Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Insights into a novel pathway of paraben-induced endotoxin affecting human sperm quality: Parabens’ dual effects of antibacterial and estrogenic activity

Linyuan Zhang
National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention
Pengkun Song
National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention
Ying Guo
Key Laboratory of Male Reproductive Health of NHC, Human Sperm Bank;National Research Institute for Family Planning & WHO Collaborating Center for Research in Human Reproduction
Chunguang Ding
National Center for Occupational Safety and Health
Jianfeng Xu
Key Laboratory of Male Reproductive Health of NHC, Human Sperm Bank;National Research Institute for Family Planning & WHO Collaborating Center for Research in Human Reproduction
Dong Yuan
Key Laboratory of Male Reproductive Health of NHC, Human Sperm Bank;National Research Institute for Family Planning & WHO Collaborating Center for Research in Human Reproduction
Peihuan Qiao
National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention
Junsheng Huo
National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention
Jian Huang
National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention
Bing Chang (bjchangbing@sohu.com)
National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention
Xiaowei Liang
National Research Institute for Family Planning & WHO Collaborating Center for Research in Human Reproduction
Gangqiang Ding
National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention

Research Article

Keywords: Exogenous chemicals, endocrine disrupting chemicals, male reproduction, gut microbiome, LPS, healthy men

Posted Date: August 1st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1895815/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Abstract

Background

The decline of human sperm quality has caused great concern. Whether exposure to parabens and the intestinal endotoxin (IE) they induce are related to sperm quality is a challenging scientific question.

Results

We measured seminal methyl-(MeP), ethyl-(EtP), propyl-(PrP), and butyl-(BuP) parabens, their metabolites PHBA and 3,4-DHB, semen parameters, and blood IE (lipopolysaccharide, LPS) of 315 healthy men. Bivariate correlation and multiple linear regression were used to evaluate their interrelationships. For the first time, we found that MeP, EtP, PrP, BuP, PHBA, and 3,4-DHB in semen were negatively correlated with sperm parameters (r = − 0.184 to − 0.438), with EtP showing the strongest correlation. LPS was detected in all plasma samples (0.021–0.195 EU/mL) and negatively correlated with sperm concentration and total sperm count with R^2 = 0.295 and R^2 = 0.236, respectively, when LPS was higher than 0.104 EU/mL. LPS was positively correlated with EtP and BuP.

Conclusion

We concluded that seminal parabens affected sperm quality possibly due to estrogenic activity. Parabens likely promote intestinal endotoxemia and affect sperm quality in healthy people by altering the composition of gut microbiota through antimicrobial activity. The finding that parabens exert dual toxicological effects on sperm quality provides novel insights for future research.

Background

The impact of environmental pollution on human reproductive health continues to be a global public health issue and a major concern. Semen quality is an important indicator of male reproductive health and could potentially be used as a biomarker for overall male health [1, 2]. Evidence shows that human semen quality has declined worldwide over the past few decades [3–5], including in China [6, 7]. Exposure to environmental chemicals is thought to be one of the important factors affecting sperm parameters [8, 9].

Parabens (PBs) are widely used as antibacterial preservatives in dietary products, personal care products, cosmetics, and pharmaceuticals [10]. PBs can enter the human body via ingestion, inhalation, and dermal absorption, where they are rapidly metabolized and excreted, mainly in urine [11, 12]. Methyl-(MeP), ethyl-(EtP), propyl-(PrP), butyl-(BuP) PBs and their metabolites PHBA and 3,4-DHB can be detected in urine, blood, semen, breast milk, adipose tissue, and other fluids and tissues of healthy people [13–17]. However, there are large differences in concentration levels in different biological media, such as urine, blood, and semen [16].

PBs have endocrine disrupting activity, particularly estrogenic and antiandrogenic activity [18, 19]. There is significant evidence from animal studies showing that PBs reduced sperm quality through estrogenic activity [20–24]. However, the correlation between PB exposure and total sperm count or sperm motility, whether the PBs are measured in urine [25–29] or semen [30, 31], has not been consistent in population studies. It is well known that seminal fluid can provide a more direct measure of testes internal concentrations than urine [32], but studies of PBs and effects in semen are scarce.

In recent years, the rise of gut microbiota research has brought increased attention to intestinal endotoxemia (IETM), which is a low grade and chronic inflammation characterized by 2–3 times higher than normal levels of intestinal endotoxin (IE) in the blood. The endotoxin is derived from the gut microbiota and is a lipopolysaccharide (LPS) component of the cell walls of Gram-negative bacteria (G−), and is synonymous with LPS. When the composition of intestinal microbiota are changed, the proportion of G − can be increased, producing more LPS [33]. Additionally, the intestinal barrier can be disrupted and its permeability increased, resulting in a "leaky gut", enabling more LPS to enter into the blood [34, 35]. IETM is closely associated with multiple adverse health outcomes [36–38], particularly male hypogonadism [39], increased sperm DNA damage and fragmentation [40], and a decreased sperm concentration and motility [41, 42]. However, these reports were limited to subjects with obesity or infertility, and biomarkers of endotoxemia, such as LPS in the blood, were not directly measured in most of the studies.

Because PBs are antibacterial preservatives, they have the ability to alter gut microbiota [43]. Our previous experiments found that BuP altered the diversity of gut microbiota and increase the proportion of G − in the intestinal tract of rats, and significantly increased LPS in the blood (Supporting Information). Researchers generally believe that PBs are more effective against G + than G− [44]. For example, PBs did not inhibit *P. aeruginosa* (G−) or *E. coli* (G−), but did inhibit *S. aureus* (G+)[45]. Although PBs have estrogenic activity [19], the estrogen receptor alpha binding properties decreased and antimicrobial properties of PBs were enhanced through a 3,5-substitution (the antimicrobial activity to G + *S. aureus* also increased 16-fold) [46]. This suggests that such structural features enable PBs to have dual biological effects on both antibacterial and estrogenic activity. It can be inferred that PBs possibly promote more LPS to release into the blood and lead to IETM by changing the composition of gut microbiota and increasing the intestinal permeability, as well as affecting sperm quality through estrogenic activity. This is an important issue researched in this study.

In this study, we collected blood and semen samples from 315 healthy men and detected LPS in blood, and the levels of PBs and their metabolites in semen. Specifically, in this study, we evaluated the correlation between PB exposure, LPS levels, and total sperm count and motility in healthy men; investigated the contribution to IE from PB exposure; and verified the hypothesis that PBs affect sperm quality through dual antibacterial and estrogenic activity effects. In particular, we provided evidence that IETM induced by PB exposure in a healthy population decreased sperm quality.

Results

Characteristics of study population

The characteristics of the subjects are listed in Table 1. The average age of the study population was 30.8 ± 5.6 years. Body weight (BW) was 71.9 ± 9.1 kg, BMI was 23.2 ± 2.6 kg/m^2, 67.0% of subjects were normal weight, and 31.1% of subjects were overweight. Waist circumference was 85.4 ± 8.3 cm. The majority of subjects were of Han ethnicity, accounting for 84.8%. Most of subjects had college degree (69.8%) and were unmarried (67.9%). Additionally, 17.5% of subjects were smokers and 13.3% of them regularly drank alcohol. All subjects had been local residents for at least 1 year, with a median of 4 years. The annual income of the subjects ranged from 40,000 to 350,000 RMB.

Table 1
Demographics of the study population (n = 315)

Characteristic	Mean ± SD	Median (5th, 95th)	N (%)
Age (y)	30.8 ± 5.6	30.0 (24.0,42.0)	
Height (cm)	175.9 ± 4.8	175.0 (170.0,185.0)	
Body weight (kg)	71.9 ± 9.1	71.0 (58.8,89.2)	
BMI (kg/m^2)	23.2 ± 2.6	22.8 (19.4,27.8)	
lean (< 18.5)			2 (0.6)
normal weight (18.5 ~ 24.9)			211 (67.0)
overweight (25 ~ 29.9)			98 (31.1)
obese (≥ 30.0)			4 (1.3)
Waist circumference (cm)	85.4 ± 8.3	86.0 (71.0,98.0)	
Race			
Han			267 (84.8)
Hui			13 (4.1)
Man			21 (6.7)
others			14 (4.4)
Education level			
Below university level			28 (8.9)
University			220 (69.8)
Graduate			67 (21.3)
Marriage			
No			214 (67.9)
Yes			101 (32.1)
Smoking status			
Non-smoker			260 (82.5)
Current smoker			55 (17.5)
Alcohol drinking status			
Seldom drank			273 (86.7)
Regularly drank			42 (13.3)
Years of local residence [a]		4.0 (1.0,30.2)	
Annual income (10000 ¥)[ab]		10 (4,35)	

Note: BMI, body mass index; SD, standard deviation.

[a] non-normal distribution, given as median and percentile.

[b] missing = 25

Concentration of PBs and metabolites in seminal plasma

In seminal plasma the detection rates (DR) of parent PBs and PHBA were all higher than 95%, except for BuP, which was 56%. 3,4-DHB had the lowest DR at 38%. Table 2 shows a summary of their levels. The concentrations of parent PBs varied greatly. MeP had the highest concentration, and PrP had a higher median concentration than EtP and a wider range. The concentration of BuP was the lowest. The concentration of PHBA was significantly higher than its individual parent PBs and higher than 3,4-DHB.

Concentration of LPS in plasma

All plasma specimens contained LPS (median, 0.063 EU/mL; interquartile range, 0.035–0.123 EU/mL) although with a high variation (Table 2). The LPS concentrations did not conform to a normal distribution, and therefore a natural logarithm conversion was used for further statistical analysis.

Table 2
Analytical results of parabens and their metabolites (ng/mL) in semen and LPS (EU/mL) in plasma (n = 315)

			Percentile						
Parameter	GM	Range	10th	25th	50th	75th	90th	LOD	DR
LPS	0.065	0.021 ~ 0.195	0.030	0.035	0.063	0.123	0.159	0.010	100%
MeP	0.696	<LOD ~ 1355.533	0.099	0.187	0.505	1.551	11.016	0.019	96%
EtP	0.465	0.062 ~ 151.056	0.102	0.124	0.226	1.140	4.642	0.021	100%
PrP	0.553	0.037 ~ 903.158	0.070	0.155	0.494	1.267	7.864	0.016	100%
BuP	0.023	<LOD ~ 0.247	<LOD	<LOD	0.018	0.040	0.071	0.015	56%
PHBA	12.451	2.284 ~ 260.596	6.190	8.411	11.020	15.1240	27.782	0.023	100%
3,4-DHB	1.027	<LOD ~ 25.903	<LOD	<LOD	1.046	2.030	4.293	0.081	38%
Note: GM, geometric mean; LOD, limit of detection; DR, detection rate;									

Semen quality

The semen quality of the subjects in this study all exceeded the lower limit of the WHO (2010) reference standards. As Table 3 shows, the median abstinence time was 5 (2–7) days. The median semen volume was 4.0 (1.5–9.6) mL. The total sperm count was 213.9 (41.6–880.0) × 10^6, the sperm concentration was 56 (15–180) × 10^6/mL, the total motility (TM) was 52.0% (32.0–88.0%), and the rate of normal morphology was 7.0% (4.0–9.0%).

