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ORIGINAL ARTICLE

Influence of Commercial Dietary Oils on Lipid Composition and Testosterone Production in Interstitial Cells Isolated from Rat Testis

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Abstract The aim of this study was to examine the influence of dietary fat on lipid composition, as well as on the steroidogenic function of interstitial cells isolated from Wistar rats that had been fed semi-synthetic diets containing four different commercial oils (S soybean, O olive, C coconut, and G grape seed). Steroidogenic enzyme activities, lipid composition, and androgen production were measured in testicular interstitial cells. Lipid analysis included measurement of the contents of major lipid subclasses (neutral lipids, polar lipids, free and esterified cholesterol), as well as principal polar and neutral lipid fatty acyl compositions. Significant differences in lipid composition were observed among the groups, most of them reflecting the specific fatty acyl composition of the diet tested. Testosterone concentration was higher in O and C groups compared with S or G. In agreement with this observation, the activity of both key enzymes involved in testosterone biosynthesis (3-β-HSD and 17-β-HSD) was higher in O and C groups with significant differences between them (O > C). A significant negative correlation was found between cellular testosterone production and cellular cholesterol ester content. Additionally, testosterone concentration directly correlated with cholesterol levels. We conclude that dietary oils qualitatively and quantitatively modified the lipid composition of interstitial cells, producing either a direct or indirect regulatory effect on testicular steroidogenic function.

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Abbreviations

C	Coconut
FC	Free cholesterol
CE	Esterified cholesterol
DBI	Double bond index
FA	Fatty acids
FAME	Fatty acid methyl esters
G	Grape seed
3β-HSD	3β-Hydroxysteroid dehydrogenase
17β-HSD	17β-Hydroxysteroid dehydrogenase
HPLC	High performance liquid chromatography
LH	Luteinizing hormone
MUFA	Monounsaturated FA
NL	Neutral lipids
O	Olive
PL	Polar lipids (phospholipids)
PUFA	Polyunsaturated FA
Rf	Relative chromatographic mobility
RIA	Radioimmunoassay
S	Soybean
SFA	Saturated FA
T	Testosterone
TL	Total lipids
TLC	Thin layer chromatography
TAG	Triacylglycerides

Introduction

It has long been known that changes in dietary fat [1–3] and/or cholesterol intake [4, 5] modify membrane

phospholipid composition in many tissues and cell types, thus influencing normal cell function. In the testis, it has been demonstrated that lipids strongly influence the histology and physiology of this tissue [6–8]. Different situations, such as essential fatty acid deficiency [9, 10] or alterations in fatty acid metabolism under diabetic conditions [10, 11], have been associated with testicular malfunction. Regarding androgen biosynthesis, it has been demonstrated that feeding rats with rapeseed oil rich in polyunsaturated fatty acids (PUFA) was associated with a high level of testosterone [12]. In contrast, decreased androgen concentration was measured in rats fed palm oil, which is rich in saturated fatty acids [12]. Total fat intake was also related to the level of sex steroid hormones. Some studies on men and rats have revealed that there was an increase in plasma androgen concentration after being fed a high-fat diet [13, 14]. In contrast, a reduction in total fat intake caused a decrease in plasma testosterone concentration [15]. Most studies on the relationship between dietary fat and testicular steroidogenesis have been carried out by analyzing the lipid composition of the whole testes. Additionally, we are unaware of any information concerning the possible relationship(s) between androgen production and a particular change in the lipid composition of interstitial cells. Therefore, the aim of this study was to evaluate the effect of diet supplementation, using four oils commercially available world wide, on lipid composition of interstitial cells and their impact on steroidogenic function.

Materials and Methods

Chemicals

Solvents (HPLC grade) were provided by Carlo Erba (Milano, Italy). Other chemicals were from Sigma Chem. Co. and Fluka Chemie AG (Buenos Aires, Argentina). Lipids used as standards were from the Serdary Research Lab (London, ON, Canada) or from Nu-Check-Prep (Elysian, MN). Collagenase was obtained from Worthington, Freehold, NJ. The commercially available oils that were added to the diets were from Molinos Río de La Plata SAIC and Platafarm SA (La Plata, Argentina).

Diets and Animal Treatment

Male Wistar rats weighing 180 ± 10 g with had been certified as specific-pathogen free (Laboratory Animal Care and Supply Facilities, Veterinary Medical School, UNLP) were used. Upon arrival, the rats were allowed to acclimatize for a week before starting the experiments. Rats were housed individually and maintained under controlled conditions at a temperature of 25 ± 2 °C with a normal photoperiod of 12 h dark and 12 h light. They were fed with standard Purina chow (Cargill type "C") from Ganave Co. (Santa Fe, Argentina) and water ad libitum throughout gestation and lactation. Clinical examinations together with body weight evaluation were performed every week during the experiment. Male pups (47 ± 4 g/animal) were divided into four groups of ten animals each, and fed specific diets ad libitum. Isocaloric diets (Table 1) were prepared in an identical manner, except for the lipid source, which was added as commercial oil (70 g/kg diet): soybean (S), olive (O), coconut (C), or grape seed (G). The fatty acyl composition of each diet is detailed in Table 2. Rats were fed according to the American Institute of Nutrition [16]. Animal maintenance and handling were conducted as recommended by NIH guidelines [17]. All procedures were approved by the local Laboratory Animal Committee, Facultad de Ciencias Médicas, UNLP, Argentina.

Experimental Design

Rats were sacrificed after being fed with the diets for 60 days. In order to avoid individual differences among animals, all rats were fasted for 24 h on day 59, giving access to the appropriate diet for 2 h, and killed by decapitation 12 h after the re-feeding period. Food intake,

Table 1 Composition of basal AIN-93 diet

Ingredients (g/kg)	
Casein, high protein	250.0
Sucrose	100.0
Corn starch	397.4
Cellulose	50.0
Commercial oil	70.0
Mineral mixture[a]	35.0
Sodium phosphate, monobasic	8.9
Potassium phosphate, monobasic	8.8
Calcium carbonate	12.5
Calcium phosphate–$2H_2O$	6.3
tert-Butylhydroquinone	0.014
Vitamin mixture[b]	10.0
Choline bitartrate	2.5
L-Cystine	3.0

