolic Equivalent of the Task (MET) | 19.7 ± 10.6           | 1.8 ± 0.6       | <0.0001* |

Results are expressed as mean ± SD. BMI; Body mass index, *; Student t test (Gaussian distribution), and *; Mann-Whitney test (non-gaussian distribution).
All semen samples from the PAG appeared normal with regards to viscosity and showed no agglutination, however various samples from SG showed moderate to high viscosity (33%) as well as isolated agglutination (47%) and moderate to abundant agglutination (13%) respectively. Among the conventional sperm parameters, total sperm motility, progressive motility and the percentage of viable sperm were significantly higher (P<0.05) in the PAG compared to the SG (Table 2). The only functional parameter that showed significant difference (P<0.05) between the PAG and SG was the percentage of moribund spermatozoa (Table 3).

### Table 2: Conventional semen parameters

| Variable                        | Physically active group n=17 | Sedentary group n=15 | P value |
|---------------------------------|------------------------------|----------------------|---------|
| Semen volume (mL)               | 4.3 ± 1.2                    | 3.5 ± 1.5            | 0.14*   |
| Sperm concentration ($\times 10^6$ sperm/mL) | 95.2 ± 47                   | 114.4 ± 63.9         | 0.37*   |
| Total sperm count ($\times 10^9$) | 353.6 (55.72-1080)          | 361.9 (100-997.4)    | 0.82*   |
| Viability (%)                   | 80.2 ± 7.2                  | 71.9 ± 10.7          | 0.01*   |
| Progressive motility (%)        | 63.0 (55.7-87.7)            | 56.8 (35.2-82.7)     | 0.03*   |
| Non-progressive motility (%)    | 3.7 (1.6-22.0)              | 5.0 (2.7-16.6)       | 0.13*   |
| Total motility (%)              | 66.5 (70.0-89.3)            | 62.3 (42.5-45.6)     | 0.03*   |
| Normal morphology (%)           | 7.3 (2.3-12.0)              | 4.8 (2.7-13.4)       | 0.52*   |
| Abnormal head (%)               | 90.2 ± 5.0                  | 89.1 ± 4.7           | 0.54*   |
| Abnormal neck/middle piece (%)  | 44.9 ± 16.0                 | 53.9 ± 18            | 0.14*   |
| Abnormal tail (%)               | 5.1 (3.3-7.1)               | 6.9 (2.5-8.7)        | 0.52*   |
| Abnormal cytoplasmic droplets (%) | 6.6 ± 4.8              | 5.7 ± 2.9            | 0.56*   |

Values are expressed as mean ± SD in data with normal distribution, and median (range) in non-normal distribution. *: Student t test (Gaussian distribution) and ^: Mann-Whitney test (non-gaussian distribution).

### Table 3: Functional seminal parameters

| Variable                          | Physically active group n=17 | Sedentary group n=15 | P value |
|-----------------------------------|------------------------------|----------------------|---------|
| $\Delta \Psi_m$ high spermatozoa (%) | 63.5 (51.5-80.6)          | 63.8 (18.9-77.1)     | 0.45*   |
| $\Delta \Psi_m$ low spermatozoa (%) | 4.0 (1.9-14.0)            | 4.4 (2.3-19.1)       | 0.54*   |
| Sperm with intact plasma membrane (%) | 68.1 (43.7-83.1)         | 65.2 (26.2-72.6)     | 0.10*   |
| Moribund sperm (%)                | 4.3 (2.0-17.0)             | 9.0 (4.6-14.4)       | 0.02*   |
| Necrotic/dead sperm (%)           | 23.5 ± 6.7                 | 28.6 ± 11.4          | 0.13*   |
| DCF positive spermatozoa (%)      | 59.3 (6.83-78.2)           | 49.3 (14.3-68.1)     | 0.09*   |
| DCF positive spermatozoa (MFI)    | 50.6 (24.4-148.8)          | 57.7 (14.6-92.2)     | 0.74*   |
| Sperm with lipid peroxidation (%) | 3.3 (0.5-18.8)             | 6.3 (0.15-33.6)      | 0.20*   |
| DNA fragmentation index (%)       | 19.6 ± 8.6                 | 17.1 ± 8.3           | 0.40*   |

