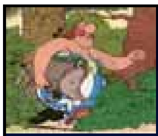
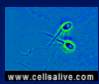


Is there an external influence

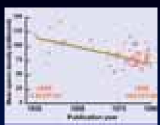
on sperm quality ?



Pr Rachel LEVY

Histologie Embryologie Cytogénétique CECOS, CHU Jean Verdier, Bondy
UMR Inserm U557 / Inra /Cnam /Paris 13, " épidémiologie nutritionnelle " - Pr. S. Hercberg

Fertility decline : myth or reality ?

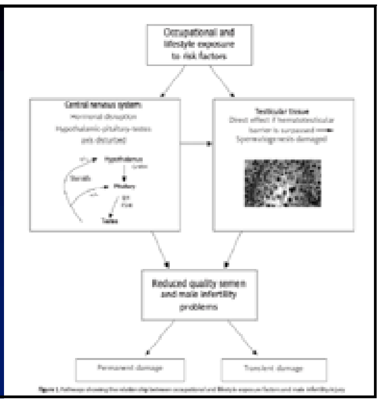


- 50 % drop of semen concentration between 1940 and 1990 (from 113 to 66 Millions/ml)

Carlsen, 1992; Swan 2000

- Controversy : geography, season, ethnic, environmental and lifestyle factors

Auger et al., 2001; Jouannet et al., 2001; Swan, 2003



Mendiola et al., 2009

External factors

Microscopic level :
- ❑Concentration
- ❑Motility
- ❑Morphology

Molecular level :
- ❑Aneuploïdy
- ❑Oxidative stress
- ❑DNA fragmentation

- ❑Drugs
- ❑Endocrine disruptors (EDs)
- ❑Smoking
- ❑Alcohol
- ❑Recreational drugs
- ❑Genital heat stress
- ❑Psychological stress
- ❑Cellular telephone use
- ❑ Weight and nutrition
- ❑....

Agents affecting the hypothalamic-pituitary-testicular axis

GnRH analogues	Leuprolide Goserelin	Azoospermia Impotence, erectile dysfunction
Androgens	Testosterone Oxandrolone Stanazolol	Azoospermia or oligospermia
Antiandrogens	Spironolactone Cimetidine Ketoconazole	Decreased spermatogenesis, testosterone deficiency, gynecomastia
5α-Reductase inhibitors	Finasteride Dutasteride	Decreased spermatogenesis (severe in 5%)
Opiates	Morphine Methadone Fentanyl	Azoospermia Impotence, erectile dysfunction
Antipsychotics	Thorazine Lithium	Elevated prolactin, testosterone deficiency, decreased spermatogenesis
Antidepressants	Fluoxetine Paroxetine	Decreased libido Anejaculation

Agents causing testicular or spermatic toxicity

Chemotherapy and radiation	Cyclophosphamide Melphalan Cisplatin Busulfan Chlorambucil Procarbazine	Azoospermia or oligospermia
Antihypertensives	Nifedipine	Possible decrease in fertility
Anti-inflammatories	Sulfasalazine Colchicine	Decreased spermatogenesis
Environmental exposure/ pesticides	Lead Cadmium Boron DBCP	Decreased spermatogenesis

Disomy and diploidy frequencies in post cisplatin, etoposide and bleomycin chemotherapy (PEB-CT) patients and in controls (De Mas, 2001)

	Post PEB-CT patients						Controls					
%	T	P	S	G	B	Mean (%)	Mean (%)	1	2	3	4	5
disomy 7	0.10	0.05	0.04	0.11	0.05	0.07	0.044	0.09	0.03	0.02	0.05	0.03
disomy 16	0.10	0.10	0.08	0.09	0.08	0.09*	0.046	0.04	0.04	0.06	0.03	0.06
disomy 18	0.06	0.04	0.03	0.06	0.03	0.044**	0.014	0.01	0.00	0.01	0.02	0.03
disomy X	0.02	0.02	0.03	0.03	0.04	0.028	0.030	0.01	0.05	0.04	0.03	0.02
disomy Y	0.01	0.04	0.02	0.07	0.02	0.032	0.008	0.00	0.00	0.01	0.01	0.02
disomy XY	0.14	0.13	0.15	0.27	0.24	**0.186*****	0.072	0.07	0.08	0.07	0.05	0.09
Diploidy[a]	0.29	0.29	0.16	0.11	0.42	**0.254*****	0.094	0.10	0.06	0.10	0.09	0.12
Diploidy[b]	0.49	0.24	0.16	0.20	0.28	**0.274*****	0.080	0.07	0.04	0.06	0.13	0.10