Table 3
Semen parameters (n = 315)

			Percentile				
Parameter	GM	Range (Min ~ Max)	10th	25th	50th	75th	90th
Abstinence (d)	5.3	2.0 ~ 7.0	4.0	5.0	5.0	7.0	7.0
Total count (10^6)	200.9	41.6 ~ 880.0	82.8	114.0	213.9	328.5	474.0
Semen volume (mL)	3.9	1.5 ~ 9.6	2.1	3.0	4.0	5.2	6.6
Concentration (10^6/mL)	51.2	15.0 ~ 180.0	23.0	32.0	56.0	80.0	102.0
PR (%)	49.0	32.0 ~ 85.0	34.0	41.0	51.0	59.0	65.0
TM (%)	50.7	32.0 ~ 88.0	35.0	44.0	52.0	60.0	65.4
IM (%)	46.5	12.0 ~ 68.0	34.0	40.0	48.0	56.0	65.0
nMor (%)	6.3	4.0 ~ 9.0	4.0	5.0	7.0	8.0	9.0
Note: GM, geometric mean; PR, progressive motility; TM, total motility; IM, immotile spermatozoa; nMor, normal morphology.							

Correlation analysis between the analyzed parameters and the general characteristics of subjects

The results of bivariate correlation analysis showed that BW, BMI, and waist circumference were significantly negatively correlated with total sperm count and concentration, while BMI and waist circumference were significantly negatively correlated with PR and TM, and positively correlated with immotile spermatozoa (IM) ($P < 0.05$). The longer the residents lived in the local area where the study was conducted, the higher were the PR and TM ($P < 0.05$, Fig. 1A), a finding which has not been previously reported. BW, BMI, and waist circumference were significantly positively correlated with the level of one or more parent PBs, and BMI was also significantly positively correlated with PHBA ($P < 0.05$). Age was positively correlated with MeP ($P < 0.05$, Fig. 1C). Plasma LPS was significantly positively correlated with age, BW, and BMI, with correlation coefficients (r) of 0.140, 0.149, and 0.125, respectively ($P < 0.05$). These results showed that the concentration of LPS increased with age and BMI. There were no correlations between other subject demographics and the plasma LPS parameter (Fig. 1D). Additional results are shown in Fig. 1A, C, and D.

Correlation between PBs and sperm parameters

There were generally weak to moderate negative correlations between total sperm count, sperm concentration, and parent paraben and metabolites (r: −0.184 to − 0.438), with median concentrations of Mep, EtP, PrP and BuP in semen from 0.018–0.505 ng/mL (Table 2 and Fig. 1B). Sperm motility parameters such as PR, TM, and IM were significantly negatively correlated with MeP, EtP, and PHBA (r: −0.186 to − 0.363). There was a significant negative correlation between PrP and TM (r = − 0.178). After adjusting confounding factors such as age, BW, BMI, waist circumference, smoking, alcohol drinking and abstinence duration, a multiple linear regression was performed, with the results shown in Table 4. Semen quality parameters showed that EtP was associated with most of the parameters, except semen volume and the rate of normal morphology (nMor). A one unit increase in the ln-transformed EtP level was associated with PR (%) (β = −1.328; 95% CI: −2.332 to − 0.323) and TM (%) (β = −1.305; 95% CI: −2.310 to − 0.300). For BuP, significant correlations were only observed with sperm concentration (β = −0.141; 95% CI: −0.220 to − 0.061) and total sperm count (β = −0.153; 95% CI: −0.249 to − 0.057). In addition, PR, TM, and IM were all significantly associated with MeP ($P < 0.05$). With a one unit change of ln-transformed levels of MeP, both PR and TM changed − 1.390 (95% CI: −2.598 to − 0.182) and − 1.341 (95% CI: −2.550 to − 0.132), respectively. Additional results are shown in Table 4.

Table 4
Estimated changes in semen quality parameters associated with 1-unit increase in ln-transformed parabens levels in semen pl

Outcome[a]	MeP β(95%CI)	P-value	EtP β(95%CI)	P-value	PrP β(95%CI)	P-value	BuP β(95%CI)	P-value	PHBA β(95%CI)	P-value	3,4-D β(95'
Semen volume (mL)	0.132 (-0.058,0.322)	0.173	0.026 (-0.132,0.184)	0.749	-0.170 (-0.351,0.011)	0.065	-0.103 (-0.359,0.153)	0.429	-0.050 (-0.433,0.333)	0.796	-0.10 (-0.2
Concentration (10^6/mL)	0.001 (-0.058,0.060)	0.980	-0.149 (-0.198,-0.100)	< 0.001	0.015 (-0.041,0.071)	0.600	-0.141 (-0.220,-0.061)	0.001	0.012 (-0.106,0.131)	0.836	-0.01 (-0.0
Total count (10^6)	0.032 (-0.040,0.103)	0.385	-0.143 (-0.203,-0.084)	< 0.001	-0.024 (-0.092,0.044)	0.486	-0.153 (-0.249,-0.057)	0.002	0.003 (-0.141,0.146)	0.970	-0.03 (-0.1
PR (%)	-1.390 (-2.598,-0.182)	0.024	-1.328 (-2.332,-0.323)	0.010	0.572 (-0.577,1.722)	0.328	0.636 (-2.261,0.990)	0.442	-2.101 (-4.534,0.332)	0.090	0.41 (-0.7
TM (%)	-1.341 (-2.550,-0.132)	0.030	-1.305 (-2.310,-0.300)	0.011	0.496 (-0.654,1.647)	0.396	0.742 (-2.369,0.885)	0.370	-1.917 (-4.352,0.517)	0.122	0.27 (-0.8
IM (%)	1.243 (0.009,2.477)	0.048	1.219 (0.193,2.245)	0.020	-0.424 (-1.599,0.750)	0.477	-0.278 (-1.385,1.941)	0.742	2.170 (-0.315,4.655)	0.087	-0.23 (-1.3
nMor (%)	-0.032 (-0.233,0.169)	0.753	-0.067 (-0.234,0.100)	0.431	-0.022 (-0.214,0.169)	0.817	-0.197 (-0.467,0.074)	0.153	0.283 (-0.122,0.688)	0.170	-0.02 (-0.2

Note: Estimated calculated using linear regression models adjusted for age, body weight, BMI, waist circumference, smoking, alcohol intake and abstinence t motility; IM, immotile spermatozoa; nMor, normal morphology.

[a] Concentration and total count were ln transformed.

Correlation between LPS and sperm parameters

LPS was negatively correlated with sperm concentration (r = − 0.164, P = 0.004), and had no significant correlation with other sperm parameters (Fig. 1F). Multiple linear regression analysis showed that a one unit increase of ln-transformed plasma LPS was associated only with sperm concentration (β = −6.842; 95% confidence interval (CI): −11.930 to − 1.034), as shown in Table 5.

Table 5
Estimated changes in semen quality parameters associated with 1-unit increase in ln-transformed plasma LPS (n = 315)

Outcome [a]	Model				
	β (95%CI)	*P*-value	R^2	F	*P*-value
Semen volume (mL)	0.179(-0.103,0.461)	0.213	0.039	1.571	0.133
Concentration (10^6/mL)	-6.842(-11.930,-1.034)	0.020	0.070	2.901	0.004
Total count (10^6)	-0.078(-0.193,0.036)	0.179	0.052	2.106	0.035
PR (%)	-0.546(-2.466,1.373)	0.576	0.051	2.067	0.039
TM (%)	-0.141(-2.060,1.779)	0.885	0.050	2.018	0.044
IM (%)	-0.177(-2.124,1.770)	0.858	0.050	2.302	0.042
nMor (%)	-0.160(-0.457,0.138)	0.292	0.015	0.601	0.777

Note: Estimated calculation using linear regression models adjusted for age, body weight, BMI, waist circumference, smoking, alcohol intake and abstinence time. PR, progressive motility; TM, total motility; IM, immotile spermatozoa; nMor, normal morphology.

[a] Concentration and total count were ln transformed.

We divided the study population into three groups based on the tertiles of LPS levels in order to better analyze the relationship between LPS and sperm parameters. The LPS tertiles were T1 = 0.039 EU/mL and T2 = 0.104 EU/mL. Therefore, group A1 included participants with plasma LPS concentrations < 0.039 EU/mL (n = 104, mean 0.032 ± 0.004 EU/mL), group A2 participants had plasma LPS concentrations 0.039–0.103 EU/mL (n = 106, mean 0.065 ± 0.019 EU/mL), and group A3 participants had plasma LPS concentrations ≥ 0.104 EU/mL (n = 105, mean 0.142 ± 0.025 EU/mL). Spearman correlations were observed between sperm concentration and LPS in group A2 and group A3, with r = − 0.227 and − 0.481, respectively (P < 0.05). For total sperm count, a significant negative correlation was only observed with LPS in group A3, with r = − 0.428 (P < 0.05, Table 6). The results of regression analysis showed that in group A3, the models for sperm concentration and LPS, and total sperm count and LPS, had statistical significance, R^2 = 0.295 and R^2 = 0.236 (P < 0.01), respectively. LPS had no correlation with other sperm parameters (data not shown). When group A1 was combined with group A2, there were no correlations between sperm concentration, total sperm count, or LPS, but when group A2 was combined with group A3, there were significant correlations (P < 0.05, Table 6). LPS concentrations greater than 0.104 EU/mL significantly correlated with sperm concentration and total sperm count, and may have affected sperm quality. Among the three groups, the mean plasma LPS concentrations between group A3 and group A1 differed by 4.6-fold, and the means between group A3 and group A2 differed by 2.2-fold.

There were statistically significant differences among the three groups in age and BW (Table S1). We found a trend towards higher age and BW with higher LPS levels. With the increase of BMI, LPS tended to increase, but there was no statistically significant difference (P = 0.076).

