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Lipids in Human Benign Prostatic Hypertrophy

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Summary. Lipids in human prostate have been the subject of limited research and the most complete studies were published forty years ago. In this study we report on the total lipid, total cholesterol, free cholesterol and triglyceride content in human benign prostatic hypertrophy. By means of thin-layer chromatography, we separated the cholesterolesters in five fractions. The effect of treatment with anti-androgens before prostatectomy was examined.

Key words: Prostate, Lipids, Cholesterol, Triglycerides, Cholesterolesters.

Introduction

The presence of lipids in human prostate was described first by Thompson [13], and later by Plenge [6]. By means of histological techniques, these authors concluded that the major part of prostatic lipids consisted of neutral fats, phospholipids, cholesterol and its esters. The lipid content of the gland was age-dependent, and increased in older patients [5, 8]. Swyer [12] determined chemically the cholesterol content of benign prostatic hypertrophy (BPH) and reported that the cholesterol concentration in BPH was twice that in normal prostate. Only in 1945, Scott [9] published a detailed chemical composition of BPH-lipids. This author could not confirm the presence of neutral lipids, but reported a content of 65% phospholipids.

These contradictory and incomplete results prompted us to study the lipids in BPH with modern techniques, and also in hypertrophied prostatic tissue following pre-operative anti-androgenic treatment. Thin-layer chromatography, the method used for separation of cholesterolesters, is described in detail.

Material and Methods

Thirty-seven prostate samples, obtained at open surgery were examined. Six BPH patients received anti-androgens prior to surgery as medication for their prostatism. From each prostate, the lateral and median lobes were separated. The tissue samples were transported to the laboratory in liquid nitrogen and stored at −76 °C. Homogenates were made following van Camp's technique [15]. The total protein concentration of the homogenates was measured by the biuret method [1]. Results are expressed in mg lipid per g protein in the homogenate. Normal prostates were not available. Determinations were carried out with chemical methods or by means of commercially available kits (Boehringer, Mannheim, FRG). All chemicals used were of analytical grade (E. Merck, Darmstadt, FRG). The techniques used will be briefly described in the subsections below.

Total Lipids. Total lipids in the homogenates have been determined with the sulphovanilline reaction [18], using a kit from Boehringer, Mannheim, FRG (Cat. no. 124,303).

Total Cholesterol. Was determined by means of three different techniques:

a) the direct Lieberman-Burchard method [16], using a kit from Boehringer (cat. no. 124,095). As hemoglobin interferes in the determination, the hemoglobin content in each tissue homogenate has been determined [4] and the cholesterol results corrected accordingly.

b) Lieberman-Burchard technique after Bloor-extraction of the homogenate [15].

c) Enzymatic determination of cholesterol by means of cholesterolesterase and cholesteroloxidase based on the Trinder reaction [14] (Boehringer kit, cat. no. 290,319).

Free Cholesterol. Has been determined following Stähler et al. [11] (Boehringer kit, cat. no. 310,328).

Triglycerides. We have used the method of Eggstein and Kuhlmann [2] (Boehringer kit, cat. no. 470,694).

Thin-layer Separation of Cholesterolesters. Cholesterolesters are extracted following the technique described by Shin [11]. The separation of the extracts took place on glass plates, covered with a layer of 0.25 mm silica gel (D.C. Fertigplatten, nr. 5,626, E. Merck, Darmstadt, FRG).

Table 1. Hemoglobin concentrations in prostate homogenates

BPH untreated	Number	Hemoglobin concentration (g%)	SEM
Lateral lobe	22	0.69	0.03
Median lobe	9	0.64	0.04
Total	31	0.67	0.02
BPH treated			
Lateral lobe	3	0.63	0.13
Median lobe	3	0.61	0.18
Total	6	0.62	0.10

SEM: standard error of the mean

Table 2. Total lipids in prostate homogenates

BPH untreated	Number	mg lipids/ g protein	SEM
Lateral lobe	22	69.66	5.56
Median lobe	9	104.38	16.13
Total	31	79.74	6.62
BPH treated			
Lateral lobe	3	103.08	25.89
Median lobe	3	117.16	23.95
Total	6	110.12	16.09

The solvent used was petroleumether : di-isoprophylether (96.6 : 1.4 v/v), in which the plate was developed 3 times to 11 cm above the application site. After drying for 30 min at room temperature, the gel was sprayed with a 20% phosphomolybdic acid solution in ethanol. After 10 min heating at 120 °C, the cholesterolesters appeared as blue spots on a yellow background [17].

