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PHOTOAGING AND CHRONOLOGICAL AGING: BIOPHYSICAL PROPERTIES OF THE PHOTOEXPOSED AND PHOTOPROTECTED SKIN

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1. Introduction

Aging is a complex, multifactorial, and inevitable process [1,2]. However, different regions of the skin can undergo varying aging processes throughout life. This variation is primarily attributed to differing levels of sun exposure, which directly influences photoaging. Typically, the face, neck, and upper body are more exposed to sunlight over a lifetime [3]. Consequently, these areas exhibit more pronounced signs of aging, such as texture changes, fine lines, wrinkles, dehydration, and pigment disorders due to solar elastosis and the degradation of collagen and elastin. Additionally, the amount of melanin and lifestyle factors, including physical inactivity, lack of sleep, high sugar intake, tobacco and alcohol consumption, and emotional stress, also play significant roles in the aging process [2].

Conversely, photoprotected areas of the body could be more affected by chronological aging, influenced by genetic predisposition and hormonal regulation. In healthy individuals, this type of aging typically begins to manifest from the third decade of life and continues throughout, exhibiting signs such as skin thinning, uneven skin tone, lax appearance, dehydration due to fat tissue loss, slower cell turnover, and reduced production of sebum, glycosaminoglycans, collagen, and elastin [2,4]. However, during the aging process, the signs of intrinsic and extrinsic aging tend to overlap, making it difficult to clearly distinguish their actions [5].

In recent years, there has been a growing interest in addressing physical signs of aging, even among younger individuals. Consequently, the market for anti-aging skincare treatments and aesthetic procedures has expanded significantly in order to find new active ingredients, increase efficacy of well-known ones, and develop beauty devices. Additionally, lifestyle choices are increasingly being adapted to align with the pursuit of a youthful appearance.

Despite the ability to classify aging in clinical practice through dermatological scales, evaluating skin properties and internal structures remains challenging. Therefore, biophysical instruments are invaluable in understanding the nuances of skin aging [3,6,7]. In this context, cutaneous bioengineering, or skin biometry, involves the study of the biological, mechanical, and functional characteristics of the skin through the precise measurement of specific variables

using scientific, non-invasive methods [6]. It can also be of significant importance in monitoring treatments, allowing a thorough evaluation of the effects on human skin using study subjects under real conditions of product use [8,9].

The variety of equipment and technologies available for studying skin biology can be considered a new era in the development of dermocosmetics, based on a robust understanding of skin physiology, its variations, and its responses to treatments, intrinsic factors, and various environmental stimuli [9].

In that way, the objective of this study was to compare the biophysical characteristics of photoprotected and photoexposed areas, evaluating skin hydration, barrier integrity, and elastic properties. This comparison aimed to understand how different parts of the same individual can age when exposed to varying environmental and behavioral factors.

2. Materials and Methods

The study was conducted in accordance with the principles of the Declaration of Helsinki, applicable regulatory requirements, including CNS Resolution No. 466/12, and approved by the Independent Ethics Committee of the Faculty of Medicine of Campinas – UNICAMP under the number CAAE: 61588016.5.0000.5404.

Up to 119 healthy female and male subjects, aged 43 to 89 and phototype I to IV presenting intact skin in the test area, who met all the inclusion criteria and none of the exclusion criteria, and agreed to the study procedures, were enrolled. Non-inclusion criteria included cognitive impairment, lack of method comprehension, active infectious diseases, suspected skin cancer, immunosuppression, pregnancy, breastfeeding, neurological and psychiatric diseases, photosensitizing diseases, collagenase disorders like lupus erythematosus, recent topical retinoic acid treatment, and use of photosensitizing or immunosuppressive medications.

2.1 Intrumental Measurements

Subjects were allowed to acclimate in a controlled environment (20°C ± 2°C, 50% ± 5% humidity) for at least 15 minutes before and during all instrumental measurements. Measurements were obtained from the volar (photoprotected skin) and dorsal (photoexposed skin) regions of the subjects' forearms.

2.1.2 Stratum Corneum Hydration

To determine, indirectly, the water content in the stratum corneum, the Corneometer CM 825 PC (Courage & Khazaka, Germany), connected to Multi Probe Adapter MPA 5, was used. The device measures skin's electrical capacitance based on its water content, providing results in arbitrary units (a.u.), where 1 a.u. equals 0.2-0.9 mg of water per gram of stratum corneum, ranging from 0 (very dry) to 120 (very hydrated). Four measurements were taken in each region of the subjects' forearms (volar and dorsal), and the average of the obtained values was calculated for each region.