Table 6
Results of correlation and regression analyze between LPS and semen parameters

Outcome variable	Group A	LPS range (EU/mL)	n	r	LPS		Model		
					β(95%CI)	*P*-value	R^2	F	*P*-value
sperm concentration (10^6/mL)	1	< 0.039	104	-0.013	0.291(-0.597,1.179)	0.517	0.069	0.875	0.540
	2	0.039–0.103	106	-0.227*	-0.267(-0.635,0.102)	0.154	0.108	1.474	0.177
	3	≥ 0.104	105	-0.481#	-1.666(-2.232,-1.099)	< 0.001	0.295	5.020	< 0.001
	1 + 2	< 0.104	210	-0.080	-0.112(-0.299,0.075)	0.240	0.069	1.852	0.069
	2 + 3	≥ 0.039	211	-0.230#	-0.227(-0.391,-0.063)	0.007	0.080	2.198	0.029
Total count (10^6)	1	< 0.039	104	0.099	0.680(-0.400,1.761)	0.214	0.068	0.861	0.552
	2	0.039–0.103	106	-0.020	-0.007(-0.416,0.402)	0.973	0.069	0.900	0.520
	3	≥ 0.104	105	-0.428#	-1.823(-2.560,-1.086)	0.001	0.236	3.704	0.001
	1 + 2	< 0.104	210	-0.094	0.018(-0.200,0.235)	0.872	0.049	1.298	0.246
	2 + 3	≥ 0.039	211	-0.137*	-0.154(-0.350,0.041)	0.120	0.051	1.358	0.217

Note: **P* < 0.05; #*P* < 0.01

Correlation between LPS and PBs

Correlation analysis showed that the concentration of plasma LPS was positively correlated with EtP (r = 0.117, P = 0.037) and BuP (r = 0.247, P < 0.001) in seminal plasma (Fig. 1E). The tertiles of EtP concentration were also calculated as T1 = 0.213 ng/mL and T2 = 0.880 ng/mL. Accordingly, the study participants were divided into three groups based on EtP concentrations; group B1: < 0.213 ng/mL, group B2: 0.213–0.879 ng/mL, and group B3: ≥ 0.880 ng/mL. The correlation between LPS and EtP in each group was analyzed. The results showed a significant correlation between LPS and EtP only in group B3, with EtP greater than 0.880 ng/mL (r = 0.480, P < 0.001, Table 7). The regression equation showed that the relationship was statistically significant for group B3, with the regression coefficient β = 1.139; 95% CI: 0.778–1.500. Next, using the median of BuP, the study participants were divided into group C1: BuP < 0.018 ng/mL, and group C2: BuP ≥ 0.018 ng/mL. Since the levels of BuP in group C1 were generally lower than the LOD, a regression equation between LPS and BuP could not be established. However, if BuP was ≥ 0.018 ng/mL and therefore detectable, a statistically significant regression equation between LPS and BuP could be established. A one-unit increase in the ln-transformed level of BuP was associated with LPS (β = 0.194; P = 0.026; 95% CI: 0.024–0.364, Table 7). Additional non-significant results are not shown.

Table 7
Results of correlation and regression analyze between LPS and parabens

Outcome variable	Group	Range (ng/mL)	n	r	Dependent variable		Model		
					β(95%CI)	P-value	R^2	F	P-value
LPS	B1	EtP < 0.213	105	0.132	0.039(-0.292,0.370)	0.816	0.056	0.822	0.571
	B2	EtP: 0.213–0.879	105	0.130	0.011(-0.104,0.126)	0.852	0.084	1.268	0.274
	B3	EtP ≥ 0.880	105	0.480#	1.139(0.778,1.500)	< 0.001	0.293	5.012	< 0.001
LPS	C1	BuP < 0.018	154	/	/	/	/	/	/
	C2	BuP ≥ 0.018	161	0.234#	0.194(0.024,0.364)	0.026	0.108	2.635	0.013
	C1 + C2	<LOD–max	315	0.247#	0.191(0.098,0.283)	< 0.001	0.085	4.090	< 0.001

Note: **P* < 0.05; #*P* < 0.01

Discussion

To our knowledge, this is the first report to show that exposure to PBs and their metabolites in human semen negatively correlated with sperm parameters. This study is also one of the few healthy population studies on the association between IE and sperm quality. We first proposed that PBs, as exogenous chemicals, were possibly contributing to an increase in IE (LPS) through antibacterial activity. Our results suggest a new pathway for the effects of PBs on male reproductive health; specifically, that PBs likely promote IETM by changing the composition of gut microbiota and intestinal permeability, thus affecting sperm quality.

PB levels in seminal plasma were associated with sperm quality

MeP, EtP, PrP, and BuP are the most commonly used representatives of PBs [10].They are easily transported into the circulation after absorption through the skin or dietary intake [47]. They are rapidly hydrolyzed by nonspecific esterases to PHBA, which is conjugated with sulfate, glucuronic acid, or glycine prior to being excreted, and is mainly eliminated through urine [21]. PHBA and its conjugates are the main PB metabolites and account for about 52.7–63.8% of all PB metabolites[12]. 3,4-DHB is a product of the hydroxylation of PHBA [48]. In this study, we detected the total state (the free state plus the binding state) of parent PBs, PHBA, and 3,4-DHB to evaluate the overall exposure level and the relationship with sperm parameters.

Characteristics of PBs in semen

In this study, the concentration of each parent PB and their metabolites in seminal plasma varied considerably (Table 2). The detection rate and level of MeP, EtP, and PrP were high (96–100%), while those of BuP were the lowest (56%), which was consistent with the previous findings of Buck Louis [30]. This may be because MeP, EtP, and PrP are the most widely used PBs, especially MeP and PrP. However, consistent with other exposure studies, we found that the concentration of PBs in semen was lower than in urine, which also differed from the concentration in blood [16, 17, 86] (Table S2), mainly because urine is the main route of excretion [21]. BuP was detected in seminal plasma at a higher rate than in urine[16, 17, 30, 49] (Table S2), which was consistent with the properties of long-chain esters, with decreased water solubility and increased fat solubility, compared to other short-chain PBs [50]. The concentration of PHBA was far higher than that of the parent PBs, and even higher than their sum (geometric mean (GM) = 12.451, range: 2.284–260.596 ng/mL, Table 2). The concentration of 3,4-DHB was high but only 38% was detected, and it was lower than PHBA. PHBA and 3,4-DHB are the metabolites of PBs, and are also derived from many other sources, such as other precursors of flavonoids and cinnamates, and natural sources (such as wine and berries) [21, 51]. Thus, both PHBA and 3,4-DHB are nonspecific exposure biomarkers. Currently, only four studies that reported PBs in semen were identified [16, 30, 31, 86], but the concentrations of PHBA and 3,4-DHB were not reported. Our study is the first to identify the exposure levels of parent PBs and their metabolites in semen of healthy men.

Relationship between PB exposure and sperm parameters

The results showed that the concentration of four PBs and two metabolites were negatively correlated (Fig. 1B) with total sperm count, sperm concentration, and sperm motility parameters. EtP and MeP had the strongest correlation with sperm motility parameters. EtP and BuP had the strongest correlation with

sperm concentration and total sperm count. Therefore, EtP may have the most important influence on sperm quality. We also found that PHBA was significantly negatively correlated with sperm quantity parameters and motility parameters, and 3,4-DHB was only correlated with sperm concentration and total sperm count (Fig. 1B). To our knowledge, no other similar studies have been reported.

Rat studies have shown that PBs caused male reproductive toxicity through estrogenic or antiandrogenic activity [22, 23, 24, 52], including pathological changes in testis and spermatogenesis disorders. An *in vitro* experiment showed that BuP promoted the generation of human sperm reactive oxygen species, leading to decreased sperm motility and increased apoptosis [53]. At present, only one study explored the relationship between semen PBs and sperm parameters, but no relationship between the two was found [31]. In our study, for the first time, a negative correlation between PBs and metabolites in semen and sperm quality parameters was found in a healthy population, with EtP showing the strongest association with sperm quality parameters.

Semen PB level is more sensitive than urine for assessing sperm quality

It was previously reported that PBs in urine were negatively associated with human sperm concentration and motility rate [25, 27], and positively associated with abnormal morphology, sperm DNA damage [25], and sperm chromosome disomy [26]. The median concentrations of Mep, EtP, PrP, and BuP in urine in these reports were 3.48–15.6 μg/L (ng/mL) [25] and 0.03–6.55 ng/mL [27]; and concentrations of PrP and BuP in urine were 3.82 μg/L and 3.45 μg/L [26], respectively. However, in our study, Mep, EtP, PrP, and BuP median concentrations in semen were 0.018–0.505 ng/mL which was clearly one to two orders of magnitude lower than the concentrations of PBs in urine. This shows that exposure levels are differentially reflected in various biological media [16]. As there were no studies on the relationship between blood PBs levels and sperm quality, we studied the effect of PB exposure on sperm parameters as measured in semen, and found the concentrations lower in semen. Therefore, the levels of PB exposure as measured in semen are more sensitive for evaluating sperm quality.

Association between intestinal endotoxin and sperm quality in healthy men

Healthy people may have endotoxemia [35], which is the presence in the plasma of endotoxin (LPS) derived from the gut. LPS has been identified as a causal or complicating factor in multiple diseases [54], including impaired reproductive health [41, 42, 55].

The measurement of LPS in serum or plasma is the most direct way to quantify endotoxemia. LPS may enter the blood through transmucosal epithelial translocation [56]. It can be detected in serum or plasma under normal physiological conditions [56, 57]. Limulus amoebocyte lysate (LAL) is the gold standard for the detection of LPS in biological samples [58]. In this study, we examined LPS concentrations in the plasma of 315 healthy men by using LAL. The detection rate of LPS was 100%. LPS was generally at a low level, with a range of 0.021–0.195 EU/mL. The mean levels of LPS in the systemic circulation of healthy humans varied over a wide range, according to previous studies. The variation can be related to the method that was used to prepare plasma and serum samples prior to assay, differences among the subjects, or the reagent kit. For example, Nádházi et al. [56] detected the plasma endotoxin level of 116 healthy donors by LAL, which was less than 1 EU/mL (0.01–1.0 EU/mL) in most of the donors. Kallio et al. [59] reported that in a nutrition cohort study of 2452 people, the serum LPS of the control group was 54.2–63.2 pg/mL, equivalent to 0.135–0.157 EU/mL. Gnauck et al. [60] summarized 44 studies that examined endotoxin levels in healthy subjects and found that the overall mean level of systemic endotoxin in healthy subjects ranged from 0.15 to 61 EU/mL, with a median of 0.32 EU/mL. Our results of LPS were lower than the above studies and the range was within the results reported by Nádházi et al. [56] We speculated that this was because the subjects in our study were young and healthy men, and men with infectious diseases were excluded. In general, our results suggested that the LAL assay could be used to effectively detect circulating LPS and may be a more sensitive health indicator for a population. The data presented here is the first reported baseline data on LPS in Chinese healthy men.