Values are expressed as mean ± SD in data with normal distribution and median (range) in non-normal distribution. DCF; 2', 7'-dichlorofluorescein, MFI; Mean fluorescence intensity, *: Student t test (Gaussian distribution), and ^: Mann-Whitney test (non-gaussian distribution).
When comparing the combined data sets from both groups, significant correlations were found between total abnormal sperm forms and spermatozoa with head defects (correlation coefficient \( r = -0.67, P < 0.01 \)), sperm with neck/middle piece defects and progressive motility (\( r = -0.56, P < 0.01 \)), ejaculation abstinence time and sperm with excess residual cytoplasm (\( r = 0.58, P < 0.01 \)), ejaculation abstinence time and non-progressive motility (\( r = -0.57, P < 0.01 \)), viable sperm and intracellular ROS production (\( r = 0.79, P < 0.01 \)), viable sperm and mitochondrial membrane potential (\( \Delta \Psi_m, r = 0.83, P < 0.01 \)), and sperm with high \( \Delta \Psi_m \) and intracellular ROS production (\( r = 0.65, P < 0.01 \)). In addition, when the PAG’s data were analyzed separately, all of the above mentioned significant correlations were found, together with a few significant correlations exclusive to the PAG. These include: ejaculation abstinence time and immotile sperm (\( r = -0.57, P < 0.01 \)), sperm concentration and normal morphology (\( r = 0.72, P < 0.01 \)), and sperm concentration and sperm with head defects (\( r = -0.63, P < 0.01 \)).

Discussion

We found differences in conventional and functional seminal parameters between physically active group and sedentary group of men. The semen parameters were better in PAG, which is in favor to adopt such a lifestyle. The average values of the conventional parameters analyzed for each group, remained above the lower limit reference values proposed by the WHO (1). The total and progressive sperm motility, sperm viability, as well as the percentage of moribund cells were significantly higher in PAG compared to SG. This is the first study in addition to conventional semen parameters, certain sperm functional parameters i.e. \( \Delta \Psi_m \) plasma membrane integrity, intracellular ROS and lipid peroxidation, were analyzed in relation to the practice of vigorous physical activity or following a sedentary lifestyle. Our results are in accordance with previous study demonstrating increased sperm motility in physical active men (41) and comparable results in a group of assisted reproduction patients classified according to their physical status (43). However, significant differences in sperm viability due to physical activity levels had not been previously reported.

Some studies reported that sperm concentration and morphology are the main parameters improved in men having moderate to vigorous physical active lifestyle (47), against being sedentary (43). Our results are not in agreement with these findings, as we did not observe any significant differences in either sperm concentration or morphology between PAG and SG. Nonetheless, a positive correlation was found between sperm concentration and normal morphology in PAG. Similar results have been reported by Munuce et al. (57) in semen samples obtained from men attending a reproductive clinic without regarding their physical status. This finding is interesting because it may be related to an increase in hormones, specially follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone, responsible to stimulate proper spermatogonia nutrition and division during the process of spermatogenesis (58, 59). This speculation is supported by the findings from previous studies where increased total and free blood plasma and serum testosterone, as well as higher FSH and LH levels have been demonstrated after continuous moderate physical activity (41).

The plasma membrane integrity is a key determinant for proper sperm interactions with other cells and their environment, therefore it is a prerequisite for successful fertilization (60). The percentage of dual stained (moribund) spermatozoa was statistically higher in SG in comparison with PAG. This sperm population have been described as slightly damaged sperm with compromised plasma membrane that have lost their ability to exclude PI, indicating a transitional phase in which the cell ultimately die (60-62). Although the biological importance of moribund sperm has not been well established, in works on bulls the percentage of moribund spermatozoa was positively correlated with the low fertility status of males, possibly compromising the availability of live sperm in the female reproductive tract (62). Furthermore, Garner and Johnson (61) have microscopically observed that the change from green to red fluorescence of some sperm, began at the posterior portion of the sperm head, proceed anteriorly and is accompanied by the progressive loss of motility until they are dead. This increased percentage of moribund spermatozoa and negative correlation between sperm with neck/middle piece defects and progressive motil-
ity in SG can be the possible explanations of significantly lower progressive and total motility in these men.

The evaluation of functional parameters has also been used to determine the levels of oxidative stress in spermatozoa. Common sedentary activities such as sitting for long, and some physical activities including running or bicycling may disrupt the intrascrotal temperature regulation (63, 64) and increase the pressure force to the testicles (46, 47, 49), leading to oxidative stress (58).