2.1.3 Skin Barrier Transepidermal Water Loss (TEWL)

Skin barrier integrity was measured by assessing transepidermal water loss (TEWL) using the Tewameter TM 300 (Courage & Khazaka, Germany), connected to software designed to measure TEWL based on the diffusion principle described by Adolf Fick in 1885, adapted by Capitani *et al.* (2012) [10] was used, with results expressed in grams per hour per square meter (g/h/m²). Higher values indicate greater evaporation of water through the stratum

corneum (in the absence of sweat). One measure of 45 seconds was taken in each region (volar and dorsal forearms), ensuring a maximum standard deviation of ±10% between them.

2.1.4 Skin Extensibility (R0) and Elasticity (R7)

To measure the skin's extensibility/firmness and elasticity, the Cutometer MPA 580 (Courage & Khazaka, Germany) coupled with a measurement probe was used. The principle of measuring the skin's elastic properties is based on suction and elongation [11,12]. After taking measurements, the R parameters are obtained. R0 represents the maximum curve amplitude and R7 represents the viscoelastic portion compared to the complete curve. For R0, the closer the value to 0, the greater the firmness, and for R7, the closer the value is to 1, the greater the elasticity. Measurements were taken in triplicate in the central and upper volar and dorsal forearm regions.

2.2 Statistical Analysis

Differences between areas: Skin hydration, transepidermal water loss (TEWL), firmness (R0) and elasticity (R7) were compared in relation to photoaged and photoprotected areas, independently, i.e. one analysis per parameter. A linear mixed-effects model was used to evaluate the effects of the variable 'Area' on each parameter. The model included 'Area' as a fixed effect and 'Subject' as a random effect to account for repeated measures within the same subject. The analysis was conducted using the lme4 package in R. Since R0 and R7 data are not normally distributed, Wilcoxon signed-rank test was applied for these parameters.

Relation with age and sex: In order to understand how each parameter (Skin hydration, transepidermal water loss (TEWL), firmness (R0) and elasticity (R7)) varies according to biological factors in photoexposed and photoprotected areas, two-way analysis of variance (ANOVA) was used to investigate the effects of age and sex, as well as their interaction on the skin parameters. Since R0 and R7 data are not normally distributed, a generalized linear model (GLMM) was conducted using the glmmTMB. A Gamma distribution with a log link function was used for R0 data and Beta distribution with a logit link function for R7. For all analyses, the parameters were considered the variable response, while sex and age were considered as predictor variables [13]. All statistical analyses were performed using the R environment.

3. Results

3.2 Differences between areas

Photoexposed and photoprotected skin differed for all parameters assessed. Photoexposed skin presented 39.3% less hydration, 7.2% higher transepidermal water loss (TEWL), 33.3% less extensibility and 16.6% less elasticity compared to photoprotected skin (Table1; Figure 1).

Table 1. Descriptive statistics and p value for assessed parameters on photoaged and photo-protected areas.

Parameters	Sample size	Photoexposed Area	Photoprotected Area	p-value
Skin Hydration (a.u)	118	21.93	30.51	<0.001
TEWL (g/h/m^2)	86	11.12	10.29	<0.001
R0 (Skin firmness)	84	0.255	0.348	<0.001
R7 (Skin Elasticity)	84	0.266	0.310	<0.001

