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Excretion Rate and Composition of Skin Surface Lipids on the Foreheads of Adult Males with Type IV Hyperlipoproteinemia

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Aim: Most of the lipids of the skin surface come from the sebaceous glands, which secrete an oily substance called sebum. Some of the sebum lipids are synthesized by sebaceous cells while some are reported to be derived from the plasma. The role of blood lipoproteins in sebum secretion rate and composition is unclear. To this end, excretion rate and composition of skin surface lipids in normo- and type IV hyperlipoproteinemic adult male subjects were compared.

Materials and Methods: Quantitative analysis of skin surface lipids of subjects with type IV hyperlipoproteinemia (n:21) and normolipoproteinemia (n:15) was performed by three successive samplings from left, middle and right zones of the forehead with a sebumeter. Skin surface lipid samples for the compositional analysis were collected from the forehead, extracted into n-hexane and analyzed by high performance thin layer chromatography (HPTLC).

Results: Skin surface lipids from type IV hyperlipoproteinemic subjects contained a higher proportion of wax ester + cholesteryl ester compared with normolipoproteinemic subjects (p:0.004). However, skin surface lipid excretion rates of normo- and hyperlipoproteinemic subjects were found to be similar.

Conclusions: Plasma triacylglycerol concentration may affect sebum wax ester+cholesteryl ester content.

Key Words: Human, hyperlipoproteinemia, sebaceous gland, sebum lipid

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Tip IV Hiperlipoproteinemili Erişkin Erkeklerde Alın Bölgesi Deri Yüzey Lipidlerinin Ekskresyon Hızı ve Kompozisyonu

Amaç: Deri yüzey lipidlerinin çoğu sebum olarak adlandırılan yağlı bir maddeyi salgılayan yağ bezlerinden köken almaktadır. Sebum lipidlerinin bir kısmı yağ bezi hücreleri tarafından sentezlenirken diğer bir kısmının ise plazmadan kaynaklanabileceği bildirilmiştir. Kan lipoproteinlerinin sebum sekresyon hızı ve kompozisyonundaki rolü açık değildir. Bu amaçla, normo- ve tip IV hiperlipoproteinemili erişkin erkeklerde deri yüzey lipidlerinin ekskresyon hızı ve kompozisyonu karşılaştırıldı.

Yöntem ve Gereç: Tip IV hiperlipoproteinemili (n:21) ve normolipoproteinemililerde (n:15) deri yüzey lipidlerinin kantitatif analizi, alnın sağ, sol ve orta bölgesinde yapılan sebumetrik ölçümlerle gerçekleştirildi. Deri yüzey lipidlerinin kompozisyonel analizi için numuneler alın bölgesinden n-heksan ile ekstrakte edilerek elde edildi ve yüksek performanslı ince tabaka kromatografisi (HPTLC) ile analiz edildi.

Bulgular: Tip IV hiperlipoproteinemili erişkin erkeklerde deri yüzey lipidlerinin normolipoproteinemililere göre daha yüksek oranda mum esteri + kolesterol esteri içerdiği tespit edildi (p:0.004). Bununla beraber, ekskresyon hızlarında istatistiksel olarak önemli bir fark tespit edilemedi.

Sonuç: Plazma triçilgliserol konsantrasyonu sebum mum esteri+kolesterol esteri içeriğini etkileyebilmektedir.

Anahtar Sözcükler: Hiperlipoproteinemi, yağ bezi, sebum, lipid

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Introduction

The lipids on the skin surface are derived from both sebaceous glands and epidermis (1,2). Sebum is an oily material secreted by the sebaceous glands onto the skin surface and mainly contains squalene, triglyceride, wax esters, cholesterol and cholesteryl esters. Sterol and sterol esters are generally derived from the epidermis while wax esters and squalene are of sebaceous origin; triacylglycerols originate from both sources (2).