[a] Contained (g/kg mix.): NaCl 184, K_2SO_4 136, $C_6H_5O_7$ $(K)_3$ 576, MgO 63, $MnCO_3$ 9.2, ferric citrate 17, $ZnCO_3$ 4.2, $CuCO_3$ 1, ammonium molybdate 0.03, Na_2SeO_3–$5H_2O$ 0.03, $CrK(SO_4)$–$12H_2O$ 1.0, KIO_4 0.05, delipidated casein 8.09

[b] Contained (g/kg mix): thiamine hydrochloride 0.5, riboflavin hydrochloride 0.5, niacin 0.5, pyridoxine 0.5, Ca-pantothenate 2.6, biotin 0.12, choline hydrochloride 50, folic acid 0.1, nicotinamide 1.26, *p*-aminobenzoic acid 0.5, inositol 50, Vitamin B12 0.006, Vitamin A 0.06, D-calciferol 0.01, α-tocopherol 1.5, menadione 2.5, ascorbate 6.0, L-methionine 2.0, delipidated casein 986.7

Table 2 Fatty acid composition of diets

Fatty acid	Diets			
	S	O	C	G
6:0			1.3 ± 0.1	
8:0			12.5 ± 0.3	
10:0			8.0 ± 0.2	
12:0			48.9 ± 1.1	
14:0		0.2 ± 0.0a	14.7 ± 0.3b	0.2 ± 0.0
15:0			0.1 ± 0.0	
16:0	11.5 ± 0.5a	15.5 ± 0.3a	6.9 ± 0.1b	7.5 ± 0.3b
16:1(n-7)	0.1 ± 0.0a	2.3 ± 0.1b	0.1 ± 0.0a	0.1 ± 0.0a
18:0	16.5 ± 0.4a	5.0 ± 0.1b	2.0 ± 0.1c	9.8 ± 0.2d
18:1(n-9)	9.7 ± 0.3a	68.5 ± 1.3b	4.0 ± 0.1c	16.8 ± 0.4d
18:1(n-7)		0.1 ± 0.0		0.1 ± 0.0
18:2(n-6)	55.1 ± 1.9a	8.2 ± 0.3b	1.3 ± 0.0c	65.1 ± 2.0d
18:3(n-6)	0.1 ± 0.0	0.1 ± 0.0		0.1 ± 0.0
18:3(n-3)	6.8 ± 0.1a	0.1 ± 0.0b		0.1 ± 0.0b
20:0			0.2 ± 0.0	
20:2(n-6)				0.1 ± 0.0
20:3(n-6)				0.1 ± 0.0
20:4(n-6)	0.1 ± 0.0			tr
20:5(n-3)	0.1 ± 0.0			
Analytical parameters				
ΣSFA	28.0 ± 0.7a	20.7 ± 0.3b	94.6 ± 0.9c	17.6 ± 0.7d
ΣMUFA	9.8 ± 0.2a	70.8 ± 0.9b	4.1 ± 0.1c	17.0 ± 0.9d
ΣPUFA	62.2 ± 1.8a	8.4 ± 0.2b	1.3 ± 0.0c	65.5 ± 1.8a
DBI	141.6 ± 3.3a	87.8 ± 1.4b	8.0 ± 0.2c	148.3 ± 4.4a
Σ(n-6)/ Σ(n-3)	7.9 ± 0.2a	83.0 ± 2.0b	∞c	654.0 ± 18d

Different letters on the same row indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

Means below 0.1% are indicated as "tr"

Results are expressed as moles % (mean ± 1 SD; $n = 6$)

S soybean oil, *O* olive oil, *C* coconut oil, *G* grapeseed oil

c-GLC of the FAME was performed as indicated in "Materials and Methods"

Σ sum of: *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *DBI* double bond index

water consumption, and body weight were individually registered during the feeding period. After sacrifice, testes were removed, weighed, and used for the isolation of interstitial cells.

Interstitial Cell Isolation

The technical procedure for interstitial cell isolation was described in detail in a previous report [18]. Briefly, cells were removed from the interstitial space of the testicular tissue by mechanical shaking with collagenase in a metabolic incubator, at 34 °C, according to the method of Suescun et al. [19]. Cells were suspended in Krebs-Ringer bicarbonate glucose (KRBG 0.1%), albumin (0.1%), pH 7.4, examined for viability (85–90%) by exclusion of trypan blue [20], and counted in a hemocytometer to adjust cell concentration. Aliquots of cell suspensions were subjected to protein determination by the micromethod of Bradford [21]. Interstitial cell preparations consisted of Leydig cells (70%) and of spermatids, spermatocytes and small cytoplasmic fragments (21%). The homogeneity of cell preparations was assessed by means of observation of smears fixed in acetone and stained with hematoxylin-eosin.

Lipid Analysis of Diets and Interstitial Cells

The fatty acid compositions of the diets were routinely checked by gas-liquid chromatography after derivatization by methanolic boron trifluoride as described below for lipid cell isolation and analysis. Total cell lipids were extracted using the Folch procedure [22]. Neutral (NL) and phospholipids (PL) were isolated from the total lipid extract by silicic acid microchromatography [23]. Absolute content of the major lipid subclasses was obtained using commercial silicagel G-60 TLC plates (Merck, Darmstadt, Germany) as previously described [24]. The total amount of lipids in each fraction was determined by either inorganic phosphorous quantification [25] or gravimetrically [26]. Lipids separated by TLC were visualized and identified using the method of Nakamura and Handa [27]. *Rf* values were as reported previously [24]. Individual lipid components were quantified densitometrically [28]. Cholesterol content (FC) was enzymatically measured [29]. Triacylglyceride content (TAG) was assayed using a kit from the Wienner Lab (Rosario, Argentina). Total fatty acyl methyl esters (FAME) were prepared by transesterification with methanolic boron trifluoride [30] and analyzed by capillary gas-liquid chromatography (c-GLC) in a Hewlett Packard HP 6890, with a terminal computer integrator system. FAMEs were identified by comparison of their relative retention times with authentic standards.

Steroidogenic Enzyme Activities

Sonicated interstitial cells were centrifuged (10,000*g*, 15 min, 1–2 °C). Aliquots of supernatants were employed to determine 3-β-hydroxysteroid-dehydrogenase (3-β-HSD) and 17-β-hydroxysteroid-dehydrogenase (17-β-HSD) enzyme activities using the method of Marugesan et al. [31].