Although the percentage of DCF positive spermatozoa in the PAG group was higher in SG, it was not significantly different. We found higher values of DCF positive sperm in comparison with previous studies (65-67) intended to evaluate the ROS/RNS production on spermatozoa using the same method. However; the oxidative stress level as depicted by lipid peroxidation measurement was discernibly lower in PAG than SG, which is in accordance with previous findings by others (16, 68). Sperm DNA integrity did not differ significantly in our study between PAG, SG and DFI remained in the range considered normal (16-24%) (69). This is in accordance with a previous report where no relation of sperm DFI was drawn in men with sedentary lifestyle in relation to their BMI and their waist circumference (70).

In addition, no mitochondrial dysfunction was detected either in the PAG or SG group despite the total and progressive motility is significantly increased in PAG. In fact, most of the spermatozoa in the semen samples from both groups had high $\Delta P_m$, which is indicative in proper mitochondrial functioning (17, 71). It may support the assumption that the rapid transition from viable to moribund sperm was influencing the loss of motility in the SG sperm rather than the viable sperm that have diminished the $\Delta P_m$ as it may be commonly related. As the higher ROS detection in the PAG was not correlated with oxidative stress generation in spermatozoa (higher lipoperoxidation-LPO-and/or altered DFI), we speculate that there must have been a balance between pro-oxidants and antioxidants molecules in the PAG volunteers’ semen samples. Possibly the practice of vigorous physical activity of volunteers, have contributed to attenuate the oxidative stress events in consequence of the higher ROS/RNS production, since it has been previously demonstrated that physical training promotes blood total antioxidant capacity (72), and also in semen, moderate to vigorous physical activity practitioners had superior levels of antioxidant enzymes in comparison with high performance-elite athletes or sedentary men (73).

Furthermore; it is known that sperm cells have a deficient ROS-scavenging system, in consequence of its limited cytosolic space. So they are very dependent on the antioxidant protection provided by the male reproductive tract (74). This is directly influenced by the men’s nutritional status and the dietary intake of antioxidant molecules since they form an essential part of the human antioxidant defense system (75).

As we did not control the diet in our volunteers, the effect of the diet cannot be ruled out, considering that, a physically active lifestyle is commonly accompanied by a healthy diet. In the light of these results, we consider convenient to include some other informational aspects, certainly related to the physically active or sedentary lifestyle and the semen quality. For instance, nutritional aspects related with dietary antioxidants intake, the determination of blood hormonal levels (mainly LH, FSH and testosterone) and the semen total antioxidant capacity evaluation, directly involved in the developmental environment of spermatozoa and the oxidative stress dynamics. On the other hand, it has also been established that if the physical training is at least moderate but regular, it may turn into an adaptation to diminish the increased amounts of ROS producing during high oxygen consumption derived from further vigorous physical activities (41, 58, 76, 77). This physical activity linked-adaptation constitutes an advantage over the possible adverse conditions associated with the practice of some previously mentioned physical activities that may negatively affect the seminal quality.

Infertility affects approximately 15% of couples of reproductive age, with significant impact on their quality of life (11, 70). As it is estimated that men contribute equally (50%) to the causes of fertility problems (29, 59), the identification and modification of some potential risk factors such as the relationship between physical activity or inactivity and semen quality, may help some couples to achieve their reproductive goals (70). The practice of vigor-
ous physical activity is clearly not the unique solution. Most of the literature regarding the relationship between physical activity, sedentarism and semen quality, have focused on elite athletes or men attending fertility clinics. However, various investigations have demonstrated the positive influence of moderate, constant exercise on the hormonal profile (41, 58, 76), libido (78), the psychological wellbeing (59) and on the body condition (30, 38, 76), which may also impact positively the male reproductive outcome. Our volunteers may be classified as recreational but vigorous physical activity practitioners and the activities performed included strength and aerobic training or vigorous occupational physical activities. This is important to clarify because the type of physical training, specially the higher intensity or constantly anaerobic training have been related to diminish seminal parameters (45-47, 49) and may influence the hormonal effect on the sperm quality, especially on the testosterone metabolism (41, 59).