The relationship between IE levels and reproductive health effects in the healthy population need to be evaluated. Previous studies have reported that animals treated with bacterial LPS showed testicular dysfunction at multiple levels [61], including steroidogenesis, spermatogenesis, and testicular cells apoptosis [62–64]. LPS *in vitro* disrupted steroidogenesis, induced apoptosis [65], disrupted the expression of the estrogen and androgen receptors [66], and induced oxidative stress of testicular cells [67, 68]. In addition, human and animal studies have shown that metabolic endotoxin associated with high-fat food or obesity could: (1) induce host immune responses, including local inflammation in the epididymis and testis, and produce a large number of pro-inflammatory cytokines (such as IL-1β, TNF-α, and IL-6) that further impaired Leydig cell function and testosterone production [69]; (2) cause germ cell apoptosis and morphological degeneration of testis; (3) upregulate the expression of nuclear factor-κB (NF-kB)/Jun N-terminal kinase (JNK) extracellular signals pathways and disrupt spermatogenesis [70]; and (4) reduce testicular mitochondrial function [42].

In this study, we found that LPS was significantly negatively correlated with sperm concentration and total sperm count, and that the correlation was more significant when LPS was higher than 0.104 EU/mL. Multiple linear regression showed that when LPS was higher than 0.104 EU/mL, the contribution rates of sperm concentration and total sperm count were higher ($R^2 = 0.295$ and $R^2 = 0.236$, respectively) ($P < 0.01$) (Table 5). In addition, the mean LPS concentration in group A2 and group A3 were 2.2- and 4.6-fold higher, respectively, than that of group A1, when LPS was significantly correlated with sperm parameters. In the report of Ding et al. [42], their analysis of 12 healthy and 48 infertile men revealed a strong negative correlation between the combined abundance of G− (*Bacteroides* and *Prevotella*) and sperm motility, as well as a strong positive association between the abundance of *Bacteroides* and the blood endotoxin concentration of patients. They also revealed that high-fat food induced microbiota dysbiosis in mice and that elevated endotoxin was associated with defects in spermatogenesis. Their study confirmed the harmful effects of IE on sperm quality. In our study, we analyzed the data of 315 healthy men and found that higher endotoxin (LPS) in plasma correlated with lower sperm quality, which supported the conclusion of Ding et al. [42] and provided additional significant evidence for population research.

PB exposure was associated with intestinal endotoxemia

There are various reasons for the increase of IE in blood and further promotion of IETM. Although excessive intake of common nutritional factors has been shown to induce IETM [33, 71, 72], we chose to focus on exploring the effects of endotoxemia induced by exogenous chemicals on reproductive health.

PBs are widely used antimicrobial preservatives [73]. Our previous study found that PBs, especially BuP, could increase the proportion of G−/G + in the intestine of rats and the levels of LPS in blood (Supporting Information Table S3; Figures S1−S9). In this study we tested PB exposure levels as measured in seminal plasma from 315 healthy sperm donors, and first analyzed the relationship between PB levels and LPS concentrations. The results showed that the levels of EtP and BuP in seminal plasma were positively correlated with the concentration of LPS in plasma ($P < 0.05$, Fig. 1E), and the higher the EtP level was, the stronger the correlation with LPS ($r = 0.480$, $P < 0.01$, Table 6). When EtP was higher than the upper tertile of 0.880 ng/mL, the regression coefficient for LPS was $\beta = 1.139$, and $R^2 = 0.293$ ($P < 0.05$, Table 6). Similarly, the concentration of BuP was associated with the concentration of LPS. We analyzed the possible reasons. First, PBs have the ability to change the diversity of gut microbiota according to their antibacterial activity. A rat study provided initial evidence that postnatal low-dose exposure to MeP was capable of modifying the gut microbiota in adolescent rats [43]. G+ (such as *Staphylococcus aureus*) are more sensitive to PBs with longer alkyl chains than G− (such as *Escherichia coli*) [44, 74], due to the significant differences in lipid composition of plasma membranes between G + and G − bacteria [75]. When PBs inhibited G+, the abundance of G − increased, and more LPS was released. Second, PBs may increase intestinal permeability. BuP had direct toxicity on human colorectal adenocarcinoma (Caco-2 cells) [45], and our previous animal experiment showed that BuP decreased the expression of the intestinal tight junction protein ZO-1 (Supporting Information Figures S8 and S9), and was speculated to contribute to increased intestinal permeability, which possibly promoted more LPS to leak into circulation.

The results also showed the total concentrations of PHBA and 3,4-DHB had no significant correlation with LPS. Although the growth and metabolism of many microorganisms were inhibited by PHBA [76], there were no specific difference in antibacterial ability against G + and G− [77]. Similarly, 3,4-DHB possessed antibacterial properties towards both G + and G− [78], so it could not influence the composition of gut microbiota.

There have been examples of other antibacterial agents that altered gut microbiota and affected endotoxin levels. It was reported that oral administration of vancomycin, a widely used G + antibiotic, resulted in dysbiosis of gut microbiota, and the absolute number of G + decreased [79, 80], while the proportion of G − increased to compensate [81, 82]. At the same time, fasting plasma LPS [83] drastically increased. Antimicrobial agent triclosan greatly disturbed the homeostasis of gut microbiota, resulting in the overproduction of LPS, and significantly increased intestinal permeability, thus promoting LPS translocation [84].

Based on a literature review, we hypothesized for the first time in a population study that IE (LPS) was associated with exposure levels of parent PBs and was detectable in semen. The above results showed that the LPS level in semen was negatively correlated with sperm concentration and total sperm count. Therefore, it is speculated that EtP and BuP affect sperm quality possibly by promoting an LPS increase through their antibacterial activity.

Limitations

This study has some limitations. First, dietary patterns and nutritious factors have potential effects on blood LPS levels [85]. To reduce the complexity of the research, dietary factors were not investigated. Previous studies showed exposure to exogenous chemicals could change the composition of gut microbiota and increase the level of LPS in blood [38]. Based on the evidence, this research did not analyze gut microbiota. Second, previous studies have shown that IE can pass through the blood-testosterone barrier and cause sperm damage, but population studies on the effects of LPS in blood on human reproduction are still limited, so our first step was to measure LPS in the blood. In the future, LPS should be measured in seminal plasma, and the difference in LPS levels between seminal plasma and blood as well as their toxicity characteristics should be explored so as to provide direct evidence for the effects of IE on sperm parameters. Finally, due to the limits of cross-sectional design, our results cannot conclude causal relationships between IE and sperm quality, between PB exposure and IE, or the definite effects of PB exposure on sperm quality. These need to be further researched in combination with animal or *in vitro* experiments to confirm the causal relationships.

Conclusions

In conclusion, we found that PB exposure is generally reflected in the semen of healthy man. Here, we reported for the first time that PB exposure as detected in semen was associated with sperm quality due to endocrine disrupting activity. We also found that the level of intestinal endotoxin in blood was associated with sperm parameters in healthy men, and PB exposure likely contributed to the increase of endotoxin. At the same time, a hypothesis was tentatively confirmed, that there is an additional pathway by which PBs affect sperm quality; that is, PBs promote IETM and affect sperm quality by altering the composition of gut microbiota and increasing intestinal wall permeability due to their antimicrobial activity. The results of this study showing that PBs have dual toxicological effects on sperm quality provide a novel insight. Further investigation and experiments to verify these findings are necessary.

Methods

Ethics approval

The study was approved by the Ethics Committee of the National Research Institute for Family Planning, China, and the Medical Ethical Review Committee of the National Institute for Occupational Health and Poison Control, China CDC. It was conducted according to the Declaration of Helsinki.

Study population

Young adult men who applied to be sperm donors were recruited at the Human Sperm Bank of the National Research Institute for Family Planning, China between March 2018 and December 2019. All donors signed informed consent during their first visit to the human sperm bank, agreeing that their biological samples and data could be used for scientific research. Under the guidance of well-trained investigators, each participant filled out a questionnaire and the demographics, life habits, and health condition of the volunteers were collected. The donors were Chinese citizen 20–45 years old in good health. Potential donors underwent laboratory testing to exclude individuals at high risk for sexually transmitted infections and genetic diseases. Donors with occupational

exposure to hazardous factors or with semen quality that did not meet the World Health Organization (WHO) range of normality were also excluded. Thus, the final sample size for this study was 315.

Semen collection and analysis

Each participant was instructed to collect ejaculate into a sterile wide-mouthed container by masturbation after 2–7 days of sexual abstinence. Semen samples were analyzed after liquefaction at 37°C within 60 min to minimize the effect of ejaculation-to-analysis delay on sperm motility. The remaining semen was centrifuged at room temperature for 20 min at 300 g. The supernatant was collected and the aliquots were stored at − 80°C until analysis. Semen parameters including volume, sperm concentration, total and progressive motility, and morphology were evaluated following WHO guidelines (2010) to ensure quality control. Normal sperm criteria were defined as a concentration $\geq 15\times10^6$/mL, PR ≥ 32%, TM ≥ 40%, normal sperm morphology forms ≥ 4%, and leukocytes $< 1\times10^6$/mL. Mycoplasma, chlamydia, and bacterial cultures were negative.

Analyses of PBs and metabolites in seminal plasma

The analytical reference standards of MeP, EtP, PrP, BuP, PHBA, 3,4-DHB, and β-glucuronidase from abalone (containing 100,000 U/mL β-glucuronidase and 20,000 U/mL sulfatase) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Formic acid, acetonitrile, ethyl acetate, and methanol (LC-MS grade) were obtained from Thermo Fisher (Shanghai, China). Chlorzoxazone as an internal standard was purchased from National Institutes for Food and Drug Control, China. Ultrapure water was produced by a Millipore Milli-Q water system (Bedford, MA, USA).

