

# EFFECT OF PHOSPHATIDYLCHOLINE LINOLEIC ACID-RICH AND GLYCOLIC ACID IN ACNE VULGARIS

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## Synopsis

Several studies have highlighted as the concentration of linoleic acid in the sebum and in the epidermal lipids seems to be strictly connected to the onset of different pathologies, and among those of acne.

It has been found also that the wax ester content of the sebum and of the epidermal acylceramides containing linoleic acid seems to be inversely proportional to the sebum production.

It has been proven that the acylceramides of the comedones and the skin surface of acne patients contain much less linoleic acid than the acylceramides from the skin surface of control subjects (6% versus 45% in the normal physiological state).

Other studies seem to suggest that a localized increase of squalene and oleic acid and contemporary reduction in levels of linoleate esterified to ceramide 1 should be considered as a causative factor in comedogenesis and acne.

The present study was conducted on 40 voluntar patients in order to determine whether the supposed overproduction of oleic acid, squalene and sebum, of an acne-affected skin could be influenced by topical application of cosmetic emulsions containing as active ingredients an high concentration of esterified linoleic acid together with glycolic acid buffered by a special mixture of aminoacids.

All the treated patients showed a significant decrease of the free fatty acids/triglycerides ratio together with decrease of the skin surface lipids.

From the other hand the obtained clinical results show that the contemporary use in the same phospholipidic emulsion of glycolic acid partially buffered with aminoacids, of salicylic acid and chlorhexidine digluconate, can effectively relieve the severity of acne ever since the first weeks of treatment.

Given the positive results obtained we believe that this cream can be considered as a new cosmeceutical and a useful aid for the normal acne therapies.

## Riassunto

Diversi studi hanno messo in luce come la concentrazione di acido linoleico nel sebo e nei lipidi dell'epidermide sembri essere strettamente correlata all'insorgere di diverse patologie, tra cui l'acne. È stato anche scoperto che il contenuto del sebo e delle acilcerammidi epidermiche contenenti acido linoleico sembrano essere inversamente proporzionali alla produzione del sebo.

È stato provato che le acilcerammidi dei comedoni e la superficie cutanea dei pazienti affetti da acne contengono molto meno acido linoleico delle acilcerammidi della superficie cutanea dei soggetti controllati (6% contro il 45% del normale stato fisiologico).

Il presente studio è stato condotto su 40 volontari con lo scopo di determinare se la supposta sovrapproduzione di acido oleico, squalene e sebo di una pelle affetta da acne potesse essere influenzata da applicazioni topiche di emulsioni cosmetiche contenenti come principi attivi un'alta concentrazione di acido linoleico esterificato insieme ad acido glicolico tamponato da una speciale miscela di aminoacidi.

Tutti i pazienti trattati hanno mostrato una riduzione significativa del rapporto acidi grassi liberi/trigliceridi, insieme ad una diminuzione dei lipidi cutanei di superficie.

D'altro canto i risultati clinici ottenuti dimostrano che l'uso contemporaneo nella stessa emulsione fosfolipidica di acido glicolico parzialmente tamponato con aminoacidi, di acido salicilico e di clorexidina digluconato possono migliorare efficacemente la gravità dell'acne già dalle prime settimane di trattamento.

Dati i positivi risultati ottenuti riteniamo che questa crema possa essere considerata, quale nuovo cosmeceutico, di utile ausilio nelle normali terapie dell'acne.

## INTRODUCTION

Several studies have highlighted as the concentration of linoleic acid in the sebum and in the epidermal lipids seems to be strictly connected to the onset of different pathologies, and among those of acne.

As it is well-known linoleic acid seems to represent a fundamental element of ceramide 1 considered the main repository of essential fatty acids in the stratum corneum, although it is also esterified to the corneocyte envelope and N-acylated to sphingosine in ceramide 2 (1-3).

It has been found also that the wax ester content of the sebum and of the epidermal acylceramides containing linoleic acid seems to be inversely proportional to the sebum production (4).

It has been proven that the acylceramides of the comedones and the skin surface of acne patients contain much less linoleic acid than the acylceramides from the skin surface of control subjects (6% versus 45% in the normal physiological state)(1,5). Other studies seem to suggest that a localized increase of squalene and oleic acid and contemporary reductions in levels of linoleate esterified to ceramide 1 should be considered as a causative factor in comedogenesis and acne (1,6-8).