Hormone Measurement

For the determination of testosterone levels in cells, interstitial cell suspensions were washed twice with cold

PBS and centrifuged (4,000*g*, 5 min). Pelleted cells were resuspended and homogenized by sonication (three 30-s bursts at 50% output in a Heat Systems Ultrasonic sonicator model W-220F from Plainview, NY) in 65 mM Tris–HCl buffer (pH 7.0) containing 10% sucrose and antiprotease inhibitor cocktail (from Sigma Chem. Co., Buenos Aires, Argentina) at the concentration recommended by the manufacturer. Cellular and plasma testosterone and plasma luteinizing hormone concentrations were determined by radioimmunoassay (RIA) using a commercial kit from Radim (Ponenzia, Italy).

Statistics

Statistical significance of data values was analyzed by one-way ANOVA followed by Tukey's test with the aid of Systat (Version 8.0 for Windows) from SPSS Science (Chicago, IL). Results were also plotted and analyzed using Sigma Scientific Graphing Software (Version 8.0) from Sigma Chem. Co. (St Louis, MO). Multivariable regression analysis was performed as described by Kleinbaum and Kupper [32].

Results

Fatty Acid Composition of Diets

Significant differences in fatty acyl composition were observed among diets (Table 2). Saturated fatty acids (SFA) represent approximately 95% of the total fatty acid (FA) content of Diet C and are mainly comprised of short or medium carbon chain fatty acids. Diet O was characterized by the highest content of monounsaturated fatty acids (MUFA), mainly oleic acid. Polyunsaturated fatty acid (PUFA) levels were high in both Diets S and G, with linoleic acid being the predominant FA. α-Linolenic acid was found in very low levels in all diets, except S (approximately 7%). As concerns the n-6/n-3 ratios, they were varied widely among diets. We observed that the relative proportion of n-6 versus n-3 fatty acyl chains decreased in the order C > G > O > S. In agreement with the fatty acyl composition of each diet, the corresponding double bond index (DBI) decreased in the order G = S > O > C.

Influence of Diets on Growth Parameters

The influence of diets on feeding parameters is listed in Table 3. Diets did not significantly influence water consumption (approximately 15 g/day). Initial body weights were similar in all groups. However, final body weights, rate of body weight gain, and food efficiency ratio were significantly elevated in Group C when compared with the others. Both absolute and relative testicular weights were significantly higher in Group O when compared to the other groups.

Changes Induced by Diets on Major Lipid Subclasses

Table 4 shows the effects caused by the diets on the absolute amount of major lipid subclasses of interstitial cells. Rats fed on Diets C and O displayed major amounts of total lipids compared with Groups S and G. The highest concentrations of phospholipids (PL) were present in Group O and those of the neutral lipids (NL) in Group C. The NL/PL ratio was three times higher in Group C with respect to those ratios observed in the other experimental groups. Group C was enriched in triacylglycerides, as well as in free cholesterol (FC). Esterified cholesterol (CE) was present in relatively small amounts in all groups. When comparing the FC/CE ratios, we observed similar values in Groups C and O, which were approximately five times higher than those calculated for the other groups.

The influence of dietary lipids on the main phospholipid subclasses is shown in Table 5. Interstitial cells from rats fed on Diet O exhibited significantly higher concentrations of PC, PE, and PI than in the other groups. Other phospholipids such as phosphatidylserine, sphingomyelin, phosphatidic acid, and phosphatidylglycerol, were present in similar amounts in all groups.

Dietary Effect on Fatty Acyl Composition of Lipid Subclasses

The relative distribution of fatty acids in PC, PE, and TAG lipids of interstitial cells is shown in Tables 6, 7, 8, respectively. We observed significant differences among groups. One of the most important changes occurred in phosphatidylcholine (PC) (Table 6) from Group C, of which 73% was composed of SFA, mainly 16:0 and 18:0, with minor amounts of short and medium carbon chain fatty acids (14:0 and 15:0). This group also showed the lowest level of both MUFA and PUFA. Group O was characterized by the highest MUFA content, mainly oleic acid, as well as the lowest levels of SFA (16:0 and 18:0). PUFA levels were similar to those observed in Groups S and C. Cells isolated from grape seed-oil fed rats were characterized by the highest amount of PUFA (45%), mainly 18:2, 20:4, and 22:5 acids of the n-6 series, with relatively low levels of SFA and MUFA. Group S was characterized by its high content of SFA followed by PUFA, especially 20:4 and 22:5 acids from the n-6 series. DBI calculated from PC fatty acyl compositional data decreased in the order G > O > S > C. Despite the fact that PE fatty acyl composition (Table 7) differs from that

Table 3 Main feeding parameters associated to diets

Parameters	Diets			
	S	O	C	G
Initial body weight (g)	40.5 ± 0.3	41.1 ± 0.4	40.0 ± 0.2	42.3 ± 0.6
Final body weight (g)	162.6 ± 5.5a	160.1 ± 4.0a	188.9 ± 4.1b	159.7 ± 5.3a
Body weight gain (g)	122.1 ± 3.3a	119.0 ± 3.7a	148.9 ± 3.1b	117.4 ± 3.5a
Rate of body weight gain (g/day)	2.1 ± 0.1a	2.0 ± 0.1a	2.5 ± 0.1b	2.0 ± 0.1a
Food efficiency ratio[a]	8.5 ± 0.2a	7.9 ± 0.3a	9.5 ± 0.2b	8.5 ± 0.2a
Absolute testicular weight (g)	1.7 ± 0.1a	2.0 ± 0.1b	1.8 ± 0.2a	1.6 ± 0.1a
Relative testicular weight (mg/g)[b]	7.6 ± 0.4a	10.5 ± 0.3b	8.9 ± 0.5a	8.0 ± 0.4a

Different letters on the same row indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

Values represent the mean ± 1 SD ($n = 10$)

S soybean oil, *O* olive oil, *C* coconut oil, *G* grapeseed oil

[a] Food efficiency ratio = [body weight gain (g)/food intake (g)] × 10^2

[b] Relative testicular weight = testis weight (mg)/body mass (g)

Table 4 Effect of dietary lipids on the absolute amount of the major lipid subclasses of interstitial cells