Quantification of parent PBs (MeP, EtP, PrP, and BuP) and their metabolites (PHBA and 3,4-DHB) were conducted by ultra-performance liquid chromatography coupled with tandem mass spectroscopy (UPLC-MS/MS) using an ACQUITY I-CLASS chromatograph (Waters, USA) coupled with a Xevo TQ-S micro mass spectrometer (Waters, USA), in combination with the MassLynx software program as previously described [86]. All experiments were performed in negative electrospray ionization mode. Analyte stock solutions (10 mg/mL) of chlorzoxazone were prepared in acetonitrile-ultra pure water (1:1, v/v). The working solutions were prepared using the standard dilution method. After thawing, 200 µL of the aliquots of seminal plasma and 20 µL of the internal standard (10 ng/mL chlorzoxazone) were mixed. The samples were extracted three times with 1.8 mL ethyl acetate. The mixtures were shaken for 60 min and then centrifuged at 12,000 × g for 5 min. The extracts were combined and concentrated to near dryness under a gentle nitrogen stream. To determine the total analytes (free plus conjugated forms), the samples were mixed with 200 µL of β-glucuronidase and incubated at 37°C for 12 h. Then, 10 µL of each sample was injected onto an analytical column (C18, 100 × 2.1 mm, Waters USA). The eluents were 0.1% formic acid in water and 0.1% formic acid in acetonitrile.

The recoveries and precision of the method were validated by spiking a known volume of native standards in pools of seminal plasma. A solvent blank sample, a blank pool sample, and quality control pools spiked with 0.25, 1.5, and 15 ng/mL of the native paraben solution were injected, and this sequence was repeated six times. The accuracy of the method was based on analyte recoveries. The intra- and inter-day precision were assessed by analyzing the blank sample and the QC samples within a single day and from six consecutive days, respectively. The LODs were 0.015–0.081 ng/mL. Calibration curves covering the concentration range from 0.1 to 100.0 ng/mL exhibited satisfactory correlation coefficients ($R^2 > 0.995$).

Detection of LPS in human plasma by limulus test

Blood was collected by venipuncture using heparin sodium vacutainers (BD Biosciences, Franklin Lakes, NJ, USA) between 8 and 10 AM for all participants. Plasma was separated and tested for LPS. Plasma LPS was assayed using the LAL chromogenic end point assay (BIOENDO, Xiamen, China) according to the manufacturer's instructions. The experiment was conducted with pyrogen-free plastic and glassware throughout. Lyophilized LAL is an aqueous extract of circulating amebocytes of Chinese horseshoe crab *(Tachypleus tridentatus*). The lysate contains a cascade of serine protease enzymes (proenzymes), which can be activated by bacterial endotoxin. Endotoxins activate the proenzymes to produce activated enzymes (coagulases), and the latter catalyzes the cleavage of a colorless substrate, releasing a yellow-colored product pNA. The released pNA can be measured photometrically at 405 nm. Alternatively, the above reaction can be stopped by addition of diazo reagents. pNA reacts with nitrite in HCl, and the product then reacts with N-(1-naphthyl)-ethylenediamine (NEDA) to form a diazotized magenta derivative with maximum absorbance at 545 nm. The absorbance is positively correlated with the LPS concentration, from which the LPS concentration was quantified.

Statistical analysis

Statistical analysis was performed using SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were conducted for the distribution of subject demographics, semen parameters, LPS concentrations (endotoxin unit [EU]/mL) in plasma, and parent PBs and metabolites (ng/mL) in seminal plasma. Continuous variables were summarized by arithmetic or GM, standard deviation (SD), and 10th–90th percentiles; categorical variables were given as percentage (%). Chi-square tests were used for comparing inter-group rates. Normality tests were used for the comparison of inter-group means. Analysis of variance was used for data consistent with the normal distribution; otherwise, the Mann-Whitney U test was used. The concentrations of parent PBs and metabolites, semen concentrations, and total sperm count showed skewed distributions, so they were transformed using the natural log (ln). The concentrations below LOD were imputed with the LOD divided by $\sqrt{2}$ before analysis.

Double-variable correlation (Pearson's correlation or Spearman's Rank correlation) was performed to analyze the correlation between subject demographics and semen parameters, concentrations of parent PBs and metabolites, and LPS levels. The cross-correlation between semen parameters, the concentration of PBs, and LPS levels were also analyzed. Heatmaps were created for better visualization of the degree of correlation by using Python V3.7.6. The possible association of semen parameters with each parent PB, PB metabolites, and LPS were further evaluated using multivariable linear regression analysis. Age, BW, BMI, waist circumference, smoking status, alcohol intake, and abstinence days were considered confounding factors [87] and adjusted. The association of LPS with each paraben and metabolite in seminal plasma were also evaluated. All tests were two-sided and values of $P < 0.05$ were considered as statistically significant.

Abbreviations

BMI: Body mass index, BuP: Butylparaben, BW: Body weight, EtP: Ethylparaben, DR: Detection rate, G−: Gram-negative bacteria, G+: Gram-positive bacteria, GM: Geometric mean, IE: Intestinal endotoxin, IETM: Intestinal endotoxemia, IM: Immotile spermatozoa, LAL: Limulus amoebocyte lysate, LOD: Limit of detection, LPS: Lipopolysaccharide, MeP: Methylparaben, nMor: Normal morphology, PBs: Parabens, PR: Progressive motility, PrP: Propylparaben, SD: Standard deviation, TM: Total motility.

Declarations

Acknowledgments

We would like to thank Dr. Longlong Fu and Donghang Li for conducting the survey and for data input and sorting, We also thank Dr. Jianfeng Li and Dr. Yuhang Zhang for their help with data analysis, statistics, and heat map production. We also thank the reviewers for their careful reviews and pertinent comments. We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Author's contributions

LYZ performed the experiments, analyzed the data, and wrote the manuscript. PKS designed the questionnaire and investigation plan. YG, JFX and DY conducted population surveys and sample collection. PHQ performed the experiments. CGD performed instrumental analysis of biological samples. JSH and JH conducted quality control and project management. BC, CGD and GQD provided the funding and idea. XWL supervised and provided population resources. BC conducted study design, conceptualized and edited the manuscript.

Funding

This work was supported by the grants from the National Natural Science Foundation of China (No.30972448) and the Key project of National Center of Occupational Safety and Health, National Health Commission of PRC (No. 2019014). This study was also funded by Study of Diet and Nutrition Assessment and Intervention Technology (No.2020YFC2006300) from Active Health and Aging Technologic Solutions Major Project of National Key R&D Program.

Availability of data and materials

Data that support the findings detailed in this study are available in the supplementary information and this article. Any other source data perceived as pertinent are available, on reasonable request, from the corresponding author.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the National Research Institute for Family Planning, China, and the Medical Ethical Review Committee of the National Institute for Occupational Health and Poison Control, China CDC. It was conducted according to the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]National Institute for Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, No.29 Nanwei Road, Xicheng District, Beijing 100050, China. [2]National Institute for Nutrition and Health, Chinese Center for Disease Control and Prevention, No.27 Nanwei Road, Xicheng District, Beijing 100050, China. [3]Key Laboratory of Male Reproductive Health of NHC, Human Sperm Bank, National Research Institute for Family Planning & WHO Collaborating Center for Research in Human Reproduction, No.12 Dahuisi Road, Haidian District, Beijing 100081, China. [4]National Center for Occupational Safety and Health, NHC, No.27 Shilong North Road, Mentougou District, Beijing, 102308, China. [5]National Research Institute for Family Planning & WHO Collaborating Center for Research in Human Reproduction, No.12 Dahuisi Road, Haidian District, Beijing 100081, China

References

1. Choy JT, Eisenberg ML. Comprehensive men's health and male infertility. Transl Androl Urol. 2020 Mar,9(Suppl 2):S239-S243. doi: 10.21037/tau.2019.08.35. PMID: 32257864, PMCID: PMC7108997.
2. Ferlin A, Garolla A, Ghezzi M, Selice R, Palego P, Caretta N, Di Mambro A, Valente U, De Rocco Ponce M, Dipresa S, Sartori L, Plebani M, Foresta C. Sperm Count and Hypogonadism as Markers of General Male Health. Eur Urol Focus. 2021 Jan,7(1):205-213. doi: 10.1016/j.euf.2019.08.001. Epub 2019 Aug 17. PMID: 31427194.
3. Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. BMJ. 1992 Sep 12,305(6854):609-13. doi: 10.1136/bmj.305.6854.609. PMID: 1393072, PMCID: PMC1883354.

4. Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, Pinotti R, Swan SH. Temporal trends in sperm count: a systematic review and meta-regression analysis. Hum Reprod Update. 2017 Nov 1,23(6):646-659. doi: 10.1093/humupd/dmx022. PMID: 28981654, PMCID: PMC6455044.
5. Sengupta P, Nwagha U, Dutta S, Krajewska-Kulak E, Izuka E. Evidence for decreasing sperm count in African population from 1965 to 2015. Afr Health Sci. 2017 Jun,17(2):418-427. doi: 10.4314/ahs.v17i2.16. PMID: 29062337, PMCID: PMC5637027.
6. Huang C, Li B, Xu K, Liu D, Hu J, Yang Y, Nie H, Fan L, Zhu W. Decline in semen quality among 30,636 young Chinese men from 2001 to 2015. Fertil Steril. 2017 Jan,107(1):83-88.e2. doi: 10.1016/j.fertnstert.2016.09.035. Epub 2016 Oct 25. PMID: 27793371.
7. Yuan HF, Shangguan HF, Zheng Y, Meng TQ, Xiong CL, Guan HT. Decline in semen concentration of healthy Chinese adults: evidence from 9357 participants from 2010 to 2015. Asian J Androl. 2018 Jul-Aug,20(4):379-384. doi: 10.4103/aja.aja_80_17. PMID: 29493550, PMCID: PMC6038153.
8. Mima M, Greenwald D, Ohlander S. Environmental Toxins and Male Fertility. Curr Urol Rep. 2018 May 17,19(7):50. doi: 10.1007/s11934-018-0804-1. PMID: 29774504.
9. Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod. 2001 May,16(5):972-8. doi: 10.1093/humrep/16.5.972. PMID: 11331648.
10. Guo Y, Kannan K. A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure. Environ Sci Technol. 2013 Dec 17,47(24):14442-9. doi: 10.1021/es4042034. Epub 2013 Nov 27. PMID: 24261694.
11. Frederiksen H, Jensen TK, Jørgensen N, Kyhl HB, Husby S, Skakkebæk NE, Main KM, Juul A, Andersson AM. Human urinary excretion of non-persistent environmental chemicals: an overview of Danish data collected between 2006 and 2012. Reproduction. 2014 Mar 4,147(4):555-65. doi: 10.1530/REP-13-0522. PMID: 24395915.
12. Moos RK, Angerer J, Dierkes G, Brüning T, Koch HM. Metabolism and elimination of methyl, iso- and n-butyl paraben in human urine after single oral dosage. Arch Toxicol. 2016 Nov,90(11):2699-2709. doi: 10.1007/s00204-015-1636-0. Epub 2015 Nov 25. PMID: 26608183.
13. Artacho-Cordón F, Arrebola JP, Nielsen O, Hernández P, Skakkebaek NE, Fernández MF, Andersson AM, Olea N, Frederiksen H. Assumed non-persistent environmental chemicals in human adipose tissue, matrix stability and correlation with levels measured in urine and serum. Environ Res. 2017 Jul,156:120-127. doi: 10.1016/j.envres.2017.03.030. Epub 2017 Mar 22. PMID: 28342347.
14. Chen MH, Yu B, Zhang ZF, Ma WL. Occurrence of parabens in outdoor environments: Implications for human exposure assessment. Environ Pollut. 2021 Aug 1,282:117058. doi: 10.1016/j.envpol.2021.117058. Epub 2021 Apr 1. PMID: 33838443
15. Fisher M, MacPherson S, Braun JM, Hauser R, Walker M, Feeley M, Mallick R, Bérubé R, Arbuckle TE. Paraben Concentrations in Maternal Urine and Breast Milk and Its Association with Personal Care Product Use. Environ Sci Technol. 2017 Apr 4,51(7):4009-4017. doi: 10.1021/acs.est.6b04302. Epub 2017 Mar 20. PMID: 28318231.
16. Frederiksen H, Jørgensen N, Andersson AM. Parabens in urine, serum and seminal plasma from healthy Danish men determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS). J Expo Sci Environ Epidemiol. 2011,21(3):262-271. doi:10.1038/jes.2010.6
17. Zhang H, Quan Q, Li X, et al. Occurrence of parabens and their metabolites in the paired urine and blood samples from Chinese university students: Implications on human exposure. Environ Res. 2020a,183:109288. doi:10.1016/j.envres.2020.109288
18. Byford JR, Shaw LE, Drew MG, Pope GS, Sauer MJ, Darbre PD. Oestrogenic activity of parabens in MCF7 human breast cancer cells. J Steroid Biochem Mol Biol. 2002 Jan,80(1):49-60. doi: 10.1016/s0960-0760(01)00174-1. PMID: 11867263.
19. Routledge EJ, Parker J, Odum J, Ashby J, Sumpter JP. Some alkyl hydroxy benzoate preservatives (parabens) are estrogenic. Toxicol Appl Pharmacol. 1998 Nov,153(1):12-9. doi: 10.1006/taap.1998.8544. PMID: 9875295.
20. Golden R, Gandy J, Vollmer G. A review of the endocrine activity of parabens and implications for potential risks to human health. Crit Rev Toxicol. 2005 Jun,35(5):435-58. doi: 10.1080/10408440490920104. PMID: 16097138.
21. Boberg J, Taxvig C, Christiansen S, Hass U. Possible endocrine disrupting effects of parabens and their metabolites. Reprod Toxicol. 2010 Sep,30(2):301-12. doi: 10.1016/j.reprotox.2010.03.011. Epub 2010 Apr 8. PMID: 20381602.
22. Boberg J, Axelstad M, Svingen T, Mandrup K, Christiansen S, Vinggaard AM, Hass U, 2016. Multiple endocrine disrupting effects in rats perinatally exposed to butylparaben. Toxicol. Sci. 152 (1), 244–256. 10.1093/toxsci/kfw079.
23. Zhang L, Ding S, Qiao P, Dong L, Yu M, Wang C, Zhang M, Zhang L, Li Y, Tang N, Chang B. n-butylparaben induces male reproductive disorders via regulation of estradiol and estrogen receptors. J Appl Toxicol. 2016 Sep,26(9):1223-1234. doi: 10.1002/jat.3291. Epub 2016 Feb 17. PMID: 26888303.
24. Zhang L, Dong L, Ding S, Qiao P, Wang C, Zhang M, Zhang L, Du Q, Li Y, Tang N, Chang B. Effects of n-butylparaben on steroidogenesis and spermatogenesis through changed E levels in male rat offspring. Environ Toxicol Pharmacol. 2014 Mar,37(2):705-17. doi: 10.1016/j.etap.2014.01.016. Epub 2014 Feb 12. PMID: 24607685.
25. Jurewicz J, Radwan M, Wielgomas B, Dziewirska E, Karwacka A, Klimowska A, Kałużny P, Radwan P, Bochenek M, Hanke W. Human Semen Quality, Sperm DNA Damage, and the Level of Reproductive Hormones in Relation to Urinary Concentrations of Parabens. J Occup Environ Med. 2017a Nov,59(11):1034-1040. doi: 10.1097/JOM.0000000000001106. PMID: 28692609.
26. Jurewicz J, Radwan M, Wielgomas B, Klimowska A, Kałużny P, Radwan P, Jakubowski L, Hanke W. Environmental exposure to parabens and sperm chromosome disomy. Int J Environ Health Res. 2017b, Oct,27(5):332-343. doi: 10.1080/09603123.2017.1339784. Epub 2017 Jun 13. PMID: 28609180.
27. Smarr MM, Honda M, Kannan K, Chen Z, Kim S, Louis GMB. Male urinary biomarkers of antimicrobial exposure and bi-directional associations with semen quality parameters. Reprod Toxicol. 2018a,77:103-108. doi:10.1016/j.reprotox.2018.02.008
28. Adoamnei E, Mendiola J, Moñino-García M, Vela-Soria F, Iribarne-Durán LM, Fernández MF, Olea N, Jørgensen N, Swan SH, Torres-Cantero AM. Urinary concentrations of parabens and reproductive parameters in young men. Sci Total Environ. 2018 Apr 15,621:201-209. doi: 10.1016/j.scitotenv.2017.11.256. Epub 2017 Nov 24. PMID: 29179076.

29. Nishihama Y, Toshima H, Yoshinaga J, Mizumoto Y, Yoneyama M, Nakajima D, Shiraishi H, Tokuoka S. Paraben exposure and semen quality of Japanese male partners of subfertile couples. Environ Health Prev Med. 2017 Mar 15,22(1):5. doi: 10.1186/s12199-017-0618-7. PMID: 29165110, PMCID: PMC5661912.
30. Buck Louis GM, Smarr MM, Sun L, Chen Z, Honda M, Wang W, Karthikraj R, Weck J, Kannan K. Endocrine disrupting chemicals in seminal plasma and couple fecundity. Environ Res. 2018 May,163:64-70. doi: 10.1016/j.envres.2018.01.028. Epub 2018 Feb 22. PMID: 29426029, PMCID: PMC5878734.
31. Smarr MM, Kannan K, Sun L, Honda M, Wang W, Karthikraj R, Chen Z, Weck J, Buck Louis GM. Preconception seminal plasma concentrations of endocrine disrupting chemicals in relation to semen quality parameters among male partners planning for pregnancy. Environ Res. 2018b Nov,167:78-86. doi: 10.1016/j.envres.2018.07.004. Epub 2018 Jul 4. PMID: 30014899.
32. Vitku J, Sosvorova L, Chlupacova T, Hampl R, Hill M, Sobotka V, Heracek J, Bicikova M, Starka L. Differences in bisphenol A and estrogen levels in the plasma and seminal plasma of men with different degrees of infertility. Physiol Res. 2015,64(Suppl 2):S303-11. doi: 10.33549/physiolres.933090. PMID: 26680493.
33. Kim SJ, Kim SE, Kim AR, Kang S, Park MY, Sung MK. Dietary fat intake and age modulate the composition of the gut microbiota and colonic inflammation in C57BL/6J mice. BMC Microbiol. 2019 Aug 20,19(1):193. doi: 10.1186/s12866-019-1557-9. PMID: 31429703, PMCID: PMC6701133.
34. An L, Wirth U, Koch D, Schirren M, Drefs M, Koliogiannis D, Nieß H, Andrassy J, Guba M, Bazhin AV, Werner J, Kühn F. The Role of Gut-Derived Lipopolysaccharides and the Intestinal Barrier in Fatty Liver Diseases. J Gastrointest Surg. 2021 Nov 3. doi: 10.1007/s11605-021-05188-7. Epub ahead of print. PMID: 34734369.
35. Mohammad S, Thiemermann C. Role of Metabolic Endotoxemia in Systemic Inflammation and Potential Interventions. Front Immunol. 2021,11:594150. Published 2021 Jan 11. doi:10.3389/fimmu.2020.594150
36. Hotamisligil GS. Inflammation and metabolic disorders. Nature. 2006 Dec 14,444(7121):860-7. doi: 10.1038/nature05485. PMID: 17167474.
37. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007 Jul,56(7):1761-72. doi: 10.2337/db06-1491. Epub 2007 Apr 24. PMID: 17456850.
38. Tu P, Chi L, Bodnar W, Zhang Z, Gao B, Bian X, Stewart J, Fry R, Lu K. Gut Microbiome Toxicity: Connecting the Environment and Gut Microbiome-Associated Diseases. Toxics. 2020 Mar 12,8(1):19. doi: 10.3390/toxics8010019. PMID: 32178396, PMCID: PMC7151736.
39. Tremellen K, McPhee N, Pearce K. Metabolic endotoxaemia related inflammation is associated with hypogonadism in overweight men. Basic Clin Androl. 2017 Mar 8,27:5. doi: 10.1186/s12610-017-0049-8. PMID: 28286655, PMCID: PMC5341351.
40. Pearce KL, Hill A, Tremellen KP. Obesity related metabolic endotoxemia is associated with oxidative stress and impaired sperm DNA integrity. Basic Clin Androl. 2019 May 13,29:6. doi: 10.1186/s12610-019-0087-5. PMID: 31114691, PMCID: PMC6513521.
41. Fan W, Xu Y, Liu Y, Zhang Z, Lu L, Ding Z. Obesity or Overweight, a Chronic Inflammatory Status in Male Reproductive System, Leads to Mice and Human Subfertility. Front Physiol. 2018 Jan 4,8:1117. doi: 10.3389/fphys.2017.01117. PMID: 29354072, PMCID: PMC5758580.
42. Ding N, Zhang X, Zhang XD, Jing J, Liu SS, Mu YP, Peng LL, Yan YJ, Xiao GM, Bi XY, Chen H, Li FH, Yao B, Zhao AZ. Impairment of spermatogenesis and sperm motility by the high-fat diet-induced dysbiosis of gut microbes. Gut. 2020 Sep,69(9):1608-1619. doi: 10.1136/gutjnl-2019-319127. Epub 2020 Jan 2. PMID: 31900292, PMCID: PMC7456731.
43. Hu J, Raikhel V, Gopalakrishnan K, Fernandez-Hernandez H, Lambertini L, Manservisi F, Falcioni L, Bua L, Belpoggi F, L Teitelbaum S, Chen J. Effect of postnatal low-dose exposure to environmental chemicals on the gut microbiome in a rodent model. Microbiome. 2016 Jun 14,4(1):26. doi: 10.1186/s40168-016-0173-2. PMID: 27301250, PMCID: PMC4906585.
44. Selvaraj KK, Sivakumar S, Sampath S, Shanmugam G, Sundaresan U, Ramaswamy BR. Paraben resistance in bacteria from sewage treatment plant effluents in India. Water Sci Technol. 2013,68(9):2067-73. doi: 10.2166/wst.2013.447. PMID: 24225110.
45. Nemes D, Kovács R, Nagy F, Mező M, Poczok N, Ujhelyi Z, Pető Á, Fehér P, Fenyvesi F, Váradi J, Vecsernyés M, Bácskay I. Interaction between Different Pharmaceutical Excipients in Liquid Dosage Forms-Assessment of Cytotoxicity and Antimicrobial Activity. Molecules. 2018 Jul 23,23(7):1827. doi: 10.3390/molecules23071827. PMID: 30041418, PMCID: PMC6100184.
46. Bergquist BL, Jefferson KG, Kintz HN, Barber AE, Yeagley AA. Disconnecting the Estrogen Receptor Binding Properties and Antimicrobial Properties of Parabens through 3,5-Substitution. ACS Med Chem Lett. 2017 Dec 15,9(1):51-55. doi: 10.1021/acsmedchemlett.7b00431. PMID: 29348811, PMCID: PMC5767889.
47. Ye X, Tao LJ, Needham LL, Calafat AM. Automated on-line column-switching HPLC-MS/MS method for measuring environmental phenols and parabens in serum. Talanta. 2008 Aug 15,76(4):865-71. doi: 10.1016/j.talanta.2008.04.034. Epub 2008 Apr 24. PMID: 18656671.
48. Wang L, Kannan K. Alkyl protocatechuates as novel urinary biomarkers of exposure to p-hydroxybenzoic acid esters (parabens). Environ Int. 2013 Sep,59:27-32. doi: 10.1016/j.envint.2013.05.001. Epub 2013 Jun 7. PMID: 23747757.
49. Smarr MM, Sundaram R, Honda M, Kannan K, Buck Louis GM. Urinary Concentrations of Parabens and Other Antimicrobial Chemicals and Their Association with Couples' Fecundity. Environ Health Perspect. 2017 Apr,125(4):730-736. doi: 10.1289/EHP189.
50. Tartaglia A, Kabir A, Ulusoy S, Sperandio E, Piccolantonio S, Ulusoy HI, Furton KG, Locatelli M. FPSE-HPLC-PDA analysis of seven paraben residues in human whole blood, plasma, and urine. J Chromatogr B Analyt Technol Biomed Life Sci. 2019 Sep 1,1125:121707. doi: 10.1016/j.jchromb.2019.06.034. Epub 2019 Jul 3. PMID: 31299363.
51. Karthikraj R, Borkar S, Lee S, Kannan K. Parabens and Their Metabolites in Pet Food and Urine from New York State, United States. Environ Sci Technol. 2018 Mar 20,52(6):3727-3737. doi: 10.1021/acs.est.7b05981. Epub 2018 Mar 7. PMID: 29512377.