The present study was conducted to determine whether the supposed overproduction of oleic acid, squalene and sebum, of an acne-affected skin could be influenced by topical application of cosmetic emulsions containing as active ingredients an high concentration of esterified linoleic acid together with glycolic acid buffered by a special mixture of aminoacids (9,10).

In order to define the activity of these emulsions, fourthy voluntar patients suffering from acne vulgaris were controlled with regard to the total quantity of sebum and free fatty acids/triglycerides ratio, and to the total number of inflammatory lesions.

Were also controlled the superficial skin-lipids and the skin hydration. These parameters surely are not normal at the level of acne-affected skin.

## MATERIALS AND METHODS

### Materials

**Vehicle:** soybean liposome containing 10% lecithin fraction with 80% phosphatidylcholine linoleic acid-rich (cream B)

**Active ingredients:** vehicle + glycolic acid buffered to pH 4.5 by a special blend of aminoacids - clorexidine digluconate and salicylic acid (cream A).

Even if the same vehicle (7) should be considered as an active ingredient having proven of being capable of increasing the presence of linoleic acid to detriment of oleic acid and squalene, as active ingredients have been utilized **glycolic acid**, aminoacid-buffered and salicylic acid for their well-known keratolytic activity (13,14). **Clorexydine digluconate** to reduce excessive presence of propionibacterium acne and of all the surface aerobic flora (15).

With this particular formulation we intended to reduce the keratinized Stratum Corneum excess through the use of salicylic and glycolic acid; to diminish the sebum and fatty acids production through the use of linoleic and buffered glycolic acid; to decrease both the aerobic and the anaerobic flora by using the clorexydine digluconate. The used soybean vehicle is capable of penetrating through the pilosebaceous follicles dragging with itself the used active ingredients.

### Patients

Forthy healthy volunteers of both sexes (20 women, 20 men) with an average age of  $16 \pm 3$  years with a mild to moderate acne vulgaris, participated in this study after providing informed consent. The nature of the study was explained to them in full.

All patients were required to have a Cunliffe score of at least 1.0 but less than 4 (11).

Exclusion criteria included patients with more than five nodules and cysts and patients who had used topical antibiotics, retinoids or ben-

zoyl peroxide in the past 14 days, systemic antibiotics in the past 30 days, systemic retinoids in the past 2 years, any other topical acne treatments including medicated soaps, creams or make up in the past 7 days, topical corticosteroids in the past 14 days or systemic corticosteroids in the past 12 weeks.

### **Test substance**

Each patient was supplied with two tubes labelled as cream A and cream B together with a cleansing cream (Mavigen® Idroschiuma).

### **Procedure**

This was a 12-week, randomized, double-blind, vehicle-controlled study. The subjects were instructed to apply the test-creams on their face twice a day for three months, and they were not allowed to use any other skin care product during the study. Each subject was used as his or her own control; the test creams (A and B) being applied on a randomized basis, on the right or on the left area of the face.

Moreover they were instructed to apply the same cream always to the designed site after washing, first thing in the morning and just before retiring in the evening. Subjects were also instructed that only the cleansing cream supplied to each at the beginning of the study should be used to cleanse the test area.

Other instructions included that the patients use no other acne treatment during the study and not to apply the creams the day of evaluations or wash their face 4 hours before evaluations.

### **Clinical evaluation**

Clinical examinations were performed on the first day (baseline), and at 2, 4, 6, 8, 10 and 12 week (end of the treatment). Clinical evaluations included individual lesion counts of open and closed comedones, papules, and pustules, and were quantified by separately calculating them by means of a transparent millimeter grid of cellophane, according to Morganti et al (16).

### **Biophysical non-invasive measurements**

Measurements were performed, on the 1st day (baseline), after 2, 4, 6, 8, 10 and 12 weeks, (end of the treatment), by means of the computerized 3C System (Dermotech, Rome, Italy) (17). This instrument measures the surface skin lipids having absorbed them by a special frosted plastic foil. The determinations were always carried out on four sites of right or left areas (forehead, cheek, chin and nose) before evaluating the patients for the calculation of inflammatory lesions. To achieve an higher degree of assurance all evaluations were performed after a 30 minutes acclimatization period in a room at 21°C to 22°C and 45% to 50 humidity, even if the 3C System automatically adjusts environmental conditions to 22°C and 50% relative humidity.