Sebum production of the forehead in adult males was reported to be between 1.25 – 3.33 mg lipid/10 cm² skin/3h (3,4,5). This considerable amount of lipid secreted by both sebaceous gland and epidermis may require the involvement of blood lipids in the sebum synthesis. Some of the sebum lipids are synthesized by sebaceous cells and others presumably acquired by uptake from the circulation (6,7). Although the role of serum lipoproteins in sebum synthesis has not yet been proposed, it can be inferred from several studies. It was reported that overexpression of apo C-I in transgenic mice, which is known to have an inhibitory role on hepatic remnant uptake, reduced relative amounts of sebum triacylglycerol and wax diesters but elevated levels of serum cholesterol and triacylglycerol, due to an accumulation of VLDL (very low density lipoprotein) remnants in the circulation (8). In addition, retinoic acid derivatives used for the treatment of severe acne were reported to act primarily by reducing sebaceous gland size and sebum production while increasing serum concentrations of apo B, total cholesterol, LDL (low density lipoprotein) cholesterol and triacylglycerols (9,10).

Type IV hyperlipoproteinemia is very common and characterized by high levels of endogenously produced triacylglycerol (VLDL) (11). This lipoprotein pattern was chosen to evaluate interaction of high plasma triacylglycerol level with sebum composition and excretion rate to shed light on the role of lipoproteins as an exogenous source of triacylglycerol in synthesis and excretion of sebum lipids. To this end, skin surface lipids in subjects with type IV hyperlipoproteinemia were quantified by a sebumeter and analyzed by high performance thin layer chromatography (HPTLC). Relative proportions of the major secretory lipids were calculated. These calculations brought to light a relative increase in wax ester + cholesteryl ester in type IV hyperlipoproteinemic compared to normolipidemic subjects.

Materials and Methods

Materials: L-phosphatidylcholine, L- α -phosphatidylethanolamine, cholesterol-3-sulfate, galactocerebrosides (type I + type II), N-palmitoyl-D-sphingosine, cholesterol, palmitic acid, triolein, cholesteryl oleate, beeswax and squalene were purchased from Sigma-Aldrich Chemie GmbH, Germany. HPTLC plates (20x10 cm, silica gel 60) were obtained from Merck KgaA, Darmstadt/Germany.

Subjects: Fifteen healthy male normolipoproteinemic subjects (average age: 41.9 \pm 4.9) were recruited from the staff of our research laboratory. Twenty- one hyperlipoproteinemic patients (average age: 40.7 \pm 11.1) were selected from those attending various clinics at Turgut Özal Medical Center, İnönü University, Malatya-Turkey.

Collection of skin surface lipids: The forehead of each volunteer was first cleaned with wet tissues followed by gentle drying with paper tissues. Skin surface lipids were sampled 3 hours after the cleaning. Skin surface lipid samples were collected from the forehead with a hexane-soaked cotton mounted on a wooden stick. The lipid was recovered by extraction into 1 ml of n-hexane. Informed consent was obtained from each subject, and the study was approved by the local ethics committee.

Separation and quantification of the forehead skin surface lipids: For separation and identification, HPTLC plates were used. Standard lipid mixture comprised L-phosphatidylcholine, L- α -phosphatidylethanolamine, cholesterol-3-sulfate, galactocerebrosides (type I + type II), N-palmitoyl-D-sphingosine, cholesterol, palmitic acid, triolein, cholesteryl oleate, beeswax and squalene. We applied 3-6 μ g per component from standards and 5-15 μ l sebum extract to HPTLC plates. TLC was conducted as described by other investigators. After separation of all lipids, the plates were sprayed with an aqueous solution of 10% copper (II) sulfate in 8% phosphoric acid and charred at 180 °C (12,13). All HPTLC plates were scanned with the Desaga Densitometer CD 60 (Desaga, Heidelberg, Germany) at 600nm. The percentage of each lipid class was calculated by the measurement of the peak height for each spot.

Measurement of excretion rate of skin surface lipids: The sebum measurement on the skin is based on the sebumeter method (Sebumeter SM 810, CK Electronic GmbH, Köln, Germany). The measurement

principle is the photometric method. The quantitative analysis of skin surface lipids in $\mu\text{g sebum}/\text{cm}^2$ of the skin was performed by successive samplings on left, right and middle zones of the forehead with a sebumeter 3h after cleaning. The average of three samplings represents the total amounts of skin surface lipids found on this zone at the time of collection.