**Conclusion**

Despite the fact that some indicators of cellular oxidative stress were higher in the PAG in comparison with the SG, no signs of developing a state of oxidative stress was observed. On the contrary, the practice of vigorous physical activity in the conditions set in our study (8 to 48 MET, in sessions of two hours minimum, with a frequency of at least 3 days a week), was significantly related to better semen parameters (increased viability, progressive and total motility and lower percentage of moribund cells), when compared to individuals following a complete sedentary lifestyle at least for a year. It can therefore be concluded that the levels of physical activity reported in this study, exert a positive effect on the semen parameters of these men or at least prevent its deterioration as a result of environmental stressors. Our findings are encouraging since they contribute to elucidate the proper intensity and frequency of physical activity which may exert a positive effect on semen quality or at least prevent its decline related to the practice of higher intensity-endurance physical activities. Future studies are required in defining the intensity and threshold to be considered as beneficial for semen quality.

**Acknowledgements**

This study was financially supported by the sustainability strategy (Reproduction Group) and Investigation Center of Exact and Natural Sciences (CIEN) of the University of Antioquia. The authors report no declaration of interest.

**References**

1. World Health Organization. WHO laboratory manual for the examination and processing of human semen. Geneva, Switzerland: WHO Press; 2010. http://apps.who.int/iris/bitstream/10665/44261/1/9789241547789_eng.pdf. (20 May 2016).
2. Eliasson R. Semen analysis with regard to sperm number, sperm morphology and functional aspects. Asian J Androl. 2010; 12(1): 26-32.
3. Bungum M, Bungum L, Giwercman A. Sperm chromat structure assay (SCSA): a tool in diagnosis and treatment of infertility. Asian J Androl. 2011; 13(1): 69-75.
4. Cardona Maya WD, Berdugo Gutierrez JA, de los Rios J, Cadavid Jaramillo AP. Functional evaluation of sperm in Colombian fertile men. Arch Esp Urol. 2007; 60(7): 827-831.
5. Mayorga-Torres BJ, Cardona-Maya W, Cadavid A, Camargo M. Evaluation of sperm functional parameters in normozoospermic infertile individuals. Actas Urol Esp. 2013; 37(4): 221-227.
6. Mayorga-Torres BJ, Camargo M, Agarwal A, du Plessis SS, Cadavid AP, Cardona Maya WD. Influence of ejaculation frequency on seminal parameters. Reprod Biol Endocrinol. 2015; 13: 47.
7. Mayorga-Torres JM, Agarwal A, Roychoudhury S, Cadavid A, Cardona-Maya WD. Can a short term of repeated ejaculations affect seminal parameters? J Reprod Infertil. 2016; 17(3): 177-183.
8. Tremellen K. Oxidative stress and male infertility: a clinical perspective. In: Agarwal A, Aitken RJ, Alvarez JG, editors. Studies on men’s health and fertility: Oxidative stress in applied basic research and clinical practice. New York: Humana Press, Springer; 2012; 325-354.
9. Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in the testes. Oxid Med Cell Longev. 2008; (1(1): 15-24.
10. Kothari S, Thompson A, Agarwal A, du Plessis SS. Free radicals: their beneficial and detrimental effects on sperm function. Indian J Exp Biol. 2010; 48(5): 425-435.
11. Henkel R. ROS and semen quality. In: Agarwal A, Aitken RJ, Alvarez JG, editors. Studies on men’s health and fertility. Oxidative stress in applied basic research and clinical practice. New York: Humana Press, Springer; 2012; 301-323.
12. Aitken RJ, Baker MA, De Iuliiis GN, Nixon B. New insights into sperm physiology and pathology, Handb Exp Pharmacol. 2010; (198): 99-115.
13. Gil-Villa AM, Cardona-Maya W, Agarwal A, Sharma R, Cadavid A. Role of male factor in early recurrent embryo loss: do antioxidants have any effect? Fertil Steril. 2009; 92(2): 565-571.