### **Measurement equipment**

**Skin surface lipids.** The skin surface lipid levels were measured with the 3C System® (Dermotech S.r.l., Rome, Italy) (17). Determination is based on photometric measurement of light transmission through a skin surface imprint obtained applying to the designed skin area a frosted plastic foil. It allows adherence of skin lipids in a 1 cm<sup>2</sup> area. The obtained readings are automatically converted into (µg/cm<sup>2</sup>).

### **Free fatty acids/triglycerides ratio**

The special and protected plastic foil was applied on the four areas with gentle pressure by 20 strokes of a gloved finger and carefully removed. Stratum corneum lipids were extracted from the frosted plastic foil using chloroform: methanol (2:1) for 2 hours at room temperature, the solvent was dried under nitrogen and the lipids were redissolved in chloroform. Lipid fractions were chromatographically separated on 0.25mm-thick layers of silica gel into their individual lipid classes and were successively separated by plastic foil contaminants,

using solid phase extraction columns, according to Cavina et al. (18). All lipid fractions, redissolved in chloroform, were stored at -20°C until required.

The isolated triglycerides and free fatty acids were then quantified separately in order to determine the free fatty acids/triglycerides ratio.

### **Skin hydration**

The hydration of the horny layer was assessed by measuring electrical capacitance of the skin surface by means of the 3C System® (17).

When the probe is applied to the skin (recording time 0.5 s), the capacitance is displayed digitally in arbitrary 3C units. The results are expressed as mean values of the measurements performed on four different right or left sites (cheek, forehead, chin and nose).

### **Statistical analysis**

Student's test was used in evaluation of all the data before and after the treatment period. All the analyses were produced using the SAS statistical package, version 5.18 (SAS Institute Inc., Cary, N.C.). Probabilities less than 0.05 were considered significant.

## **RESULTS**

### **Clinical evaluation**

Of the 40 patients enrolled 36 patients (90%) completed the study. Four patients (3 active treated, 1 vehicle-treated) dropped from the study for the following reasons: two for skin irritation (active-treated), due probably to glycolic acid, and two for personal reasons. The mean results of the clinical parameters evaluated are shown in Table I and Fig.1.

Both the treatments, cream A and cream B, had significantly reduced all the acne lesion counts, even if cream A (active) has proven more effective than cream B (vehicle) and having a quicker activity. In fact after the first month of treatment all the inflammatory le-

sions improved as follows: A treatment (A cream - active) 33% on average if compared with the basic values;

B treatment (B cream - vehicle) about 17%.

After three months of treatment improvements were noticed on all the lesions counts with the A cream (active) and they resulted very high, equal to about 80% (Tab. I and Fig. 1).



FIG 1

The vehicle also, however, has proven to be, as it was expected, highly effective (46%) towards all the checked parameters. This fact has further proven the activity carried out by the high concentration of the linoleic acid in the liposomal emulsion.

**Table I**

**MEAN INFIAMMATORY COUNTS IN MILD ACNE TREATED BY  
PHOSPHATIDYLCHOLINE-CREAM GLYCOLIC ACID ENRICHED**  
n=36

ACNE LESIONS COUNTS	Baseline	Week 4	Week 8	Week 12 change	Reduction
<b>Open Comedones</b>					
Baseline	34.3				
Active (cream A)		22	15 (55%)	12 (35%)	-65%
Vehicle (cream B)		27	20	17	-50%
<b>Closed Comedones</b>					
Baseline	43				
Active (cream A)		33	25	20	-53%
Vehicle (cream B)		38	32	26	-39%
<b>Pustoles</b>					
Baseline	7				
Active (cream A)		5	3	1	-86%
Vehicle (cream B)		6	4	3	-57%
<b>Papules</b>					
Baseline	37				
Active (cream A)		20	15	12	-67%
Vehicle (cream B)		29	20	18	-51%

All p values are significant ( $p < 0.01$ ) as to groups and highly ( $p < 0.005$ ) significant as baseline values.