Results

As results obtained are expressed in lipids/g protein, we first determined the total protein content of the homogenates. The mean value obtained was 4.76 g% (SEM = 0.11). There was no significant difference between lateral and median lobes, nor was there a difference between untreated and anti-androgen treated BPH.

The Lieberman-Burchard reaction for the total cholesterol determination is influenced by the hemoglobin content of the homogenates. Results of the hemoglobin determination in 37 prostate homogenates are summarized in Table 1.

From these values were estimated the concentration of serum proteins in the homogenates. The hemoglobin values account for ± 0.30 g serum protein; that means, that on a total protein content of 4.76 g% in the homogenates, 6.3% of these proteins are due to blood contamination.

Table 3. Total cholesterol in prostate homogenates (Lieberman-Burchard technique)

BPH untreated	Number	mg cholesterol/ g protein	SEM
Lateral lobe	22	13.40	1.68
Median lobe	9	18.02	3.03
Total	31	14.74	1.50
BPH treated			
Lateral lobe	3	11.39	4.58
Median lobe	3	15.76	5.10
Total	6	13.58	3.22

The results of the total lipid determination are given in Table 2. No significant difference could be stated between untreated and treated BPH.

The cholesterol determination with the direct Lieberman-Burchard method gives results for cholesterol which are influenced by the hemoglobin concentration of the homogenate. This can easily be corrected by calculating the cholesterol interference of the hemoglobin present.

A series of known hemoglobin concentrations was analyzed for "total cholesterol" by the direct Lieberman-Burchard technique, and the values found substracted from those of the determinations on the homogenate. Results obtained are given in Table 3.

In order to avoid the hemoglobin interference in the cholesterol determination, we carried out the Lieberman-Burchard technique after extraction of the cholesterol with Bloor-reagens (ethyl alcohol-ether 3 : 1, v/v).

The organic layer was evaporated and the residue disolved in chlorophorm. Addition of acetic anhydride and sulfuric acid (10 : 1, v/v) produces a colored complex, with an absorption maximum at 670 mm. Although it might be expected that all the proteins, including hemoglobin, would have been precipitated by the alcohol-ether mixture, this was not the case, as the chlorophorm extract of the evaporated residue was stained light red. The results for the corrected total cholesterol values in the prostate homogenates were similar to those obtained by the direct Lieberman-Burchard technique.

The enzymatic cholesterol determination is simple to carry out and should give exact values. The absorption of the formed 4-(p-benzoquinone mono imino)-phenazone changed with time. Therefore, the extinction values were read after 10 min incubation at room temperature, effecting reproducible results. These are summarized in Table 4.

Table 4. Enzymatic cholesterol determination in prostate homogenates

BPH untreated	Number	mg cholesterol/ g protein	SEM
Lateral lobe	13	8.19	1.23
Median lobe	7	11.08	1.80
Total	20	9.20	1.04
BPH treated			
Lateral lobe	3	9.84	4.51
Median lobe	3	8.74	4.87
Total	6	9.29	4.52

Table 5. Free cholesterol in prostatic homogenates

BPH untreated	Number	mg free cholesterol/g protein	SEM
Lateral lobe	9	3.99	0.38
Median lobe	22	4.02	0.59
Total	31	4.00	0.33
BPH treated			
Lateral lobe	3	4.61	0.66
Median lobe	3	3.93	1.22
Total	6	4.27	0.84

Table 6. Triglycerides in prostate homogenates

BPH untreated	Number	mg triglycerides/ g protein	SEM
Lateral lobe	11	19.56	3.19
Median lobe	3	19.21	4.36
Total	14	19.49	2.60
BPH treated			
Total	4	23.39	6.24