14. El-Taieb MA, Herwig R, Nada EA, Greilberger J, Marberger M. Oxidative stress and epididymal sperm transport, motility and morphological defects. Eur J Obstet Gynecol Reprod Biol. 2009; 144 Suppl 1: S199-203.
15. Aitken RJ, Smith TD, Jobling MS, Baker MA, De Iuliiis GN.
Oxidative stress and male reproductive health. Asian J Androl. 2014; 16(1): 31-38.
16. Aitken RJ, Wingate JK, De Iuliis GN, McLaughlin EA. Analysis of lipid peroxidation in human spermatozoa using BODIPY C11. Mol Hum Reprod. 2007; 13(4): 203-211.
17. Köppers AJ. Mitochondria as a source of ROS in mammalian spermatozoa. In: Agarwal A, Aitken RJ, Alvarez JG, editors. Studies on men’s health and fertility. Oxidative stress in applied basic research and clinical practice. New York: Humana Press, Springer; 2012; 21-40.
18. Gil-Villa AM, Cardona-Maya W, Agarwal A, Sharma R, Cadavid A. Assessment of sperm factors possibly involved in early recurrent pregnancy loss. Fertil Steril. 2010; 94(4): 1465-1472.
19. Rodriguez E, Gil-Villa AM, Aguirre-Acevedo DC, Cardona-Maya W, Cadavid AP. Evaluation of atypical semen parameters in individuals whose couples had a history of early recurrent embryo death: in search for a reference value. Biometica. 2011; 31(1): 100-107.
20. Bungum M. Sperm DNA integrity assessment: a new tool in diagnosis and treatment of fertility. Obstet Gynecol Int. 2012; 2012; 531042.
21. Agarwal A, Desai NR, Ruffoli R, Carpi A. Lifestyle and testicular dysfunction: a brief update. Biomed Pharmacother. 2008; 62(8): 550-553.
22. Ivell R. Lifestyle impact and the biology of the human scrotum. Reprod Biol Endocrinol. 2007; 5: 15.
23. Ahmad G, Moinard N, Esquerre-Lamare C, Mieussent R, Bujan L. Mild induced testicular and epididymal hyperthermia alters sperm chromatin integrity in men. Fertil Steril. 2012; 97(3): 546-552.
24. Sharpe RM. Environment, lifestyle and male infertility. Baillieres Best Pract Res Clin Endocrinol Metab. 2000; 14(3): 489-503.
25. Mendiola J, Torres-Cantero AM, Agarwal A. Lifestyle factors and male infertility: an evidence-based review. Arch Med Sci. 2009; 5(1A): S3-S12.
26. Kumar S, Kumari A, Murarka S. Lifestyle factors in deteriorating male reproductive health. Indian J Exp Biol. 2009; 47(8): 615-624.
27. Jurevic J, Radwan M, Sobala W, Ligocka D, Radwan P, Bochenek M, et al. Lifestyle and semen quality: role of modifiable risk factors. Syst Biol Reprod Med. 2014; 60(1): 43-51.
28. Bujan L, Daudin M, Charlet JP, Thouneau P, Mieussent R. Increase in scrotal temperature in car drivers. Hum Reprod. 2000; 15(6): 1355-1357.
29. Du Plessis SS, Cabel S, McAlister DA, Sabanegh E, Agarwal A. The effect of obesity on sperm disorders and male infertility. Nat Rev Urol. 2010; 7(3): 153-161.
30. Blair SN. Physical inactivity: the biggest public health problem of the 21st century. Br J Sports Med. 2009; 43(1): 1-2.
31. Bernstein MS, Morabia A, Slutzkis D. Definition and prevalence of sedentarism in an urban population. Int J Public Health. 1999; 48(6): 862-867.
32. Fox M. What is sedentarism? J Acad Nutr Diet. 2012; 112(8): 1124-1128.
33. Magnusdottir EV, Thorsteinssson T, Thorsteinsdottir S, Heimisdottir M, Olafsdottir K. Persistent organochlorines, sedentary occupation, obesity and human male subfertility. Hum Reprod. 2005; 20(1): 208-215.
34. Brownson RC, Boehmer TK, Luke DA. Declining rates of physical activity in the United States: what are the contributors? Annu Rev Public Health. 2005; 26: 421-443.
35. Janevic T, Kahn LG, Landsbergs F, Cirillo FM, Cohn BA, Liu X, et al. Effects of work and life stress on semen quality. Fertil Steril. 2014; 102(2): 530-538.
36. Bayol S, Brownson C, Loughna PT. Electrical stimulation modulates IGF binding protein transcript levels in C2C12 myotubes. Cell Biochem Funct. 2005; 23(5): 361-365.