Lipid subclass	Diets			
	S	O	C	G
Total Lipids[a]	86.5 ± 4.2a	107.6 ± 5.2b	128.1 ± 4.4c	80.0 ± 3.1a
Phospholipids (PL)[b]	55.5 ± 3.0a	72.3 ± 2.3b	44.1 ± 3.2c	59.3 ± 3.1a
Neutral lipids (NL)[c]	29.3 ± 1.5a	36.2 ± 3.1b	79.5 ± 2.3c	33.3 ± 2.1a
Triacylglycerides[d]	12.4 ± 0.6a	16.1 ± 1.4a	39.7 ± 1.1b	9.5 ± 0.3c
Free cholesterol (FC)[e]	17.8 ± 0.8a	23.8 ± 0.8b	31.1 ± 1.0c	15.3 ± 0.5a
Esterified cholesterol (CE)[e]	2.0 ± 0.1a	0.8 ± 0.1b	1.1 ± 0.1b	2.7 ± 0.2c
NL/PL	0.53 ± 0.02a	0.50 ± 0.03a	1.80 ± 0.08b	0.56 ± 0.02a
FC/CE	8.9 ± 0.2a	29.8 ± 0.5b	28.3 ± 0.7b	5.7 ± 0.2c

S soybean oil, *O* olive oil, *C* coconut oil, *G* grapeseed oil

Results represent the mean ± 1 SD, of four independent determinations assayed in triplicate

Different letters on the same row indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

[a] pg/mg protein

[b] pmol inorganic phosphate/mg protein

[c] pmol tripalmitin/mg protein

[d] pmol linoleoyl-cholesterol/mg protein

of PC, the distribution pattern among different diets was similar. In summary, according to analytical parameters, SFA were the predominant fatty acids in Group C, MUFA were the most abundant acyl chains in Group O, and PUFA enriched the glycerolipids isolated from Groups S or G derived cells. Table 8 shows the fatty acyl composition of triacylglycerides (TAG) of interstitial cells. Group C exhibited the highest levels of SFA, as well as the lowest amount of MUFA. Group O was characterized by the highest levels of MUFA and the lowest levels of SFA. In Groups S and G, SFA amounts were quite similar. Group G exhibited the highest level of PUFA, similar to that of Group O, followed by Groups S and C. However, the DBI in TAG decreased in the order O > G = S > C.

Dietary oils also influenced the fatty acyl composition of cholesterol esters (CE) (Table 9). In this case, the composition of the diet exerted a complex influence on the analytical profile, which did not reflect the relative abundance of the acyl chains in the oil. For example, the proportion of SFA was similar among groups and even less abundant (considering stearic acid level) in Group C, which was fed the most saturated oil. MUFA content was elevated in the olive-supplemented group, however, the other groups had similar proportions of oleic and palmitoleic acids. The

Table 5 Effect of dietary lipids on the main phospholipid subclasses from interstitial cells

Lipid subclass	S	O	C	G
Phosphatidylcholine[a]	25.3 ± 1.8a	34.4 ± 2.0b	21.0 ± 2.1c	29.7 ± 1.7a
Phosphatidylethanolamine[a]	18.6 ± 1.4a	23.5 ± 1.7b	11.1 ± 1.1c	18.0 ± 2.0a
Phosphatidylserine	5.5 ± 0.4	5.9 ± 0.2	5.5 ± 0.3	5.0 ± 0.2
Sphingomyelin	3.3 ± 0.2	4.0 ± 0.1	3.4 ± 0.2	3.1 ± 0.1
Phosphatidic acid	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.0	0.9 ± 0.1
Lysophospholipids	1.7 ± 0.1a	2.2 ± 0.1b	1.5 ± 0.1a	2.2 ± 0.1a
Phosphatidylinositol	2.3 ± 0.2a	3.8 ± 0.2b	1.5 ± 0.2c	2.1 ± 0.2a
Phosphatidylglycerol	0.6 ± 0.0	0.7 ± 0.1	0.4 ± 0.0	0.5 ± 0.1

S soybean oil, *O* olive oil, *C* coconut oil, *G* grapeseed oil

Main phospholipid subclasses separated by HP-TLC as described in "Materials and Methods"

Values represented pmol of inorganic phosphate/mg cellular protein (mean ± 1 SD, $n = 6$)

Different letters on the same row indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

[a] These fractions include plasmanyl- and plasmenyl-species

Table 6 Effect of dietary lipids on the fatty acyl composition of phosphatidylcholine (PC) from interstitial cells

Fatty acids	S	O	C	G
14:0	tr a	tr a	3.1 ± 0.1b	0.1 ± 0.0a
15:0	tr a	tr a	0.2 ± 0.0b	tr a
16:0	44.4 ± 1.9a	29.9 ± 2.1b	64.1 ± 2.0c	33.3 ± 2.5b
16:1(n-7)	1.8 ± 0.2a	1.6 ± 0.1a	tr b	1.0 ± 0.2a
18:0	4.1 ± 0.1a	3.0 ± 0.1b	5.5 ± 0.1c	4.6 ± 0.0a
18:1(n-9)	18.0 ± 0.6a	37.5 ± 1.4b	2.0 ± 0.1c	15.1 ± 0.4a
18:2(n-6)	3.0 ± 0.1a	2.5 ± 0.1a	0.1 ± 0.0b	12.8 ± 2.2a
18:3(n-3)	0.2 ± 0.0a	tr b	tr b	0.1 ± 0.0c
20:3(n-6)	0.7 ± 0.1a	0.5 ± 0.1a	tr b	0.8 ± 0.1a
20:4(n-6)	11.5 ± 0.6a	10.0 ± 0.5a	4.3 ± 0.1b	15.5 ± 0.5c
22:4(n-6)	0.5 ± 0.0	0.1 ± 0.0b	tr c	1.0 ± 0.1d
22:5(n-6)	14.0 ± 1.0a	14.6 ± 0.7a	20.6 ± 0.6b	13.9 ± 0.4a
22:6(n-3)	0.6 ± 0.1a	0.3 ± 0.0b	0.1 ± 0.0c	0.7 ± 0.1a
Analytical parameters				
ΣSFA	48.5 ± 1.8a	32.9 ± 1.2b	72.9 ± 2.0c	38.0 ± 2.1b
ΣMUFA	19.8 ± 0.9a	39.1 ± 1.9b	2.0 ± 0.1c	16.1 ± 1.2a
ΣPUFA	30.5 ± 2.0a	28.0 ± 2.2a	25.1 ± 1.0a	44.8 ± 1.8b
DBI	139.5 ± 10.0a	160.8 ± 9.5b	121.0 ± 11.2c	186.3 ± 5.3d
Σ(n-6)/Σ(n-3)	37.1 ± 1.5a	92.3 ± 1.8b	251.0 ± 3.3c	55.0 ± 1.2d

S soybean oil, *O* olive oil, *C* coconut oil, *G* grapeseed oil

c-GLC of PC-FAME was performed as described in "Materials and Methods"

Values are expressed as mol% (mean ± 1 SD, $n = 6$)

Different letters on the same row indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

Means below 0.1% are indicated as "tr"

Some minor components have been omitted; Σ sum of: *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *DBI* double bond index

content of arachidonate (20:4n-6) was increased in Diet C, which was depleted in linoleic acid, while it was decreased in the other diets, especially those rich in MUFA (O). The content of 22:6n-3 fatty acid was approximately constant among groups and independent of the diet composition, while the amount of 22:5n-6 was significantly different and not associated with the acyl composition of the dietary oil. For example, Group C had a 22:5n-6 level similar to that of Group G. The changes observed in the relative amounts of PUFA agreed with the DBI values calculated for each diet.