Values are expressed as percentages.
[A] Chromosomes 7 and 16 two-colour FISH. [B] Chromosomes X, Y and 18 three-colour FISH.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

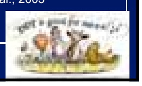
DIETHYLSTILBESTROL AND SEMEN QUALITY

Authors	Cohort	DES+/DES-	Cryptorchidism (DES+/DES-)	Other genital abnormalities (DES+/DES-)	Semen quality (DES+/DES-)
Bibbo (1977)	Dieckmann Cohort	163/168	NA	25,1%/6,5% **(p<0,005)**	28%/0% **(p<0,05)** 26%/0% **(p<0,01)**
Andonian (1979)	SF Bay area cohort	24/24	NA	13%/8%	17%/20% 9%/17%
Gill (1979)	Dieckmann cohort	308/307	5,5%/0,3% **(p<0,005)**	31,5%/7,8% **(p<0,005)**	18%/8% **(p<0,05)** 20%/8%
Shy (1984)	Seattle cohort	51/29	8%/0% **(p=0,07)**	35%/4% **(p=0,0006)**	21%/0% **(p<0,025)**

Human semen quality and EDCs

	Effects	Authors
organochlorine compounds	↘ SC	Dallinga et al., 2002
organochlorine compounds (PCBs et p,p' DDE)	↘ SQ	Hauser et al., 2002
organochlorine compounds (CB-153)	↘ SM (CASA)	Richthoff et al., 2003
organochlorine compounds	↗ Testicular cancer	Hardell et al., 2003
organochlorine compounds (CB-153 et p,p' DDE)	No change	Rignell-Hydbom et al., 2004
Pesticides	↘ SC	Abell et al., 2000
Pesticides	↘ SQ	Swan et al., 2003
Phthalates	↘ SC ↘ SM	Duty et al., 2003

SQ : semen quality, SC : sperm count; SM : sperm motility



Environmental exposure to phthalates and DNA damage in human sperm using the neutral comet assay

- Population without identified sources of exposure to phthalates
- 168 men recruited : semen + urine sample
- 8 phthalate metabolites measured in urine by using HPLC and mass spectrometry
- COMET ASSAY

Table 3. Adjusted regression coefficients for a change in comet assay parameters associated with an IQR increase in phthalate monoester levels[a] (n = 141).

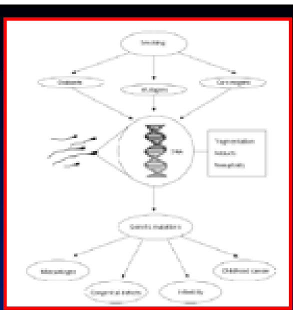
p=0,015

Phthalate monoester	IQR (ng/mL)	Coefficients[b] for comet assay parameters[c]: Comet extent (95% CI)	Tail% (95% CI)	TDM (95% CI)
MEP	443	3.61 (0.74 to 6.47)*	−0.17 (−0.81 to 0.47)	1.17 (−0.05 to 2.38)
MBzP	11.7	2.45 (−1.07 to 5.97)	0.05 (−0.07 to 0.62)	1.06 (−0.42 to 2.54)
MBP	20.3	−0.31 (−0.80 to 0.19)	−0.02 (−0.13 to 0.09)	−0.12 (−0.32 to 0.09)
MEHP	11.9	−0.19 (−1.54 to 1.16)	−0.01 (−0.30 to 0.29)	0.08 (−0.48 to 0.65)
MMP	9.3	2.20 (−1.51 to 5.90)	−0.12 (−1.24 to 0.40)	0.93 (−0.62 to 2.49)

The first human data to demonstrate that urinary MonoEthyl Phthalate, at environmental levels, is associated with increased DNA damage in sperm

Duty et al., 2003

NO SMOKING



Microscopic level :

- ❑Sperm concentration
- ❑Sperm motility
- ❑Sperm morphology

Molecular level :

- ❑Sperm aneuploïdy
- ❑Seminal oxidative stress
- ❑Sperm plasma membrane phospholipids asymmetry
- ❑Sperm DNA fragmentation

Smoking

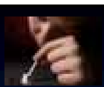
NO SMOKING

Maternal smoking :

- ❑Adverse and irreversible effect on semen quality in male descendants
- ❑Higher risk of birth defects and childhood cancer