37. Blair SN, LaMonte MJ, Nichaman MZ. The evolution of physical activity recommendations: how much is enough? Am J Clin Nutr. 2004; 79(5): 913S-920S.
38. World Health Organization. Global recommendations on physical activity for health. Geneva, Switzerland: WHO Press; 2010. Available from: http://www.who.int/publications/2010/9789241599979_eng.pdf. (20 May 2016).
39. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000; 32(9 Suppl): S498-504.
40. Wise LA, Cramer DW, Hornstein MD, Ashby RK, Missmer SA. Physical activity and semen quality among men attending an infertility clinic. Fertil Steril. 2011; 95(3): 1025-1030.
41. Vaamonde D, Da Silva-Grigoletto ME, Garcia-Manso JM, Barrera N, Vaamonde-Lemos R. Physically active men show better semen parameters and hormone values than sedentary men. Eur J Appl Physiol. 2012; 112(9): 3267-3273.
42. Gaskins AJ, Mendiola J, Afeiche M, Jorgensen N, Swan SH, Chavarro JE. Physical activity and television watching in relation to semen quality in young men. Br J Sports Med. 2015; 49(4): 256-260.
43. Guarnizo MC, Niebla ED, Garcia SM, González MR, Gordillo J, Fernández de Velasco JDJ, et al. Influence of physical activity and body mass index on sperm quality: analysis in assisted reproduction patients. Rev Assoc Est Biol Rep. 2011; 16(1): 25-34.
44. Parnt T, Grau Ruiz R, Kunovac Kallak T, Ruiz JR, Davey E, Hreinsson J, et al. Physical activity, fatness, educational level and snuff consumption as determinants of semen quality: findings of the ActARTudy study. Reprod Biomed Online. 2015; 31(1): 108-119.
45. Arce JC, De Souza MJ, Pescatello LS, Luciano AA. Subclinical alterations in hormone and semen profile in athletes. Fertil Steril.1993; 59(2): 398-404.
46. De Souza MJ, Arce JC, Pescatello LS, Scherzer HS, Luciano AA. Gonadal hormones and semen quality in male runners. A volume threshold effect of endurance training. Int J Sports Med. 1994: 15(7): 383-391.
47. Vaamonde D, Da Silva-Grigoletto ME, Garcia-Manso JM, Vaamonde-Lemos R, Swanson RJ, Oehninger SC. Response of semen parameters to three training modalities. Fertil Steril. 2009; 92(6): 1941-1946.
48. Bagatell CJ, Bremmer WJ. Sperm counts and reproductive hormones in male marathoners and lean controls. Fertil Steril. 1990; 53(4): 688-692.
49. Lucia A, Chicharro JL, Perez M, Serratos L, Bандres F, Legido JC. Reproductive function in male endurance athletes: sperm analysis and hormonal profile. J Appl Physiol (1985). 1996; 81(6): 2627-2636.
50. Mínguez-Alarcón L, Chavarro JE, Mendiola J, Gaskins AJ, Torres-Cantero AM. Physical activity is not related to semen quality in young healthy men. Fertil Steril. 2014; 102(4): 1103-1109.
51. Stoy J, Hjollund NH, Mortensen JT, Burr H, Bonde JP. Semen quality and sedentary work position. Int J Androl. 2004; 27(1): 5-11.
52. Lalinde Acevedo PC, Mayorga Torres JM, Cardona Maya WD. Relation between physical activity, sedentarism and semen quality (Relación entre la actividad física, el sedentarismo y la calidad seminal). Rev Chil Obstet Ginecol. 2014; 79(4): 326-328.
53. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity
questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003; 35(6): 1381-1395.
54. Cardona-May W, Berdugo J, Cadavid A. Comparing the sperm concentration determined by the makler and the neubauer chambers. Actas Urol Esp. 2008; 32(4): 443-445.
55. Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, et al. Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertil Steril. 1986; 46(6): 1118-1123.
56. Evenson DP. Sperm chromatin structure assay (SCSA(R)). Methods Mol Biol. 2013; 927: 147-164.
57. Munuce MJ, Cardona-May A, Werta CL. Is there an association between sperm normal morphology and their kinetic displacement? Actas Urol Esp. 2006; 30(6): 591-597.