Table 7 Effect of dietary lipids on the fatty acyl composition of phosphatidylethanolamine (PE) from interstitial cells

Fatty acids	S	O	C	G
14:0	0.1 ± 0.0a	tr a	3.1 ± 0.1b	0.1 ± 0.0a
15:0	0.2 ± 0.0a	tr b	0.2 ± 0.0a	tr b
16:0	30.4 ± 2.1a	21.5 ± 2.2a	39.4 ± 2.3b	23.7 ± 2.2c
16:1(n-7)	0.8 ± 0.1a	1.1 ± 0.1a	tr b	0.9 ± 0.1a
18:0	9.8 ± 0.3a	7.0 ± 0.4a	18.3 ± 0.7b	7.6 ± 0.2a
18:1(n-9)	10.3 ± 0.4a	26.6 ± 0.5a	1.2 ± 0.1b	12.2 ± 0.5c
18:2(n-6)	2.5 ± 0.2a	3.0 ± 0.1a	0.1 ± 0.0b	6.2 ± 0.7c
18:3(n-3)	0.4 ± 0.0a	0.1 ± 0.0b	tr c	0.2 ± 0.0d
20:3(n-6)	1.0 ± 0.2a	0.6 ± 0.1b	0.1 ± 0.1c	0.9 ± 0.1a
20:4(n-6)	15.2 ± 0.5a	12.0 ± 0.4b	11.1 ± 0.2b	17.2 ± 2.3a
22:4(n-6)	1.1 ± 0.1a	0.7 ± 0.3b	0.1 ± 0.0c	1.5 ± 0.1a
22:5(n-6)	20.3 ± 1.8a	23.8 ± 1.1a	25.1 ± 1.2b	21.0 ± 0.5a
22:6(n-3)	3.2 ± 0.2a	2.1 ± 0.1b	1.0 ± 0.0c	2.7 ± 0.1d
Analytical parameters				
ΣSFA	40.5 ± 1.8a	28.5 ± 1.0b	57.7 ± 2.0c	31.4 ± 1.0b
ΣMUFA	11.1 ± 0.5a	27.7 ± 1.1b	1.2 ± 0.1c	13.1 ± 0.4a
ΣPUFA	43.7 ± 1.2a	42.3 ± 2.1a	37.5 ± 1.3b	49.7 ± 1.3c
DBI	214.8 ± 10.0a	221.4 ± 9.0a	178.7 ± 7.7b	238.0 ± 9.3c
Σ(n-6)/Σ(n-3)	11.1 ± 1.1a	18.2 ± 0.7b	36.5 ± 1.1b	13.0 ± 0.5c

S soybean oil, *O* olive oil, *C* coconut oil, *G* grapeseed oil

c-GLC of PC- FAME was performed as described in "Materials and Methods"

Values are expressed as mol% (mean ± 1 SD, $n = 6$)

Different letters on the same row indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

Means below 0.1% are indicated as "tr"

Some minor components have been omitted; Σ sum of: *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *DBI* double bond index

Influence of Dietary Lipids on Steroidogenic Function and Testosterone Production of Interstitial Cells

The activities of the two key enzymes for testosterone biosynthesis, 3-β-hydroxysteroid-dehydrogenase (3-β-HSD) and 17-β-hydroxysteroid-dehydrogenase (17-β-HSD), were significantly affected by the diets tested. Both enzyme activities were significantly decreased in interstitial cell homogenates from rats fed Diets S and G compared with the levels observed in Group O or C preparations (Fig. 1).

Testosterone concentrations in interstitial cell homogenates are shown in Fig. 2a. The highest androgen concentrations were found in cells from Groups O and C. The decrease in cellular testosterone concentration was in the order, O = C > S = G. Figure 2b shows the plasmatic levels of free testosterone and luteinizing hormone. The highest levels of testosterone were observed when the rat diet was supplemented with Oils O or C. In Groups S or G, plasma testosterone concentration was significantly lower. Luteinizing hormone followed the opposite trend (Fig. 2b) with Groups S and G containing the highest levels and Groups O and C the lowest.

In Fig. 3 we have represented the linear correlation coefficient (r^2) between testosterone production and free or esterified cholesterol. Therefore, testosterone concentration directly correlated with free cholesterol (Fig. 3a). Additionally, a significantly negative correlation was found between cellular testosterone production and CE content (Fig. 3b).

Discussion

The control of testicular function is a complex process that requires the functional integrity of the seminiferous tubules and Leydig cells with a suitable multihormonal stimulation. The steroidogenic capacity of Leydig cells is essential for spermatogenesis. However, not only hormonal aspects are important in testis physiology; lipid composition is also crucial. Several factors have demonstrated that nutrition is an environmental factor of major importance [33]. Other

Table 8 Effect of dietary lipids on the fatty acyl composition of triacylglycerides (TAG) from interstitial cells