52. Guerra MT, Sanabria M, Leite GA, Borges CS, Cucielo MS, Anselmo-Franci JA, Foster WG, Kempinas WG. Maternal exposure to butyl paraben impairs testicular structure and sperm quality on male rats. Environ Toxicol. 2017 Apr,32(4):1273-1289. doi: 10.1002/tox.22323. Epub 2016 Jul 22. PMID: 27444704.
53. Li D, Yuan D, Zhang L, Qiao P, Liang X, Chang B. Increase of apoptosis and decrease of sperm motility induced by oxidative stress after exposed to butyl p-hydroxybenzoate. Wei Sheng Yan Jiu. 2017 Mar,46(2):196-200. Chinese. PMID: 29903093.
54. Guerville M, Boudry G. Gastrointestinal and hepatic mechanisms limiting entry and dissemination of lipopolysaccharide into the systemic circulation. Am J Physiol Gastrointest Liver Physiol. 2016 Jul 1,311(1):G1-G15. doi: 10.1152/ajpgi.00098.2016. Epub 2016 May 5. PMID: 27151941.
55. Tremellen K. Gut Endotoxin Leading to a Decline IN Gonadal function (GELDING) - a novel theory for the development of late onset hypogonadism in obese men. Basic Clin Androl. 2016 Jun 22,26:7. doi: 10.1186/s12610-016-0034-7. PMID: 27340554, PMCID: PMC4918028.
56. Nádházi Z, Takáts A, Offenmüller K, Bertók L. Plasma endotoxin level of healthy donors. Acta Microbiol Immunol Hung. 2002,49(1):151-7. doi: 10.1556/AMicr.49.2002.1.15. PMID: 12073822.
57. Selkirk GA, McLellan TM, Wright HE, Rhind SG. Mild endotoxemia, NF-kappaB translocation, and cytokine increase during exertional heat stress in trained and untrained individuals. Am J Physiol Regul Integr Comp Physiol. 2008 Aug,295(2):R611-23. doi: 10.1152/ajpregu.00917.2007. Epub 2008 Jun 18. PMID: 18565834.
58. Munford RS. Endotoxemia-menace, marker, or mistake? J Leukoc Biol. 2016 Oct,100(4):687-698. doi: 10.1189/jlb.3RU0316-151R. Epub 2016 Jul 14. PMID: 27418356, PMCID: PMC5014740.
59. Kallio KA, Hätönen KA, Lehto M, Salomaa V, Männistö S, Pussinen PJ. Endotoxemia, nutrition, and cardiometabolic disorders. Acta Diabetol. 2015 Apr,52(2):395-404. doi: 10.1007/s00592-014-0662-3. Epub 2014 Oct 19. PMID: 25326898.
60. Gnauck A, Lentle RG, Kruger MC. Chasing a ghost?--Issues with the determination of circulating levels of endotoxin in human blood. Crit Rev Clin Lab Sci. 2016,53(3):197-215. doi: 10.3109/10408363.2015.1123215. Epub 2016 Jan 6. PMID: 26732012.
61. O'Bryan MK, Schlatt S, Phillips DJ, de Kretser DM, Hedger MP. Bacterial lipopolysaccharide-induced inflammation compromises testicular function at multiple levels in vivo. Endocrinology. 2000 Jan,141(1):238-46. doi: 10.1210/endo.141.1.7240. PMID: 10614644.
62. Izvolskaia MS, Tillet Y, Sharova VS, Voronova SN, Zakharova LA. Disruptions in the hypothalamic-pituitary-gonadal axis in rat offspring following prenatal maternal exposure to lipopolysaccharide. Stress. 2016,19(2):198-205. doi: 10.3109/10253890.2016.1149695. Epub 2016 Mar 3. PMID: 26941006.
63. Sadasivam M, Ramatchandirin B, Ayyanar A, Prahalathan C. Bacterial lipopolysaccharide differently modulates steroidogenic enzymes gene expressions in the brain and testis in rats. Neurosci Res. 2014 Jun,83:81-8. doi: 10.1016/j.neures.2014.02.011. Epub 2014 Mar 1. PMID: 24594480.
64. Wang H, Yang LL, Hu YF, Wang BW, Huang YY, Zhang C, Chen YH, Xu DX. Maternal LPS exposure during pregnancy impairs testicular development, steroidogenesis and spermatogenesis in male offspring. PLoS One. 2014 Sep 25,9(9):e106786. doi: 10.1371/journal.pone.0106786. PMID: 25255222, PMCID: PMC4177809.
65. Ramatchandirin B, Sadasivam M, Kannan A, Prahalathan C. Sirtuin 4 Regulates Lipopolysaccharide Mediated Leydig Cell Dysfunction. J Cell Biochem. 2016 Apr,117(4):904-16. doi: 10.1002/jcb.25374. Epub 2015 Sep 22. PMID: 26365714.
66. Wang G, Cheng S, Zhang S, Zhu Y, Xiao Y, Ju L. LPS impairs steroidogenesis and ROS metabolism and induces PPAR transcriptional activity to disturb estrogen/androgen receptor expression in testicular cells. Mol Biol Rep. 2020 Feb,47(2):1045-1056. doi: 10.1007/s11033-019-05196-6. Epub 2019 Nov 18. PMID: 31741264.
67. Hu W, Shi L, Li MY, Zhou PH, Qiu B, Yin K, Zhang HH, Gao Y, Kang R, Qin SL, Ning JZ, Wang W, Zhang LJ. Adrenomedullin protects Leydig cells against lipopolysaccharide-induced oxidative stress and inflammatory reaction via MAPK/NF-κB signalling pathways. Sci Rep. 2017 Nov 28,7(1):16479. doi: 10.1038/s41598-017-16008-x. PMID: 29184072, PMCID: PMC5705677.
68. Metukuri MR, Reddy CM, Reddy PR, Reddanna P. Bacterial LPS-mediated acute inflammation-induced spermatogenic failure in rats: role of stress response proteins and mitochondrial dysfunction. Inflammation. 2010 Aug,33(4):235-43. doi: 10.1007/s10753-009-9177-4. PMID: 20087639.
69. Tremellen K, McPhee N, Pearce K, Benson S, Schedlowski M, Engler H. Endotoxin-initiated inflammation reduces testosterone production in men of reproductive age. Am J Physiol Endocrinol Metab. 2018 Mar 1,314(3):E206-E213. doi: 10.1152/ajpendo.00279.2017. Epub 2017 Nov 28. PMID: 29183872, PMCID: PMC5899218.
70. Leisegang K, Henkel R, Agarwal A. Obesity and metabolic syndrome associated with systemic inflammation and the impact on the male reproductive system. Am J Reprod Immunol. 2019,82(5):e13178. doi:10.1111/aji.13178
71. Zhou Y, Wei Y, Yan B, Zhao S, Zhou X. Regulation of tartary buckwheat-resistant starch on intestinal microflora in mice fed with high-fat diet. Food Sci Nutr. 2020 May 12,8(7):3243-3251. doi: 10.1002/fsn3.1601. PMID: 32724589, PMCID: PMC7382121
72. Zhang Y, Liang XF, He S, Chen X, Wang J, Li J, Zhu Q, Zhang Z, Li L, Alam MS. Effects of High Carbohydrate Diet-Modulated Microbiota on Gut Health in Chinese Perch. Front Microbiol. 2020b Sep 15,11:575102. doi: 10.3389/fmicb.2020.575102. PMID: 33042089, PMCID: PMC7523390.
73. Nowak K, Ratajczak-Wrona W, Górska M, Jabłońska E. Parabens and their effects on the endocrine system. Mol Cell Endocrinol. 2018 Oct 15,474:238-251. doi: 10.1016/j.mce.2018.03.014. Epub 2018 Mar 27. PMID: 29596967.
74. Loeffler M, Schwab V, Terjung N, Weiss J, McClements DJ. Influence of Protein Type on the Antimicrobial Activity of LAE Alone or in Combination with Methylparaben. Foods. 2020 Mar 2,9(3):270. doi: 10.3390/foods9030270. PMID: 32131440, PMCID: PMC7143257.
75. Flasiński M, Kowal S, Broniatowski M, Wydro P. Influence of Parabens on Bacteria and Fungi Cellular Membranes: Studies in Model Two-Dimensional Lipid Systems. J Phys Chem B. 2018,122(8):2332-2340. doi:10.1021/acs.jpcb.7b10152
76. Cho JY, Moon JH, Seong KY, Park KH. Antimicrobial activity of 4-hydroxybenzoic acid and trans 4-hydroxycinnamic acid isolated and identified from rice hull. Biosci Biotechnol Biochem 1998,62(11): 2273–2276. https://doi.org/10.1271/bbb.62.2273