58. Lanfranco M, Minetto MA. Endocrinology of physical activity and sport. In: Constantini N, Hackney AC, editors. The male reproductive system, exercise, and training: endocrine adaptations. 2nd ed. New York: Springer; 2013; 121-132.
59. du Plessis SS, Kashou A, Vaamonde D, Agarwal A. Is there a link between exercise and male factor infertility. Open Reprod Sci J. 2011; 3: 105-113.
60. Kim S, Lee YJ, Kim YJ. Changes in sperm membrane and ROS following cryopreservation of liquid boar semen stored at 15 degrees C. Anim Reprod Sci. 2011; 124(1-2): 118-124.
61. Garner DL, Johnson LA. Viability assessment of mammalian sperm using SYBR-14 and propidium iodide. Biol Reprod. 1995; 53(2): 276-284.
62. Shojaei H, Kroetsch T, Wilde R, Blondin P, Kastelic JP, Thundathil JC. Moribund sperm in frozen-thawed semen, and sperm motion end points post-thaw and post-swim-up, are related to fertility in Holstein AI bulls. Theriogenology. 2012; 77(5): 940-951.
63. Jung A, Schuppe HC. Influence of genital heat stress on semen quality in humans. Andrologia. 2007; 39(6): 203-215.
64. Koskela R, Zaprudina N, Vuorikari K. High scrotal temperatures and chairs in the pathophysiology of poor semen quality. Pathophysiology. 2005; 11(4): 221-224.
65. Mahfouz R, Sharma R, Lackner J, Aziz N, Agarwal A. Evaluation of chemiluminescence and flow cytometry as tools in assessing production of hydrogen peroxide and superoxide anion in human spermatozoa. Fertil Steril. 2009; 92(2): 819-827.
66. Mahfouz RZ, du Plessis SS, Aziz N, Sharma R, Sabanegh E, Agarwal A. Sperm viability, apoptosis, and intracellular reactive oxygen species levels in human spermatozoa before and after induction of oxidative stress. Fertil Steril. 2010; 93(3): 814-821.
67. Sullivan MJ, Beltz TG, Johnson AK. Astamatin potentiality of drinking induced by blood-borne angiotensin: evidence for mediation by endogenous brain angiotensin. Brain Res. 1990; 510(2): 237-241.
68. Barbonetti A, Vassallo MR, Cinque B, Filipponi S, Mastrostefano P, Cifone MG, et al. Soluble products of Escherichia coli induce mitochondrial dysfunction-related sperm membrane lipid peroxidation which is prevented by lactobacilli. PLoS One. 2013; 8(12): e83136.
69. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl. 2002; 23(1): 25-43.
70. Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF, Louis GMB. The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. Hum Reprod. 2014; 29(2): 193-200.
71. Wang X, Sharma RK, Gupta A, George V, Thomas AJ, Falcone T, et al. Alterations in mitochondria membrane potential and oxidative stress in infertile men: a prospective observational study. Fertil Steril. 2003; 80 Suppl 2: 844-850.
72. Child RB, Wilkinson DM, Fallowfield JL, Donnelly AE. Elevated serum antioxidant capacity and plasma malondialdehyde concentration in response to a simulated half-marathon run. Med Sci Sports Exerc. 1998; 30(11): 1603-1607.
73. Hajizadeh Maleki B, Tartibian B, Eghtehal M, Asri-Rezaei S. Comparison of seminal oxidants and antioxidants in subjects with different levels of physical fitness. Andrology. 2013; 1(4): 607-614.
74. Aitken RJ, De Iuliis GN. On the possible origins of DNA damage in human spermatozoa. Mol Hum Reprod. 2010; 16(1): 3-13.
75. Agarwal A, Prabakaran SA, Said TM. Prevention of oxidative stress injury to sperm. J Androl. 2005; 26(6): 654-660.
76. Redman LM. Physical activity and its effects on reproduction. Reprod Biomed Online. 2006; 12(5): 579-586.
77. Kraemer WJ, Ratamess NA. Hormonal responses and adaptations to resistance exercise and training. Sports Med. 2005; 35(4): 339-361.
78. Wogatzky J, Wirleitner B, Stecher A, Vanderzwalmen P, Neyer A, Spitzer D, et al. The combination matters--distinct impact of lifestyle factors on sperm quality: a study on semen analysis of 1683 patients according to MSOME criteria. Reprod Biol Endocrinol. 2012; 10: 115.