Fatty acids	S	O	C	G
12:0		tr a	0.5 ± 0.1b	
14:0	0.8 ± 0.1a	0.4 ± 0.0b	2.4 ± 0.2c	
15:0	0.1 ± 0.0a	tr b	0.1 ± 0.0	tr
16:0	28.8 ± 1.6a	22.9 ± 1.4b	44.8 ± 3.5c	25.3 ± 2.2b
16:1(n-7)	6.0 ± 0.2a	3.9 ± 0.1b	0.7 ± 0.1c	tr d
18:0	5.1 ± 0.2a	4.8 ± 0.2a	14.2 ± 0.6b	5.3 ± 0.1a
18:1(n-9)	19.7 ± 0.8a	24.7 ± 2.0b	8.0 ± 0.3c	21.1 ± 1.5a
18:1(n-7)	0.2 ± 0.0a	tr b	0.6 ± 0.1c	0.1 ± 0.0a
18:2(n-6)	10.0 ± 0.5a	11.1 ± 0.5b	5.1 ± 0.2c	21.0 ± 0.8d
18:3(n-6)	tr a	0.1 ± 0.0a	tr a	0.3 ± 0.0b
18:3(n-3)	0.2 ± 0.0a	tr b	tr b	tr b
20:2(n-6)	tr a	0.3 ± 0.0b	1.0 ± 0.1c	0.9 ± 0.1c
20:3(n-6)	0.3 ± 0.0a	2.5 ± 0.2b	0.1 ± 0.0a	1.3 ± 0.1c
20:4(n-6)	4.3 ± 0.1a	tr b	2.5 ± 0.1c	8.0 ± 0.2d
22:4(n-6)	3.1 ± 0.1a	2.8 ± 0.1a	0.2 ± 0.0b	4.5 ± 0.1c
22:5(n-6)	19.2 ± 1.1a	24.6 ± 0.9b	18.1 ± 1.9a	11.7 ± 0.4c
22:6(n-3)	1.1 ± 0.1a	1.0 ± 0.1a	0.4 ± 0.0b	0.2 ± 0.0c
Analytical parameters				
ΣSFA	34.8 ± 2.0a	28.1 ± 1.7b	62.0 ± 3.3c	30.6 ± 2.1b
ΣMUFA	25.9 ± 1.5a	28.6 ± 1.3b	9.3 ± 0.8c	21.2 ± 1.1d
ΣPUFA	38.2 ± 2.3a	42.4 ± 2.5b	27.4 ± 1.8c	47.9 ± 3.0b
DBI	178.7 ± 10.1a	199.4 ± 8.0b	125.5 ± 7.7c	184.8 ± 5.9a
Σ(n-6)/Σ(n-3)	28.5 ± 2.2a	42.4 ± 1.9b	68.5 ± 3.0c	239.5 ± 5.2d

S soybean oil, *O* olive oil, *C* coconut oil, *G* grapeseed oil

c-GLC of PC- FAME was performed as described in "Materials and Methods"

Values are expressed as mol% (mean ± 1 SD, $n = 6$)

Different letters on the same row indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

Means below 0.1% are indicated as "tr"

Some minor components have been omitted; Σ sum of: *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *DBI* double bond index

authors have demonstrated that dietary fats can modulate steroidogenic function of mammalian testis [12, 34]. However, little is known about the changes induced by dietary oils on lipid composition of interstitial cells and their eventual correlation with testosterone biosynthesis.

In this study, we demonstrated that commercial oils added to diets were able to affect lipid composition of interstitial cells, mainly Leydig cells, and that they markedly influenced their androgen biosynthesis capacity. The oils tested were selected by taking into account their massive consumption over the world and also because they were obtained from vegetable sources where cholesterol was absent. Differences among oils reflected their characteristics as a source of precursor FA, which, in turn, were then metabolized to MUFA and PUFA of the different fatty acid series.

Despite the fact that Oils S and G had the highest content of linoleic and/or linolenic acids they differed in the relative proportion of n-6 and n-3 fatty acid components. It was demonstrated that the ratio n-3/n-6 was important not only for cardiovascular disease prevention but also for a normal spermatogenesis [6]. However, the relationship between this analytical parameter and the androgenic capacity of interstitial cells is a matter that is still unsolved. We clearly demonstrated that oil-supplemented diets strongly modified interstitial cell lipid composition. Especially important were the differences produced in the absolute amount of NL and PL, and in the ratios of FC/CE. Moreover, FA patterns of these major lipids strongly differed in their fatty acyl chains being SFA, MUFA and PUFA, concentrated in Groups C, O and S/G, respectively. Changes observed in the major lipid subclasses induced by the commercial oils were in accordance with those found for tissues other than testis [35].

The importance of the FA modifications observed in the main lipid subclasses is their capacity to influence the androgen metabolism. Testicular lipids have an active metabolism, and they arise from both dietary sources and from the processes of synthesis, elongation, desaturation, interconversion, esterification, and oxidation by the testicular tissue itself. Moreover, it is known that dietary fats can affect the testicular fatty acid composition in a similar way to that seen in hepatic tissue [7]. Also, certain dietary fats can modulate not only fatty acyl composition of testicular lipids, but also testosterone metabolism. It was demonstrated that diets rich in PUFAs depress Leydig cell function in rats [12]. In agreement with this report, our data demonstrated that diets supplemented with Oils S and G caused reductions in 3β- and 17β-HSD enzyme activities compared with Oils O and C. Decreased enzyme activities

Table 9 Effect of dietary lipids on the fatty acyl composition of cholesterol esters (CE) from interstitial cells

Fatty acids	S	O	C	G
14:0	2.3 ± 0.1a	1.9 ± 0.2a	4.5 ± 0.2b	1.0 ± 0.1c
15:0	1.0 ± 0.3	0.8 ± 0.2	0.7 ± 0.2	0.7 ± 0.1
16:0	17.6 ± 1.0a	18.4 ± 0.7a	19.2 ± 0.9a	13.3 ± 0.5b
16:1 (n-7)	0.3 ± 0.1a	1.5 ± 0.1b	0.4 ± 0.1a	0.3 ± 0.0a
18:0	23.0 ± 1.2a	25.4 ± 1.2b	19.9 ± 0.8c	21.8 ± 0.9a
18:1 (n-9)	15.3 ± 0.5a	20.0 ± 1.0b	13.0 ± 0.2c	14.4 ± 0.3c
18:2 (n-6)	1.1 ± 0.1a	1.2 ± 0.3a	0.8 ± 0.1a	3.8 ± 0.1b
18:3 (n-3)	0.3 ± 0.1a	tr b	tr b	tr b
20:3 (n-6)	1.5 ± 0.1a	1.3 ± 0.2a	1.0 ± 0.2a	3.1 ± 0.1b
20:4 (n-6)	8.6 ± 0.6a	5.1 ± 0.2b	12.3 ± 0.4c	11.7 ± 0.8c
22:2 (n-6)	1.6 ± 0.1a	1.8 ± 0.1a	0.8 ± 0.1b	1.3 ± 0.1a
22:3 (n-6)	0.1 ± 0.0a	tr a	0.1 ± 0.0a	0.2 ± 0.0b
22:4 (n-6)	0.9 ± 0.1a	0.1 ± 0.0b	0.5 ± 0.0c	0.7 ± 0.1a
22:5 (n-6)	13.8 ± 0.8a	9.5 ± 0.5b	16.7 ± 1.0c	16.2 ± 0.5c
22:5 (n-3)	0.2 ± 0.0a	0.2 ± 0.0a	tr b	0.1 ± 0.0b
22:6 (n-3)	12.0 ± 1.4	12.5 ± 0.8	10.1 ± 0.7	11.2 ± 1.0
Analytical parameters				
ΣSFA	43.9 ± 1.2a	46.5 ± 1.1a	44.3 ± 1.3a	36.8 ± 1.1a
ΣMUFA	15.6 ± 1.0a	21.5 ± 0.7b	13.4 ± 0.3c	14.7 ± 0.5a
ΣPUFA	40.1 ± 1.3a	31.7 ± 1.5b	42.3 ± 1.1a	48.3 ± 1.0c
DBI	205.8 ± 8.1a	175.7 ± 6.0b	185.2 ± 9.9c	233.1 ± 8.4d
Σ(n-6)/Σ(n-3)	2.2 ± 0.2a	1.5 ± 0.1b	3.1 ± 0.2c	3.3 ± 0.1c