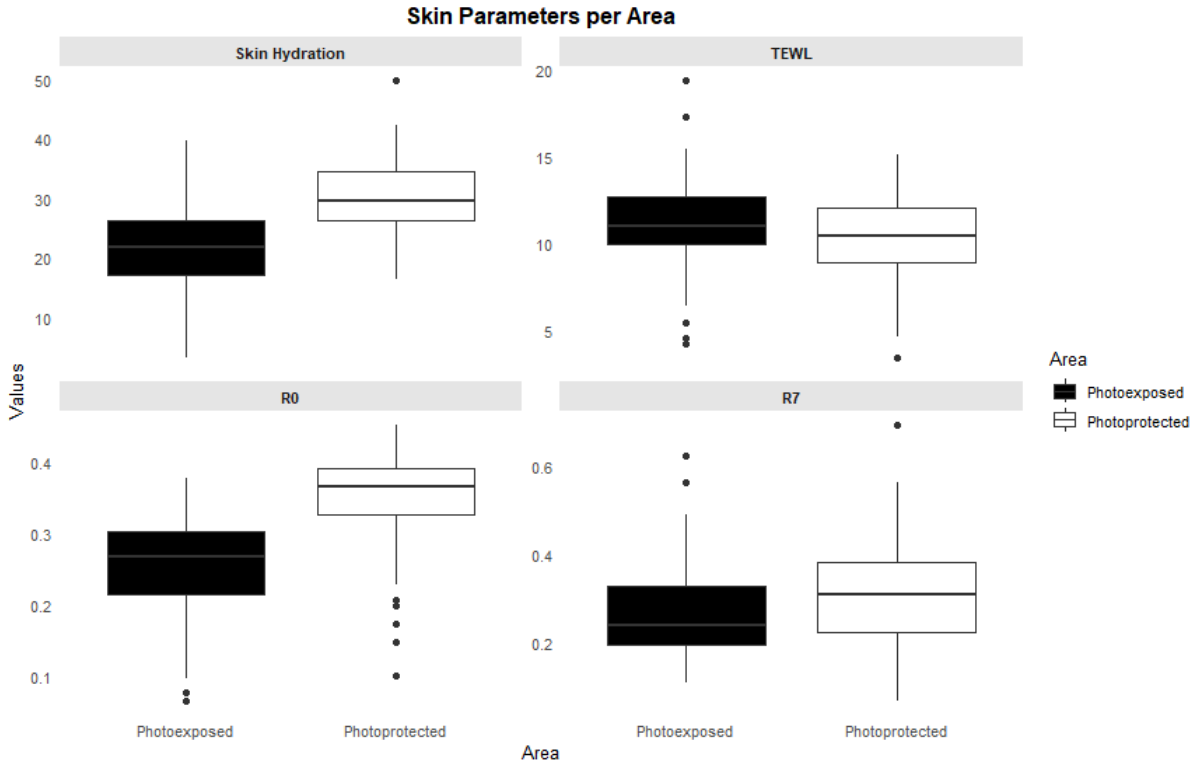

Figure 1. Evaluation of Skin Biophysical Properties. The graphs show the data from Stratum Corneum Hydration (device: Corneometer CM 825), Cutaneous Barrier Integrity - Transepidermal Water Loss (TEWL) (device: Tewameter TM 300) and Evaluation of skin firmness (R0) and Elasticity (R7) (device: Cutometer MPA 580 (Courage and Khazaka, Germany)).

3.3 Relation with age and sex

In photoprotected skin, while the hydration level, TEWL and skin extensibility (R0) did not vary according to age and sex, a relation between R7 and age was observed ($p < 0.001$), in which the higher the age the lower the skin elasticity (Figure 2).