Alcohol

- *In vivo*, sperm alteration, not related to nutritional or hepatic status
  - Morphology, motility, concentration ….
  - Maturation arrest, SCOS Pajarinen et al. 1994
  - Progressive alcohol-induced sperm alterations resulting in spermatogenic arrest reversible after alcohol withdrawal Vicari et al., 2002; Mills et al. 2007 ; Sermondade et al., 2009
- *In vitro*, reduction of sperm motility and morphology, with a dose-related response
- Increased risk for XY sperm aneuploïdy
- Possible synergistic effect of alcohol and smoking



Recreational drug use

- Cocaine

In vivo : association of cocaine use and sperm concentration, motility and morphology

Bracken et al., 1990

In vitro : a decrease in straight line velocity and linearity

Yelian et al., 1994

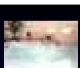
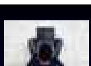
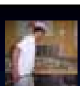
- Cannabis :

In vitro : reduced sperm progressive motility and acrosome reaction

Whan et al., 2006

In vivo : potent inhibitor of mitochondrial 02 consumption in human sperm

Badawy et al., 2009

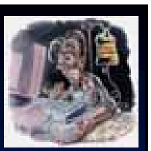




Genital heat stress

- Heat exposure reduces sperm quality Hjollund et al., 2000 and 2002; Jung et al., 2007
- Sedentary postures increases scrotal temperature :
  - Car drivers Bujan et al., 2000
  - Heated floor Song et al., 2006
  - Wet heat (Jacuzzi or hot baths) Shefi et al., 2007
  - Laptop computer users Sheynkin et al., 2005

REVERSIBLE

Psychological stress



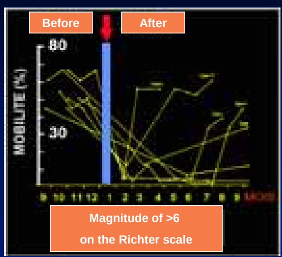
- General population :

Small effect Hjollund et al., 2004

- Infertile couples :

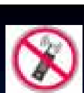
Weak association between psychological factors and impaired semen quality Zorn et al., 2008

Kobe earthquake and reduced sperm motility



Fukuda et al., 1996

Cellular telephone use

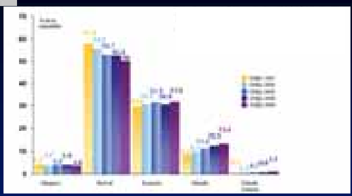


- Escalating concerns about the adverse effects of cell phones on human health and male reproductive system
- Negative effects on sperm parameters of prolonged used of cell phones : sperm count, motility, viability and morphology

Fejes et al., 2005, Wdowlak et al., 2007, Agarwal et al., 2008

- Impact of radiofrequency electromagnetic waves on semen quality ?

Weight and nutritional habits

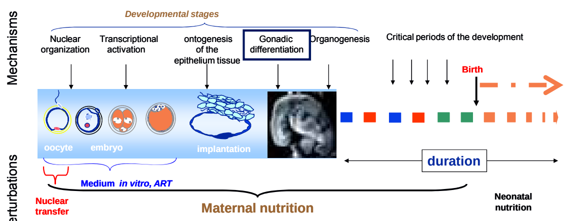


❑ France :

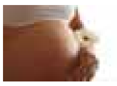
- 67% M 35-74 years overweight with 20.6%>30
- 54% M 35-44 years overweight or obese
- 3% (1965) to 16% (2000) children overweight or obese

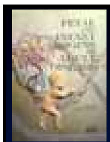
Monitoring national du risque artériel, MONA LISA 2005-2007

Nutritional imprinting



From Pascale Chavatte-Palmer, INRA





DEVELOPMENTAL ORIGIN OF ADULT DISEASE

Barker's hypothesis (1980)

Maternal Malnutrition | Placental abnormality | *Stress in utero*

⇓

Restriction in the foetal growth

⇓

Structure and/or altered function
(ß cells, muscle, adipocytes, kidney, ….)