### **Skin surface lipids**

All the patients showed at baseline an higher content of surface lipids (casual level) ( $175 \pm 50 \mu\text{g}/\text{cm}^2$ ).

At week 2 the values already decreased of about 70% for both the vehicle and active cream. As seen in Fig. 2 and Tab. II both the vehicle (B cream) and the active cream (A cream) are able to reduce the skin lipids casual level since the first month of treatment (- 70%) proving a remarkable topic effectiveness.

These values seem to prove what stated by Ghyzcy et al (7): phospholipidic emulsions, when rich in linoleic acid, are able to reach the cells of the sebaceous gland inducing them to reduce the sebum secretion.

The high percentage of linoleic acid, entering into competition with the oleic acid, due to a mechanism yet unknown, would act as a "brake", reducing its excessive presence also at level of the surface lipidic film.

**Table II**

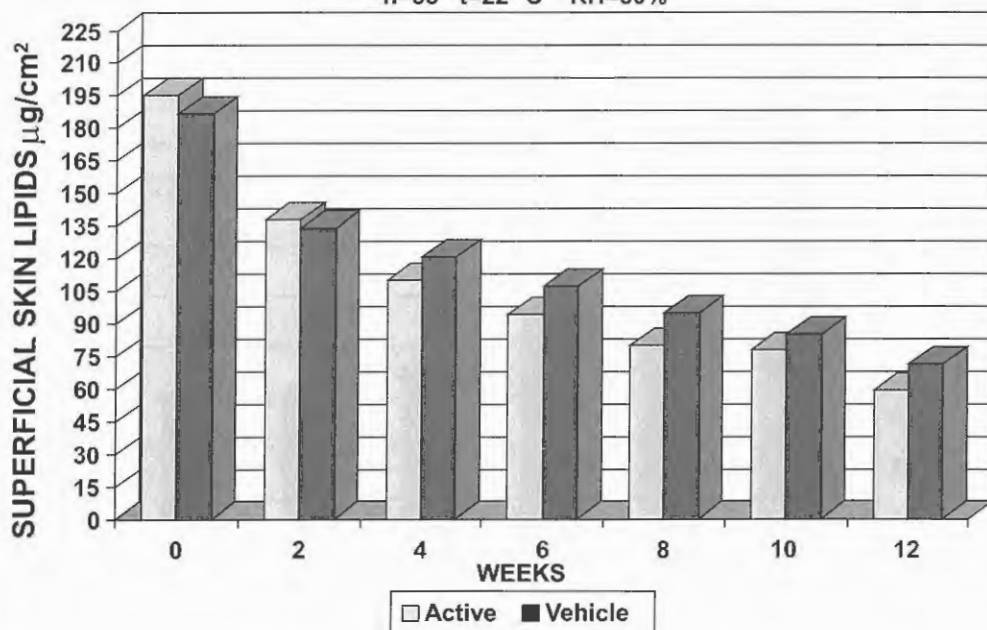
**REDUCTION OF SURFACE SKIN LIPIDS BY  
A PHOSPHATIDYLCHOLINE-CREAM GLYCOLIC ACID ENRICHED**  
( $\mu\text{g}/\text{cm}^2$ ) n=36 t=22°C RH=50%

	Weeks						
	0	2	4	6	8	10	12
Active baseline	195±50	138±33	110±24	94±24	80±18	78±21	59±16
Vehicle baseline	187±48	134±35	121±31	107±27	95±25	85±22	71±18

All p values are not significant as to groups and highly significant ( $p < 0.005$ ) as to baseline values.

**REDUCTION OF SURFACE SKIN LIPIDS BY A PHOSPHATIDYLCHOLINE-CREAM GLYCOLIC ACIDS ENRICHED**

n=36 t=22 °C RH=50%



All p values are not significant as to groups and highly significant ( $p < 0.005$ ) as to baseline values