Agarose gel electrophoresis: Agarose gel electrophoresis was used for electrophoretic separation of serum lipoproteins. The electrophoresis was performed on pre-made agarose gels according to manufacturer's instruction (Beckman Instruments, Inc., Fullerton, CA 92634-3100, USA). After electrophoresis, the lipoprotein pattern was visualized by staining and quantitated by densitometry.

Serum lipid analysis: Serum triacylglycerol, total cholesterol, and high density lipoprotein (HDL)-cholesterol analyses were performed by an automatic analyzer (Olympus AU 600, Olympus Diagnostica GmbH, Lismeehan, O'Callaghan's Mills, Co. Clare, Ireland).

Quantitative determination of serum apolipoprotein A-I and B: Immunonephelometric determination of human serum apolipoprotein A-I and B was carried out by Behring Nephelometer System (Dade Behring Marburg GmbH, Marburg-Germany).

Statistical analysis: Statistical analysis was conducted by SPSS for Windows version 11.0 program. All data were reported as means \pm standard deviation (SD). Normality for continued variables in groups was determined by the Shapiro Wilks test. The variables did not show normal distribution ($P < 0.05$). Thus, Mann-Whitney U test was used for comparison of variables between the studied groups. A value of $P < 0.05$ was considered significant.

Results

1. Excretion rates of forehead skin surface lipids in normo- and type IV hyperlipoproteinemic subjects: The sebumetric measurements on the foreheads of the subjects were performed between 08:00 and 10:00 am at room temperature and relative humidity of $21.7 \pm 0.8^\circ\text{C}$ and $51.4 \pm 9.3\%$, respectively. For all measurements of skin parameters, it is important to keep constant ambient conditions. The optimum room conditions are 20°C and 40-60% relative humidity.

Sebum value in $\mu\text{g sebum}/\text{cm}^2$ skin/3h of normolipoproteinemic subjects was found to be 110.3 ± 43.7 (n:15), which was slightly lower compared to that in patients with type IV hyperlipoproteinemia (120.7 ± 45.6 ; n:19). However, the difference was not statistically significant. The sebum values were within the normal range (100-220) indicated for healthy skin at normal room conditions (20°C and 40-60% air humidity).

2. Lipid composition of forehead skin surface lipids by HPTLC: Skin surface lipids were sampled from the forehead and recovered by extraction into hexane and analyzed. Lipids were separated into the following lipid classes by HPTLC: squalene, wax esters + cholesteryl ester, triacylglycerol and free fatty acids. The percentage of each lipid class was calculated from densitometric scanning pattern of the charred plates by the measurement of the densitometric peak height for each spot. The percentage of each lipid class and their peak height rates in lipids from foreheads of normo- and hyperlipoproteinemic subjects are shown in Table 1.

It can be seen that the percentage of wax ester + cholesteryl ester in skin surface lipids of type IV hyperlipoproteinemic subjects was increased as compared to that in normolipoproteinemic subjects. Accordingly, there is a decrease in the ratio of triacylglycerol/wax ester + cholesteryl ester, while wax ester + cholesteryl ester/squalene ratio increased significantly. Since it has been demonstrated that wax esters are of sebaceous gland origin, this represents a change in the lipid composition of sebum.

3. Serum lipid, apolipoprotein A-I, B and lipoprotein profiles of normo- and type IV hyperlipoproteinemic subjects: Agarose gel electrophoresis of serum lipoproteins was performed and the percentage distribution of serum lipoproteins was evaluated by densitometric analysis at 600nm (Table 2). Densitometric analysis of Sudan Black B-stained lipoprotein bands revealed a marked increase in pre- β lipoproteins (VLDL) without chylomicrons, whereas α -lipoproteins (HDL) values were reduced in the patient group. In all subjects, sera were subjected to agarose gel electrophoresis. Lipoprotein electrophoresis showed a type IV pattern (familial hypertriacylglycerolemia), which is characterized by high levels of VLDL. Serum triacylglycerol and cholesterol levels were clearly higher compared to that in normolipoproteinemic subjects,

Table 1. Percentage compositions and ratios of skin surface lipid constituents of normo- and type IV hyperlipoproteinemic subjects quantified by peak heights on chromatograms.