Predictive adaptive response

Obesity
Ageing

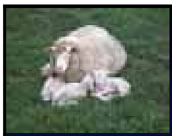
⇓

Infertility | CV Disease | Type 2 diabetes | Hypertension

SERTOLI

METABOLIC SYNDROME

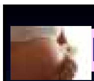
From Pascale Chavatte-Palmer, INRA, 2009



Low maternal nutrition during pregnancy reduces the number of Sertoli cells in the newborn lamb

« the number of Sertoli cells : 43.0 +/- 2.5 for HighME v. LowME 34.5 +/- 2.0 x 10^8 ; P=0.018) per testis »

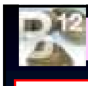
Martin et al. 2002



MATERNAL OBESITY

Is maternal obesity related to semen quality in the male offspring? A pilot study

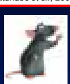
❑Danish study of 1984-87 : 347/5109 sons in 2006



MATERNAL DIET

Maternal vitamin B12 deficiency affects spermatogenesis at the embryonic and immature stages in rat

Watanabe et al., 2003; 2007

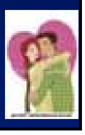


- ❑ Germinal cells of the embryo
- ❑ Sperm : OAT
- ❑ Reversibility ?

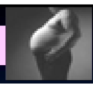
ANDROLOGIA

Prevalence of low serum cobalamin in infertile couples

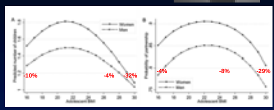
- ❑ 35.5% of 172 M and 23.3% 223 F
- ❑ >43.3% of couples
- ❑ 39% of M with OAT



BMI IN ADOLESCENCE



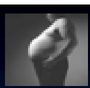
Body Mass Index in Adolescence and Number of Children in Adulthood



- 583 teenagers from 1980 (12, 15, 18 years) to 2001 (33, 36 et 39 years)

Lower fertility associated with obesity and underweight: the US National Longitudinal Survey of Youth

- 6091 young americans recorded from 17-24 years in 1981 to 2004 (47 years)



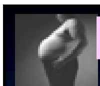
BMI AND FERTILITY

- Retrospective epidemiological studies
  - USA : 1329 couples, 1/4 infertile
    - dose-response curve BMI and infertility OR = 1.12
    - Cut-off BMI>32

    Sallmen et al. 2006
  - Denmark: 47835 couples with living birth
    - dose-response curve BMI et hypofertility, overweight OR=1.15, obesity OR=1.49

    Ramlau-Hansen et al. 2007
  - Norway: 26303 couples with pregnancy
    - dose-response curve BMI et hypofertility, overweight OR=1.19, obesity OR=1.36
    - Cut-off BMI>35

    Nguyen et al. 2007



BMI AND SPERM QUALITY

A controversial issue !!

	Population	N	Results
Jensen et al. 2004	**Healthy males** (military service)	1558	BMI>25 : ↘[sperm]
Fejes et al. 2005	Male from infertile couples	81	↘[sperm] and mobility correlated to weight, TT, TH
Magnusdottir et al. 2005	Male from infertile couples	72	3x more obese male in case of infertility factor
Koloszar et al. 2005	Male from infertile couples **With sperm N**	274	↘[sperm] if BMI>30
Kort et al. 2006	Male from infertile couples	520	↘mobility and ↗ DNA fragmentation with BMI
Hammoud et al. 2008	Male from infertile couples	526	More OAT related to obesity
Chavarro et al., 2009	Male from infertile couples	483	↘[sperm] if BMI>35 et ↗ DNA fragmentation
Pauli et al. 2008	**Fertiles + infertiles**	87	No impact
Aggerholm et al. 2008	**Fertiles + infertiles**	2139	No impact
Li et al. 2009	**Healthy males**	1346	No impact

BMI AND SPERM QUALITY

TABLE 2

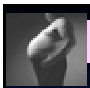
Slope of BMI and *P* value of linear multivariate regressions on seminal parameters from patients attending an andrology laboratory.

Seminal parameters	Normal (n = 251)	Overweight (n = 388)	Obese (n = 155)	BMI (slope)	P value
Seminal volume (mL)	3.2 ± 0.1	3.1 ± 0.1	3.1 ± 0.1	−0.01	0.526
Sperm concentration (× 10^6/mL)	43.7 ± 1.9	44.2 ± 1.8	43.0 ± 3.2	−0.45	0.162
Motility (% of total motile spermatozoa)	51.4 ± 1.2	50.2 ± 1.0	46.6 ± 1.7	−0.49	0.007
Rapid motility (% of rapid spermatozoa)	39.8 ± 1.2	38.8 ± 0.9	35.9 ± 1.6	−0.41	0.019
Viability (% of dead spermatozoa)	16.9 ± 0.6	17.8 ± 0.5	19.0 ± 1.0	0.10	0.321
Kruger's morphology (% of normal spermatozoa)	8.3 ± 0.4	8.4 ± 0.3	8.7 ± 0.5	0.001	0.973
OMS morphology (% of normal spermatozoa)	19.3 ± 0.7	19.7 ± 0.6	20.5 ± 1.0	0.06	0.552
HOS (% of reactive spermatozoa)	79.3 ± 0.9	78.1 ± 0.8	76.1 ± 1.7	−0.16	0.306
Nuclear maturity (% of mature nuclei sperm)	66.9 ± 1.2	66.8 ± 1.0	66.7 ± 1.5	−0.03	0.886
Alpha-glucosidase (mg%)	71.7 ± 3.5	65.0 ± 2.4	62.6 ± 3.5	−0.99	0.033
Fructose (mg%)	333.6 ± 8.1	329.4 ± 6.8	351.6 ± 9.6	2.27	0.049
Citric acid (mg%)	460.9 ± 10.4	443.9 ± 8.8	449.6 ± 12.1	−0.44	0.769