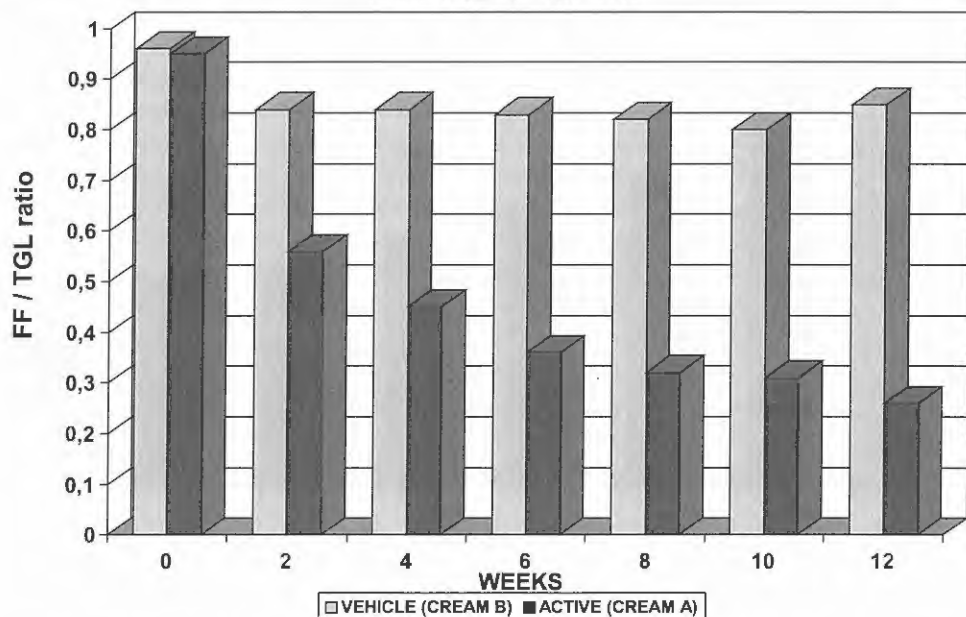
**Table III**

**EFFECT OF TOPICAL APPLICATION OF PHOSPHATIDYLCHOLINE-CREAM  
GLYCOLIC ACID ENRICHED ON FREE FATTY ACIDS/TRIGLYCERIDES RATIO**  
n=36 t=22°C RH=50%

Product	number of patients	Weeks						
		0	2	4	6	8	10	12
Vehicle (cream B)	36	0.96	0.84	0.84	0.88	0.82	0.80	0.85
Active (cream A)	36	0.95	0.56	0.45	0.36	0.32	0.31	0.26

All p values are highly significant both as to groups ( $p < 0.005$ ) and to baseline values.

**EFFECT OF TOPICAL APPLICATION OF A PHOSPHATIDYLCHOLINE-CREAM  
GLYCOLIC ACID ENRICHED ON FREE FATTY ACIDS / TRIGLYCERIDES RATIO**  
n=36 t=22°C RH=50%



All p values are highly significant ( $p < 0.005$ ) both as to groups and to baseline values

Fig 3



**Table IV**

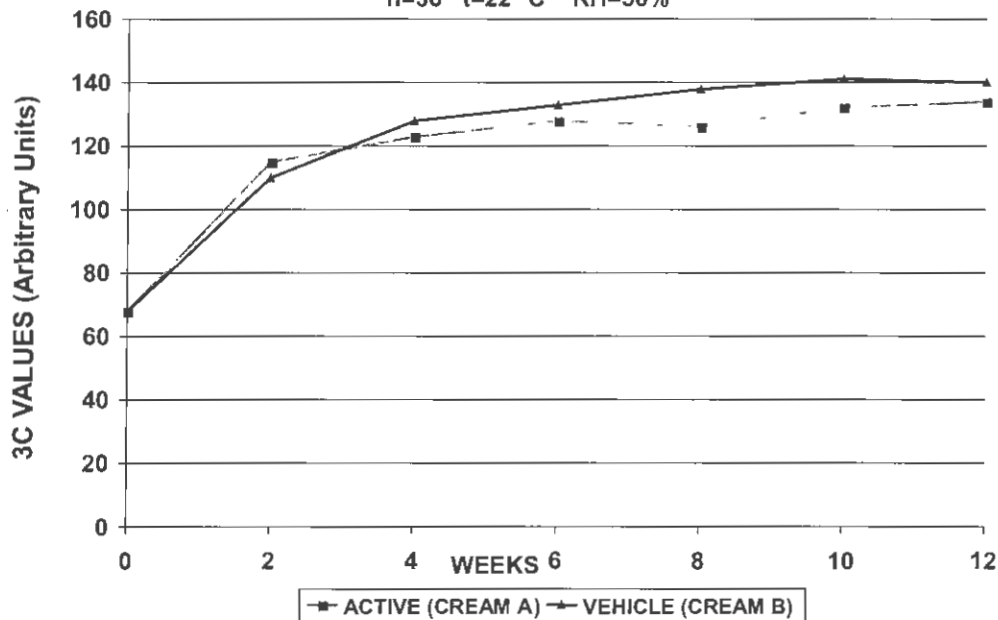
**MEAN VALUES OF SKIN HYDRATION IN ACNE PATIENTS TREATED BY  
A GLYCOLIC ACID ENRICHED PHOSPHATIDYLCHOLINE CREAM**  
n=36 t=22°C RH=50%