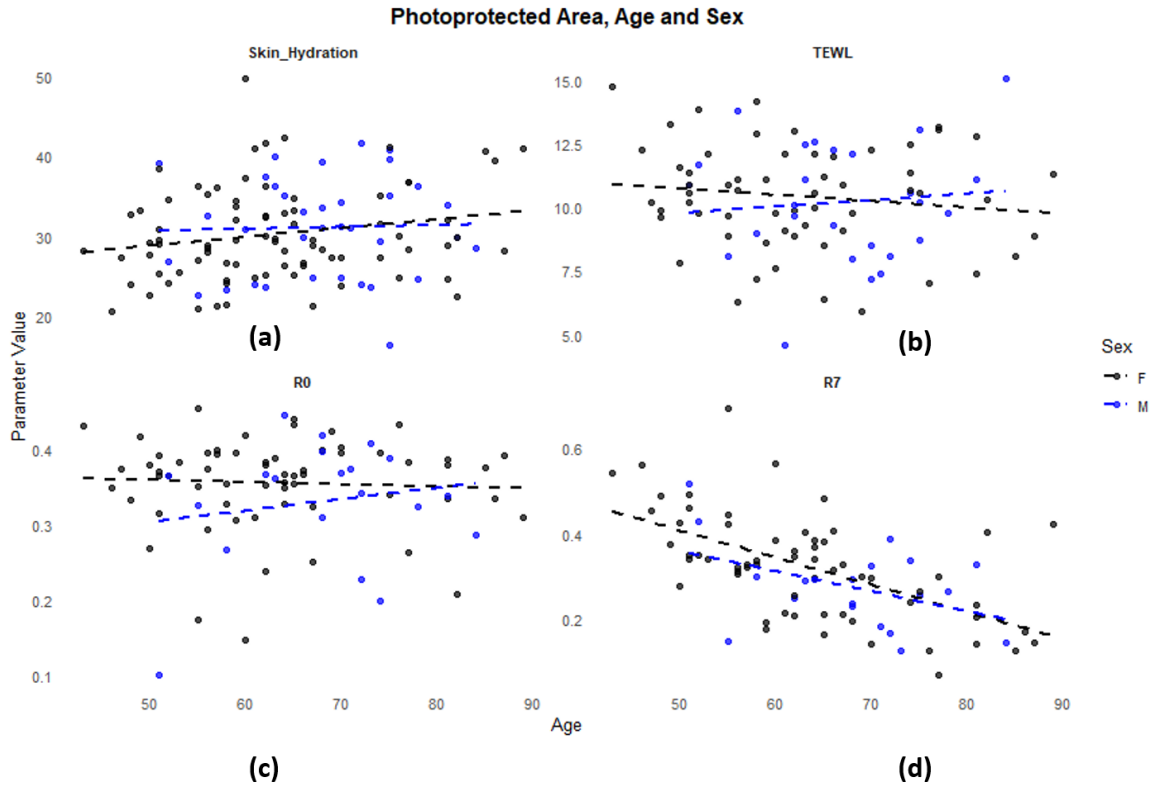

Figure 2. Relation among the biophysical properties of the photoprotected skin *versus* age, and sex, respectively. Data represented: **(a)** Stratum Corneum Hydration (device: Corneometer CM 825), **(b)** Cutaneous Barrier Integrity - Transepidermal Water Loss (TEWL) (device: Tewameter TM 300), **(c)** Evaluation of skin firmness (R0) and **(d)** Elasticity (R7) (device: Cutometer MPA 580, Courage and Khazaka, Germany). The black and blue dots represent females and males, respectively, and the serrated lines represent the tendency of the data according to age.

For photoexposed skin, TEWL and R0 did not vary according to these factors. In photoaged skin, hydration levels differed according to age (p=0.001) and Sex (p=0.004), in which higher skin hydration was observed with age and females presented higher hydration than males. In relation to elasticity (R7), the parameter variation was related only to Sex (p=0.049), in which males presented a higher skin elasticity compared to females (Figure 3).