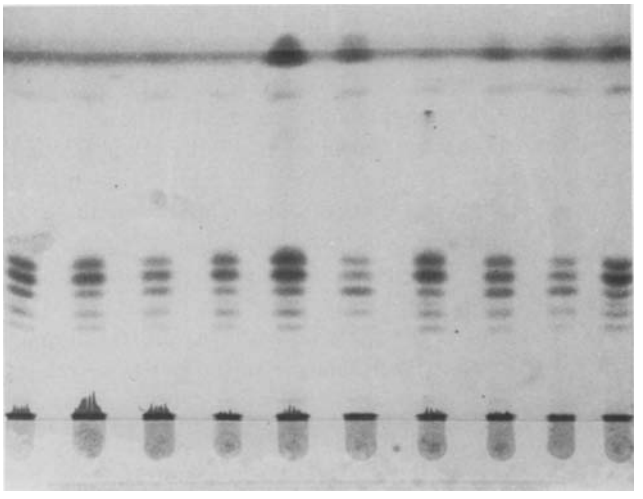

Fig. 1. Thin-layer chromatography of cholesterolesters extracted from prostate homogenates. Solvent: petroleum-benzin : di-isopropyl-ether 98.6 : 1.4 (v/v)

The enzymatic determination of free cholesterol was determined in 37 prostate homogenates. Results obtained are given in Table 5.

The determination of the total triglyceride content of prostatic homogenates following Eggstein and Kuhlman [2] was difficult. The principle of the reaction is:

$$\text{Triglycerides} \xrightarrow{\text{lipase/esterase}} \text{glycerol} + 3\ \text{R-COOH}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerkinase}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{ADP} + \text{phosphoenol pyruvate} \xrightarrow{\text{pyruvatekinase}} \text{pyruvate} + \text{ATP}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{lactic dehydrogenase}} \text{NAD}^+ + \text{lactate}.$$

The absorption rate $NADH/NAD^+$ was not stable and changed with time. It is likely that prostate contains interfering enzymes, therefore the prostate enzymes were denatured by heating (10 min at 65 °C). This treatment caused a turbid liquid, so that we had to carry out the determination on the centrifuged homogenate. Results were much better and are given in Table 6.

The results of the separation by means of thin-layer chromatography of the cholesterolesters are represented in Fig. 1. Ten homogenates were examined (7 untreated and 3 treated BPH). In all cases, 6 fractions were detected.

The fraction which remained on the application site was free cholesterol. The second fraction was the most pronounced. Comparison with standard solutions of known cholesterolesters showed that the cholesterolesters in prostatic tissue are:

fraction 1: saturated cholesterolesters
fraction 2: saturated and mono-unsaturated cholesterolesters
fraction 3: mono-unsaturated cholesterolesters
fraction 4: di-, tri- and tetra-unsaturated cholesterolesters
fraction 5: poly-unsaturated cholesterolesters
fraction 6: unknown

Discussion

The results obtained in this study give information relating to the lipid-pattern of BPH.

It should, however, be emphasized that the methods available for lipid determinations of human serum have to be adapted carefully for prostate homogenates.

Statistical analysis of the results obtained showed:

a) that there is no significant difference of the lipids studied between lateral and median lobes

b) there is no statistical difference in the lipid pattern between untreated and anti-androgenic treated prostate

There is a high content of free cholesterol compared with the concentration of total cholesterol. This ratio is different to that found in human serum.

Preliminary results on prostate extracts following the Folch et al. [3] technique and separation by means of thin-layer chromatography, showed that about 11 lipid fractions can be detected. Comparing their Rf-values with those of known lipids suggests that besides the lipids determined in this study, prostate contains sulfatides, sphingomyelin, lecithine, phosphatidylethanolamine amongst other fractions.

The fractions of the cholesterolesters in prostate homogenates have still to be determined in prostatic cancer. Further studies will isolate these fractions by gaschromatography, coupled with a masspectrometer.

The studies of Schaffner [7] on prostatic cholesterol metabolism, with the accent on regulation and alteration have to be confirmed by these techniques. These authors stated that the polyene macrolide antibiotic candicidin, acts as an hypocholesterolemic agent on the prostate gland, accompanied by a marked reduction of the size of the prostate gland.

More interesting is the finding that in older patients a part of the cholesterol is transformed in epoxycholesterols. For both cholesterol 5β,6β-epoxide and in particular cholesterol 5α,6α-epoxide it is postulated that they may be a significant factor in the pathogenesis of prostate cancer.

In conclusion, this report on the lipid content of prostatic homogenates, may provide a new means of assessing drugs for the treatment of BPH. The role of prostatic lipids in the pathogenesis of cancer of the prostate is suggested.

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