77. Gutiérrez S, Morán A, Martínez-Blanco H, Ferrero MA, Rodríguez-Aparicio LB. The Usefulness of Non-Toxic Plant Metabolites in the Control of Bacterial Proliferation. Probiotics Antimicrob Proteins. 2017 Sep,9(3):323-333. doi: 10.1007/s12602-017-9259-9. PMID: 28357646.
78. Kalinowska M, Gołębiewska E, Świderski G, Męczyńska-Wielgosz S, Lewandowska H, Pietryczuk A, Cudowski A, Astel A, Świsłocka R, Samsonowicz M, Złowodzka AB, Priebe W, Lewandowski W. Plant-Derived and Dietary Hydroxybenzoic Acids-A Comprehensive Study of Structural, Anti-/Pro-Oxidant, Lipophilic, Antimicrobial, and Cytotoxic Activity in MDA-MB-231 and MCF-7 Cell Lines. Nutrients. 2021 Sep 4,13(9):3107. doi: 10.3390/nu13093107. PMID: 34578985, PMCID: PMC8466373.
79. Chae S, Kim DJ, Cho JY. Complex influences of gut microbiome metabolism on various drug responses. Transl Clin Pharmacol. 2020 Mar,28(1):7-16. doi: 10.12793/tcp.2020.28.e3. Epub 2020 Mar 30. PMID: 32274377, PMCID: PMC7136083.
80. Sunwoo J, Ji SC, Kim AH, Yu KS, Cho JY, Jang IJ, Lee S. Impact of Vancomycin-Induced Changes in the Intestinal Microbiota on the Pharmacokinetics of Simvastatin. Clin Transl Sci. 2020 Jul,13(4):752-760. doi: 10.1111/cts.12761. Epub 2020 Mar 29. PMID: 32058642, PMCID: PMC7359932.
81. Vrieze A, Out C, Fuentes S, Jonker L, Reuling I, Kootte RS, van Nood E, Holleman F, Knaapen M, Romijn JA, Soeters MR, Blaak EE, Dallinga-Thie GM, Reijnders D, Ackermans MT, Serlie MJ, Knop FK, Holst JJ, van der Ley C, Kema IP, Zoetendal EG, de Vos WM, Hoekstra JB, Stroes ES, Groen AK, Nieuwdorp M. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. J Hepatol. 2014 Apr,60(4):824-31. doi: 10.1016/j.jhep.2013.11.034. Epub 2013 Dec 6. PMID: 24316517.
82. Kim AH, Lee Y, Kim E, Ji SC, Chung JY, Cho JY. Assessment of Oral Vancomycin-Induced Alterations in Gut Bacterial Microbiota and Metabolome of Healthy Men. Front Cell Infect Microbiol. 2021 May 27,11:629438. doi: 10.3389/fcimb.2021.629438. PMID: 34123865, PMCID: PMC8190408.
83. Bakker GJ, Schnitzler JG, Bekkering S, de Clercq NC, Koopen AM, Hartstra AV, Meessen ECE, Scheithauer TP, Winkelmeijer M, Dallinga-Thie GM, Cani PD, Kemper EM, Soeters MR, Kroon J, Groen AK, van Raalte DH, Herrema H, Nieuwdorp M. Oral vancomycin treatment does not alter markers of postprandial inflammation in lean and obese subjects. Physiol Rep. 2019 Aug,7(16):e14199. doi: 10.14814/phy2.14199. PMID: 31423751, PMCID: PMC6698488.
84. Zhang P, Zheng L, Duan Y, Gao Y, Gao H, Mao D, Luo Y. Gut microbiota exaggerates triclosan-induced liver injury via gut-liver axis. J Hazard Mater. 2022 Jan 5,421:126707. doi: 10.1016/j.jhazmat.2021.126707. Epub 2021 Jul 21. PMID: 34315018.
85. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes. 2008 Jun,57(6):1470-81. doi: 10.2337/db07-1403. Epub 2008 Feb 27. PMID: 18305141.
86. Yu L, Peng F, Yuan D, Zhang L, Guo Y, Chang B, Shi X, Ding C, Liang X. Correlation study of parabens in urine, serum, and seminal plasma of adult men in Beijing, China. Environ Sci Pollut Res Int. 2021 Aug,28(30):41120-41126. doi: 10.1007/s11356-021-13625-y. Epub 2021 Mar 28. PMID: 33774781.
87. Pan Y, Cui Q, Wang J, Sheng N, Jing J, Yao B, Dai J. Profiles of Emerging and Legacy Per-/Polyfluoroalkyl Substances in Matched Serum and Semen Samples: New Implications for Human Semen Quality. Environ Health Perspect. 2019 Dec,127(12):127005. doi: 10.1289/EHP4431. Epub 2019 Dec 16. PMID: 31841032, PMCID: PMC6957285.

Figures

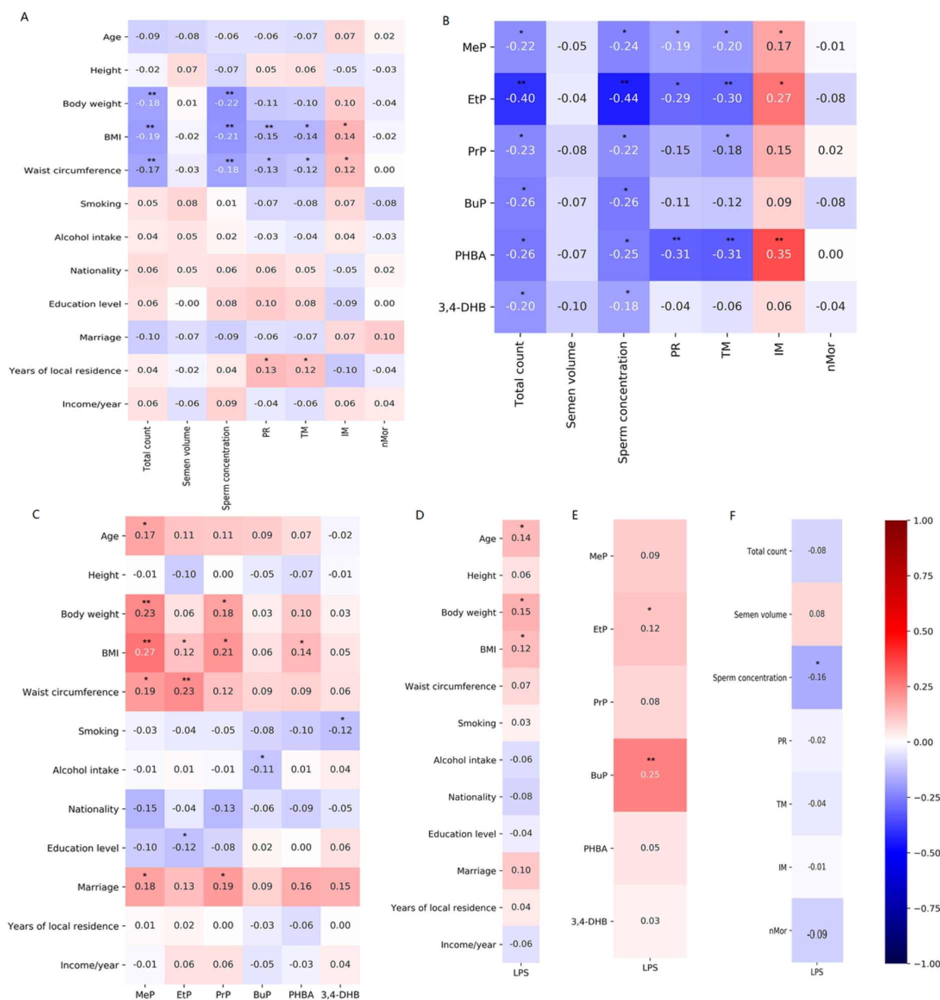

Figure 1

Heatmap for the correlation coefficient of bivariate correlation analysis among 315 healthy men from Beijing, China. Figure 1A, C, D. showed the results of correlation analysis between semen parameters, the concentration of PBs in semen, the concentration of LPS in plasma and the general characteristic of subjects, respectively. Figure 1B. showed the results of correlation between PBs and semen parameters. Figure 1E. showed the results of correlation between LPS and PBs. Figure 1F. showed the results of correlation between LPS and semen parameters. Red: positive correlation. Blue: negative correlation. Abbreviations: BMI, body mass index, PR, progressive motility, TM, total motility, IM, immotile spermatozoa, nMor, normal morphology, MeP: methylparaben, EtP: ethylparaben, PrP: propylparaben, BuP: butylparaben, PHBA: *p*-hydroxybenzoic acid, 3,4-DHB: 3,4-dihydroxybenzoic acid, LPS: lipopolysaccharide. *p-value <0.05. **p-value <0.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.docx