	Normolipoproteinemic subjects	Type IV hyperlipoproteinemic subjects	p value
%FFA	22.7 ± 4.3 (n=15)	22.5 ± 4.8 (n=21)	NS
%TG	30.1 ± 6.0 (n=15)	28.8 ± 5.9 (n=21)	NS
%WE+CE	31.7 ± 3.6 (n=15)	35.7 ± 3.9 (n=21)	0.004
%SQ	15.6 ± 5.7 (n=15)	13.1 ± 2.5 (n=21)	NS
TG/FFA	1.39 ± 0.44	1.38 ± 0.53	NS
TG/WE+CE	0.96 ± 0.19	0.82 ± 0.22	0.048
TG/SQ	2.28 ± 1.15	2.31 ± 0.79	NS
WE+CE/SQ	2.30 ± 0.89	2.85 ± 0.81	0.042

FFA: Free fatty acids. TG: Triacylglycerol. WE: Wax ester. CE: Cholesteryl ester. SQ: Squalene. NS: Not significant.

Table 2. Serum lipid, lipoprotein and apolipoprotein compositions of normo- and type IV hyperlipoproteinemic patients.

	Normolipoproteinemic subjects	Type IV hyperlipoproteinemic subjects	p value
% LDL	36.9 ± 9.9 (n=14)	34.3 ± 11.2 (n=18)	NS
% VLDL	29.6 ± 9.0 (n=14)	43.3 ± 13.6 (n=18)	0.005
% HDL	33.6 ± 7.7 (n=14)	22.4 ± 6.9 (n=18)	0.0001
HDL-Cholesterol (mg/dL)	43.4 ± 10.1 (n=14)	37.7 ± 10.8 (n=21)	NS
Cholesterol (mg/dL)	228.7 ± 37.2 (n=15)	80.5 ± 83.4 (n=21)	0.020
Triacylglycerol (mg/dL)	187.1 ± 76.0 (n=15)	664.0 ± 270.3 (n=21)	0.0001
Apo A-I (g/L)	1.26 ± 0.43 (n=13)	1.26 ± 0.37 (n=14)	NS
Apo B (g/L)	1.33 ± 0.31 (n=15)	1.29 ± 0.28 (n=14)	NS

NS: Not significant.

whereas HDL cholesterol was under the normolipidemic level.

Mean fasting plasma lipid, lipoprotein and apolipoprotein concentrations for the normolipoproteinemic and type IV hyperlipoproteinemic subjects are shown in Table 2. Apo A-I and apo B concentrations were not significantly different in type IV compared to normolipidemic patients.

Discussion

Human skin surface lipids are a very complex mixture, which has been analyzed using various techniques. The average percentage composition of the mixture was reported to be 19.5-63.6% triacylglycerol, 0-11.5% free fatty acids, 9.4-29.0% wax ester + cholesteryl ester, 0-1.5% cholesterol, 12-16.7% squalene and some other minor lipid fractions (1,14-17), which were close to our results.

Sebaceous glands are under hormonal control. Androgens and estrogens act on the gland antagonistically (18,19). As a result, sebaceous gland activity varies with sex. In the present study, female adults were excluded in order to avoid the effect of hormonal variation on sebum synthesis and composition. The sebaceous glands in men show age-related differences in their activity. Sebaceous secretion is low in children and increases until the late teens under the influences of androgens, after which no significant change takes place until late in life (4,14). It follows that no significant variation in the composition or synthesis of sebum can be expected in men aged between 30 to 50 years. Therefore, in our study, differences in sebum composition of normo- and hyperlipoproteinemic subjects can be attributed to differences in their blood lipid/lipoprotein level.