Note: Men enrolled in this study attended the Andrology and Reproductive Laboratory in Córdoba, Argentina. Values are shown as mean ± SEM. n = number of seminal samples evaluated (one per patient).

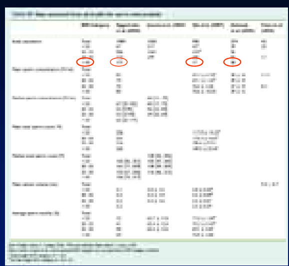
Martini. Overweight and seminal quality. Fertil Steril 2009.

META-ANALYSIS



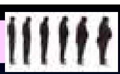
Heterogeneity

- **Threshold BMI**
- **Fertility status**
- **Ethnics**
- **Indicators**
- **Other lifestyle factors**



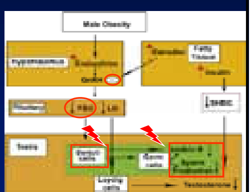
CONCLUSIONS: [illegible]

MacDonald et al. 2009



MECHANISMS

- **Secondary subclinical hypogonadotropic hypogonadism**
- **Insulin (abdominal fat levels) and leptin**
- **Direct testicular impact**
- **Toxics and EDs in the fatty tissue**
- **Scrotal temperature**
- **Polygenic obesity**
  - Heredity of the alimentary behavior
  - « obesogenic » environment
  - FTO, PTER, MC4R, MAF, NPC1



Strain 1982, Magnusdottir 2005, Winters 2006, Chavarro 2009

NUTRITIONAL HABITS

< 2/3 of Recommended Nutritional Contribution (RNC)	Deficiencies (biological tags)

< 2/3 of Recommended Nutritional Contribution (RNC)

VITAMIN
- Vitamin C : 20% of adults (H)
- Vitamin A : 30-45% adults
- Vitamin E : 40-60%

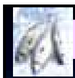
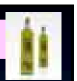
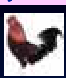
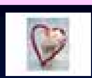
MINERAL
- Copper and Zinc : 25-50%
- Calcium : 8% of M
- Magnesium : 18% of M

Deficiencies (biological tags)

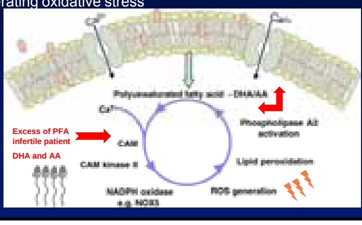
VITAMIN
- Vitamin B1: 22% of adults
- Vitamin B2 : 22% of M
- Vitamin B6 : 15% of M
- Vitamin C : 12% of M
- Vitamin D : 12% of M

MINERAL
- Copper: 15% of M
- Selenium : 30% of adults

Polyunsaturated fatty acid PFA

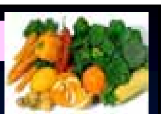





- ω6/ω3 ratio in sperm related to food
- PFA on sperm membrane
  - providing necessary fluidity during fertilization
  - generating oxidative stress



Wathes and Aitken 2007

FOLATE (B9)



Folate and human reproduction

The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility

I.M.W.Ebisch, C.M.G.Thomas, W.H.M.Peters, D.D.M.Braat and R.P.M.Steegers-Theunissen

Impact of folate and homocysteine metabolism on human reproductive health

Low folate in seminal plasma is associated with increased sperm DNA damage

MALE FACTOR

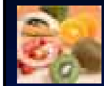
Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial

ORIGINAL ARTICLE

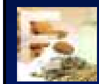
Does folic acid and zinc sulphate intervention affect endocrine parameters and sperm characteristics in men?