S soybean oil, *O* olive oil, *C* coconut oil, *G* grapeseed oil

c-GLC of PC-FAME was performed as described in "Materials and Methods"

Values are expressed as mol% (mean ± 1 SD, $n = 6$)

Statistical analyses were performed as indicated in "Materials and Methods"

Different letters on the same row indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

Means below 0.1% are indicated as "tr"

Some minor components have been omitted; Σ sum of: *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *DBI* double bond index

were in agreement with an inhibition in testosterone production by Leydig cells. This pattern of response was also reflected in peripheral plasma [34].

We also observed that Diets C and O had the best comparative performance in testosterone production despite their very different fatty acyl composition. The role of SFA and MUFA in steroidogenic function is a controversial issue. Oleic acid was provided predominantly by olive oil. This fatty acid has regulatory functions in many tissues [36–41] and it is accepted that its endogenous biosynthesis is insufficient for normal cell function. Thus, this acid must be obtained from dietary lipids [42]. However, previous reports have suggested that excessive oleic acid supplementation appears to inhibit testosterone synthesis by decreasing cholesteryl esterase activity [43]. This finding contradicts previous experimental evidence, indicating a stimulating effect of MUFA and an inhibitory effect exerted by n-3 PUFA [44]. Similar controversial results were observed in the relative amount of saturated fatty acids [45]. Other researchers have also reported that FA could inhibit steroidogenesis at one of the steps preceding the conversion of cholesterol to pregnenolone [46]. Taking into account all these previous results, it is likely that both excessive PUFA and/or MUFA may be deleterious for testosterone production. However, our findings indicate that a diet enriched in MUFA (olive oil) may be important to keep androgenic synthesis within the normal values whereas PUFA diets appear to depress Leydig cell function. Such a conclusion denotes the importance of dietary lipids in Leydig cell function.

The availability of free cholesterol from cholesterol esters is influenced not only by the quantity and quality of FA but also by hormonal levels. In rat Leydig cells, the cholesterol side-chain cleaving enzyme is subjected to LH regulation in a complex way, which depends on both the time and the intensity of the gonadotrophic stimulus [47].

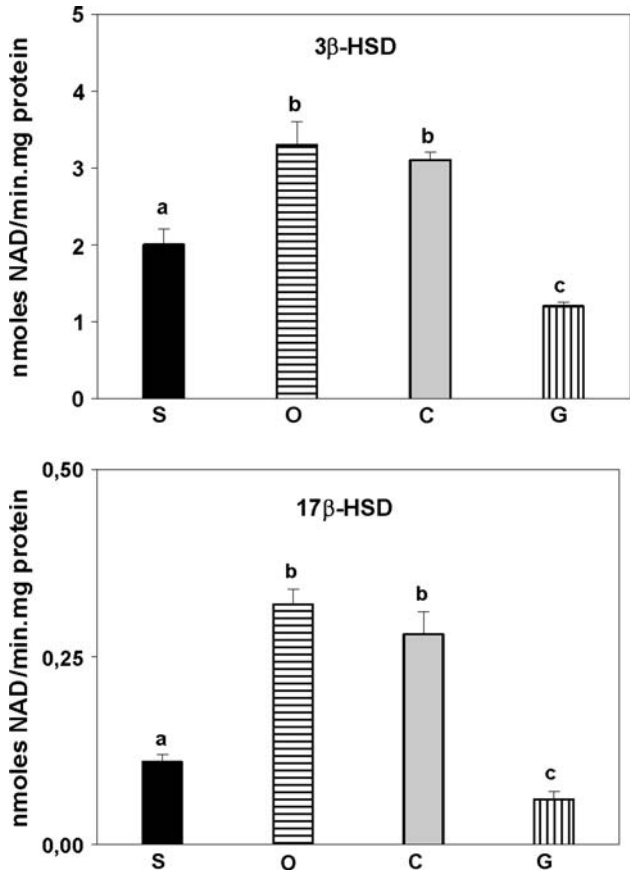

Fig. 1 Activities of the two key enzymes for testosterone biosynthesis: 3-beta-hydroxysteroid-dehydrogenase (*3β-HSD*) and 17-beta-hydroxysteroid dehydrogenase (*17β-HSD*) in interstitial cells from rats fed different diets (*S* soybean, *O* olive, *C* coconut, and *G* grape-seed oil-supplemented diet). Results presented as mean ± 1 SD, $n = 10$. Different *superscript letters* indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