	Weeks						
	0	2	4	6	8	10	12
Active	78±11	115±17	123±14	128±21	126±19	132±20	134±24
Vehicle	74±9	110±	128±16	133±17	138±18	141±23	140±22

All p values are not significant as to groups and highly significant ( $p < 0.005$ ) as to baseline values.

**MEAN VALUES OF SKIN HYDRATION IN ACNE PATIENTS TREATED BY A  
PHOSPHATIDYLCHOLINE-CREAM GLYCOLIC ACID ENRICHED**

n=36 t=22 °C RH=50%



All p values are not significant as to groups and highly significant ( $p < 0.005$ ) as to baseline values

Fig 4

The contemporary presence of the glycolic acid would facilitate, moreover, the transcutaneous penetration of phospholipids, improving in this way their activity.

### **Free fatty Acids/Triglycerides ratio**

This particular phospholipidic emulsion has proven to be highly active also towards another parameter of the acne development, namely the presence of free fatty acids, that are the main cause of the abnormal intrafollicular keratinization. Free fatty acids have an inflammatory effect and become potential irritants, which when released into the dermis cause cysts to form.

As seen in Fig. 3 and Tab. III the free fatty acids/triglycerides mean ratio decreases of 41% just after two weeks of treatment, and further reduces up to about 71% after three months.

During this time period it seems that this activity is performed only by the active cream (A cream) for the probable antiseptic activity carried out by the glycolic acid, and particularly by the salicylic acid and the clorexidine digluconate. In fact, as it has been proven elsewhere, there is a significant reduction of propionibacterium acnes colonies, with a subsequent drop in the production both of enzyme esterase and of free fatty acids (19).

### **Skin hydration**

All the patients showed a significant increase in moisture content and maintained these values until the end of the study. The results are shown in Table IV and Fig. 4.

## **DISCUSSION**

In acne, as known, defects in the keratinization of the "epithelial" lining of the pilosebaceous follicle block egress of sebum, leading to "plugging".

Propionibacterium acnes, exerts lipolytic effect on stagnant sebum, releases free fatty acids and causes inflammation when they escape from the pilosebaceous follicle.

In this study, as also suggested from Ghyczy et

al (7) the use of soybean derived phosphatidylcholine seems to be able to normalize excess of sebum.

All the treated patients showed a significant decrease of the free fatty acids/triglycerides ratio together with decrease of the skin surface lipids (Tab III and Fig. 3).

From the other hand the obtained clinical results show that the contemporary use in the same phospholipidic emulsion of glycolic acid partially buffered with aminoacids, of salicylic acid and clorexidine digluconate, can effectively relieve the severity of acne ever since the first weeks of treatment. It has been noted an high reduction of all the inflammatory acne lesions such as small or large solid bumps, pus pimples, "blackheads" and whiteheads (Fig. 1 and Tab. I). The use of the glycolic acid, even if in rather high concentration (10%) did not cause clear irritating forms or stinging activity.

Only two patients had to stop immediately the treatment, considering it not suitable for their skin type.

The high bearableness of the used compound is due, in our opinion, both to the strong presence of phospholipids and to the special buffer we already used to neutralize the aggressiveness of the glycolic acid (12).

Unlike all the acne treatments utilized, this phospholipidic preparation not only reduces in drastic way the presence of lipids and acneic lesions, but rehydrates the skin since the first period of application, maintaining it elastic (Fig. 2, 4 and Tab. II, IV).

Given the positive results obtained we believe that this cream can be considered as a new cosmetic and a useful aid for the normal acne therapies.

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