The association of folate, zinc and antioxidant intake with sperm aneuploidy in healthy non-smoking men

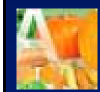
VITAMINS



- ❑ Sperm count, motility, morphology

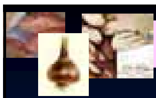


- ❑ Fertilization
- ❑ Sperm count, DNA, motility, morphology

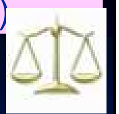


- ❑ Spermatogenesis, spermiation, steroïdogenesis
- ❑ Excess : from oligozoospermy to complete meiosis arrest
- ❑ Deficiency: early arrest BUT reversible, AR

Livera et al.,2002; Zervos et al., 2005; Ghyselinck et al., 2006



SELENIUM (Se)

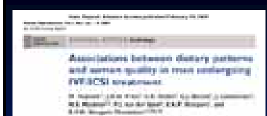


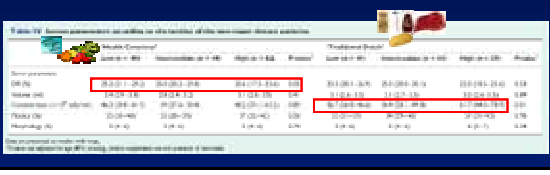
- ❑ Positive correlation between [Se] and [sperm] and motility
- ❑ Deficiency in Se : weight reduction of testis and morphological alteration of sperm cells, not compensated by E vitamin or any other antioxidants!

Zinc (Zn)

- ❑Essential for the spermatogenesis : DNA synthesis, AR, antioxidant…..
- ❑High seminal concentration +++, lower for infertile M

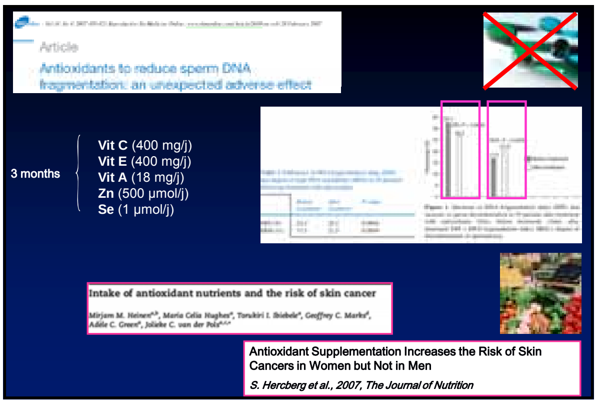
DIETARY PATTERN AND SEMEN QUALITY

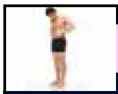




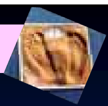

Food intake and its relationship with semen quality: a case-control study
OAT
BMI 23,2
N
BMI 23,5


Soy food and isoflavone intake in relation to semen quality parameters among men from an infertility clinic
❑Inverse relation between soy food intake and sperm concentration
❑Overweight and obese
❑High sperm concentration

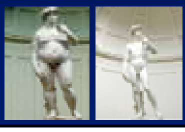

Article
Antioxidants to reduce sperm DNA fragmentation: an unexpected adverse effect
3 months
Vit C (400 mg/j)
Vit E (400 mg/j)
Vit A (18 mg/j)
Zn (500 µmol/j)
Se (1 µmol/j)
Intake of antioxidant nutrients and the risk of skin cancer
Antioxidant Supplementation Increases the Risk of Skin Cancers in Women but Not in Men
S. Hercberg et al., 2007, The Journal of Nutrition

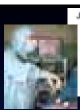


DIET…



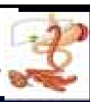
- No effect on free or total testosterone T Leenen et al. 1994
- Increase of free and total T, without effect on E2 Niskanen et al 2004
- Increase of free and total T, without effect on sexual life Kaukua et al 2003
- Improvement of the sexual function Esposito et al 2009





J Clin Endocrin Metab. First published ahead of print January 27, 2009 as doi:10.1210/jc.2008.1598

Effect of Roux-en-Y Gastric Bypass Surgery on the Sex Steroids and Quality of Life in Obese Men



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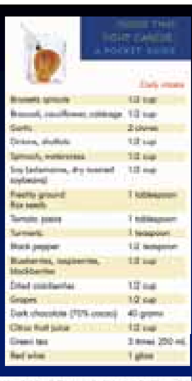
Hammoud et al 2009

Clinical Review

Role of nutrition in preventing cancer



FOODS THAT FIGHT INFERTILITY?


ANNE GEDDES