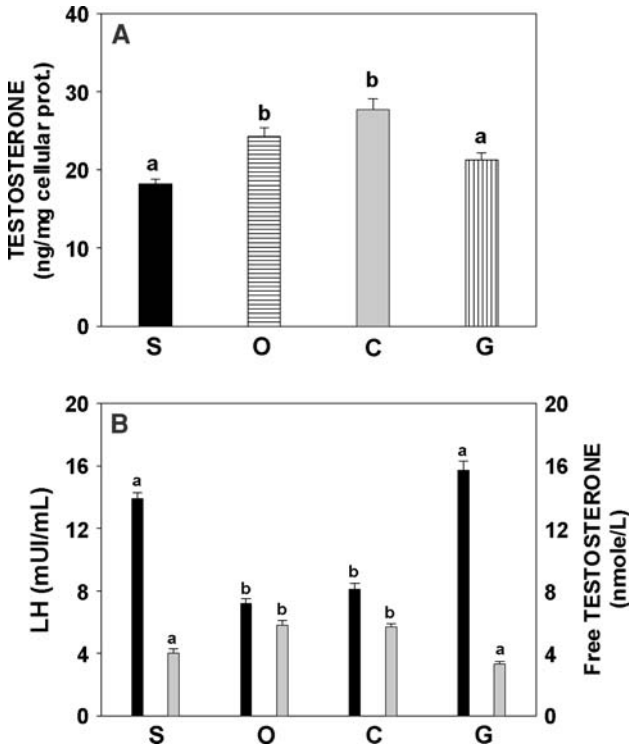

Fig. 2 Hormonal levels measured by RIA methodology for interstitial cells (**a**) or plasma (**b**) from rats fed different diets (*S* soybean, *O* olive, *C* coconut, and *G* grape-seed oil-supplemented diet). Values of free testosterone for interstitial cells were expressed as ng/mg cellular protein, and they were the mean ± SD of ten independent determinations. *Bars* of **b** correspond to the concentration of LH (*black* mUI/mL) or free testosterone (*gray* nmol/L) in peripheral plasmas and they are the mean ± SD of ten independent determinations. Statistical analyses were performed as indicated in "Materials and methods". In all cases different *superscript letters* indicate values significantly different ($P < 0.01$) as determined by one-way ANOVA and Tukey's post hoc test

As described in our paper, LH levels were elevated in Diets S and G compared to the other experimental groups. This stimulation may adversely affect the activity of the cholesteryl-side chain cleavage enzyme, and consequently, it may contribute to the inhibition of androgen production by decreasing the concentration of the precursor.

We also observed that the side chain of the esterified cholesterol is strongly influenced by the type of lipid present in the diet. This finding was in agreement with previous experimental evidence indicating that the composition of the cholesteryl esters in testis is more strongly affected than that of the other lipid subclasses [7].

The lipid changes we observed could also be involved in other mechanism(s) of action which depend on the physicochemical properties of interstitial cell membranes. It is well known that alterations in lipid proportions and/or fatty acyl chains acylated to complex lipids strongly influence several processes that occur in the cytoplasm and in the inner cell membranes, such as mitochondrial membranes [48–51]. Receptor-mediated signal transduction and even free cholesterol transport to the inner mitochondrial membrane are examples of processes that should be affected by lipid composition. It is well documented that the phospholipid content and the type of fatty acids acylated to phospholipids were both influenced by dietary manipulation and caused alterations on membrane-mediated gonadotropin action in testicular tissues [7].

Previous reports from other laboratories have described the stimulating effect of arachidonyl-CoA or free arachidonate on the activity of StAR protein and cholesterol transport into the mitochondria for androgen production [52]. Arachidonate involved in these regulatory processes would be obtained from the cholesteryl ester pool via the ACS4/Acot2 system [53] without the intervention of phospholipase A_2 [54]. The proportion of arachidonyl-side chains within the esterified cholesterol pool would probably regulate the proportion of free cholesterol available for testosterone biosynthesis. This speculation was not evident

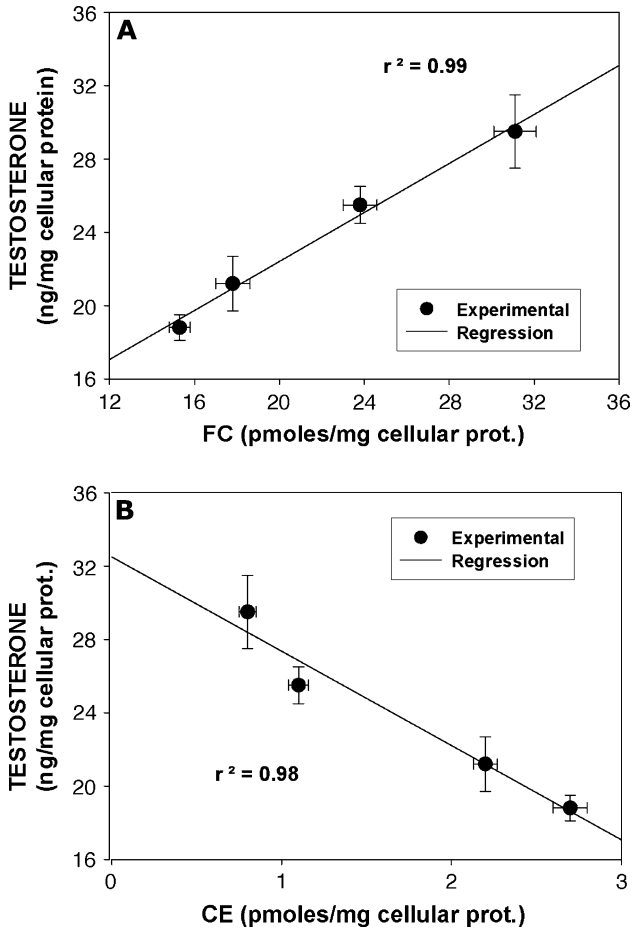

Fig. 3 Linear correlation coefficient (r^2) for regression plots between testosterone production by interstitial cells and (**a**) free cholesterol (*FC*) or (**b**) cholesterol ester (*CE*) levels. Data from Figure 1 and Table 4 were plotted and analyzed using the software described in "Materials and Methods"

from the compositional data. However, we demonstrated that the level of esterified cholesterol increased in the order O > C > S > G, while the level of the acylated cholesterol increased in the opposite way (G > S > C > O). Moreover, a positive linear correlation between androgen production and FC was evident, while this correlation became negative when CE was considered as the independent variable.

Data obtained form other laboratories also provide substantial support for the hypothesis that fat ingestion is sexually differentiated in humans. The regulatory stimuli controlling the consumption of fat arise from oral, gastric, intestinal, hepatic, and adipose sites [55]. However, environmental factors may modify this physiological behavior. In the past decades, a progressively significant worldwide decline in semen quality and androgenic performance was reported [56–59]. Within the environmental factors that determine this phenomenon, increasing pollutants for example [59–61], we think that lipid composition of the diet should be considered as one of the most important. Future research in this area may contribute to the understanding of the mechanism(s) involved and also the prevention of the damage induced by an inadequate diet.

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