In our HPTLC analysis, we were unable to separate wax esters and cholesteryl esters satisfactorily. Therefore, these lipid fractions were considered as a whole (wax ester + cholesteryl ester). Since the contribution of cholesteryl esters to sebum lipids is very limited (1-2%) (1,17), peak heights for wax esters + cholesteryl esters could almost entirely represent wax esters. Wax ester content or the wax ester-to-cholesterol ester ratio of sebum reflects differences in sebaceous gland activity. During periods of increased activity, the sebaceous glands would be expected to synthesize wax esters more than that of the sterol esters that are produced primarily by the epidermis (2). Cooper et al. (20) reported that at high rates of dermal lipogenesis in male subjects, there was a small but significant increase in wax ester relative to triglyceride and squalene. Based on our results, a relative increase in wax esters + cholesteryl esters can be seen in the sebum of hyperlipoproteinemic subjects. This may represent an increase in sebaceous gland activity in these subjects. Although there was a slight increase in sebumetric measurements of hyperlipoproteinemic compared to the normolipidemic subjects, this was found to be statistically insignificant and can be a result of high variations between subjects.

Forehead skin produces a considerable amount of lipid, which was reported to be between 1.25-3.33 mg lipid/10 cm² skin/3h (3,4,5). It is therefore conceivable to consider the involvement of blood lipids/lipoproteins as an exogenous lipid source in sebum synthesis. Some of the sebum lipids are synthesized by sebaceous cells while

some of the free fatty acids (6) and cholesterol (7) were reported to be derived from the plasma. Wax esters are the major component of mammalian sebum and synthesized by wax synthase enzymes, which conjugate a long chain fatty alcohol, synthesized by fatty acyl-CoA reductase, to a fatty acyl-CoA via an ester linkage (21,22). Substrate preferences of fatty acyl-CoA reductase or additional factors (22), endogenous lipid versus exogenous lipid, may influence the wax ester content of sebum.

Type IV hyperlipoproteinemia is characterized by the production of large VLDL with abnormally high triglyceride content. Plasma LDL cholesterol and apo B-100 concentrations are normal (11). However, some investigators reported that apo B increased in type IV hyperlipoproteinemia (23,24). Apo A-I was normal (25) or below the normal level (23), and HDL cholesterol is often decreased (11,26). Blood lipid, lipoprotein and apolipoprotein patterns of hyperlipoproteinemic subjects in our study are valid for type IV hyperlipoproteinemia.

Interaction of blood lipoproteins with sebum secretion rate and composition can be inferred from various studies. Overexpression of apo C-I, which is known to have an inhibitory role on hepatic remnant uptake, reduced sebum triacylglycerol and wax esters significantly. Elevated levels of serum cholesterol and triacylglycerol, due to an accumulation of VLDL remnants in the circulation, were also reported to occur in apo C-I transgenic mice (8). Smythe et al. (27) found that 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity in sebaceous glands was downregulated by exogenous cholesterol, LDL. Association between sebaceous gland activity and the serum lipid level can also be deduced from several works on retinoic acid metabolism. Retinoic acid derivatives (isotretinoin, 13-cis retinoic acid) used for the treatment of severe acne were reported to act primarily by reducing sebaceous gland size and sebum production while increasing serum concentrations of apo B, total cholesterol, LDL cholesterol and triacylglycerol (9,10). Contradictory data is also available. It was reported that inhibitors of cholesterol synthesis (aluminium nicotinate and clofibrate) had no effect on sebum production in patients with acne (28). However, they did not analyze the sebum lipid composition.

The question that arises is what is the link between hyperlipoproteinemia (type IV) and the increase in

proportion of wax esters + cholesteryl esters in sebum? The answer may lie in remnant-like particles, which were reported to be isolated from the plasma of type IV hyperlipoproteinemic subjects (29,30). Whether these particles can be taken up by the sebaceous glands is currently unknown.

In conclusion, the relative proportion of wax esters + cholesteryl esters in sebum of type IV hyperlipoproteinemic subjects is increased. It appears that plasma triacylglycerol concentration affects sebum wax ester+cholesteryl ester content. Sebum lipid composition could be reflective of blood lipid/lipoprotein profile, which

can make it possible for blood lipids to be estimated through skin surface lipid analysis. Moreover, viscosity and hydrophobic properties of sebum result mainly from lipid composition. Variation in sebum lipid composition may alter its protective properties against wetting and microorganisms and, as a result, against skin disorders.

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