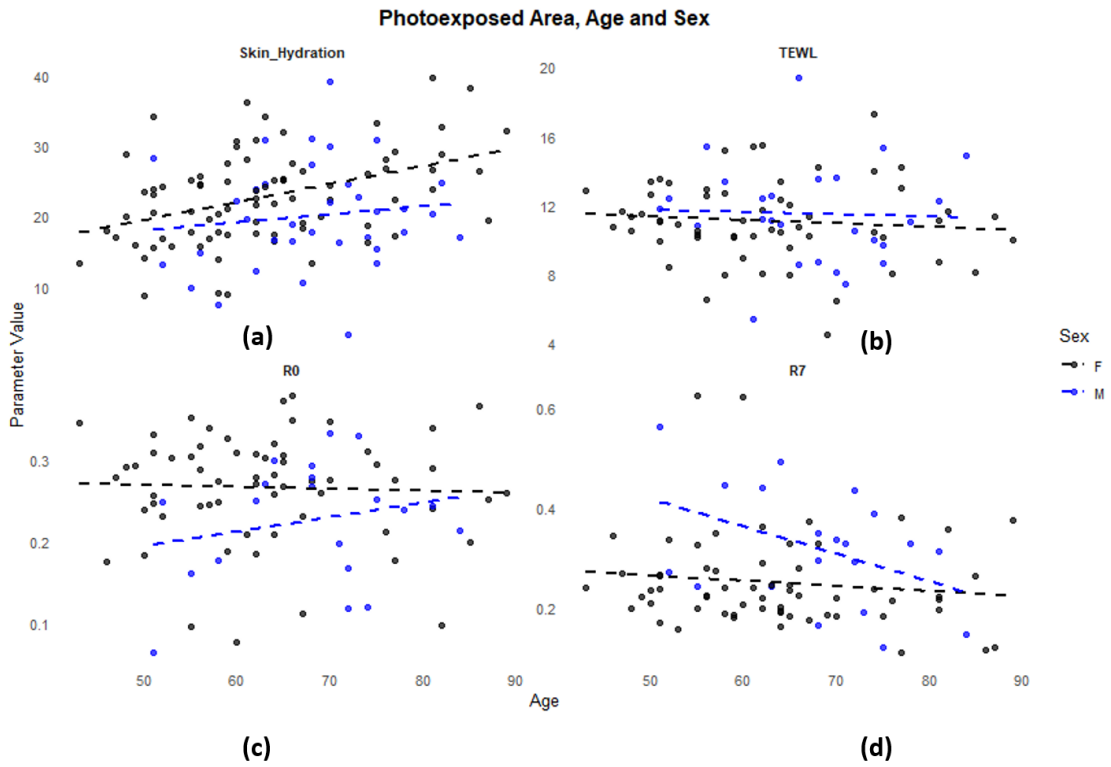

Figure 3. Relation among the biophysical properties of the photoexposed skin *versus* age, and sex, respectively. Data represented: **(a)** Stratum Corneum Hydration (device: Corneometer CM 825), **(b)** Cutaneous Barrier Integrity - Transepidermal Water Loss (TEWL) (device: Tewameter TM 300), **(c)** Evaluation of skin firmness (R0) and **(d)** Elasticity (R7) (device: Cutometer MPA 580, Courage and Khazaka, Germany). The black and blue dots represent females and males, respectively, and the serrated lines represent the tendency of the data according to age.

4. Discussion

Regions of body that are exposed to the sun present distinct biophysical properties values than photoprotected ones and these properties may or may not be related to chronological aging process and hormonal differences. Photoprotected skin regions presented higher skin hydration, lower transepidermal water loss (TEWL), higher skin extensibility (R0) and elasticity (R7) than photoexposed area. These results suggest a better skin barrier function and elastic properties in photoprotected skin compared to photoexposed skin. In that way, a lower hydration and higher TEWL in photoexposed skin is an indicative of a disfunctional skin barrier.

Chronic sun exposure can cause various changes in the skin. The epidermis, for example, loses its function due to damage to the integrity of the skin barrier, the reduction of the natural moisturizing factor (NMF), and the lipid bilayer, causing issues such as increased transepidermal water loss, and consequent aggravation of xerosis, increased transdermal drug delivery, increased sensitivity to irritants, and development of pruritus. These components, in addition to forming the barrier and helping in water retention in the cells, lubricate the structure, promoting mobility [14]. The UV radiation can also leads to solar elastosis, characterized by the degradation of elastic fibers and the replacement of collagen fibers with disorganized elastic fibers, and this process results in thicker, less elastic skin. Over time, elastosis compromises the skin's barrier function, increasing TEWL and reducing hydration [15,16].

It is expected that skin characteristics vary throughout age, with a worsening of the properties with aging and gender, due to hormonal and cosmetic-related actions. Surprisingly, for photoprotected areas, properties such as hydration, transepidermal water loss and skin extensibility (firmness) did not vary with age and sex, but skin elasticity was reduced with chronological aging. On the other hand, for photoexposed skin, higher hydration was observed with age, and females presented higher hydration than males. In relation to elasticity, unlike protected areas, in photoexposed regions, males presented a higher skin elasticity compared to females, but age was not an important factor. This indicates that elasticity is determined by sun exposure and that the chronological aging doesn't reflect the observed elasticity level.

Considering that R0 indicates the amount of skin suctioned by the probe and R7 is the proportion of the skin's return in relation to the amplitude after suction, both parameters are interconnected and offer complementary insights. Generally, lower values of R0 indicate higher firmness [17,18], which might suggest that the photoexposed area is firmer compared to photoprotected ones. However, since the elasticity (measured by R7) was also reduced in the sun-exposed region, the lower R0 values in this context can be an indicative of a rigid skin, reflecting a reduction of skin extensibility. This underscores the harmful effects of UV radiation on skin elasticity.The increase of skin's stiffness can be related to the collagen decrease [19] and can be higher on the population with more UV exposure, varying by geographic region and climate, with areas closer to the equator—more exposed to chronic sun exposure—being particularly susceptible to more rigidity on the skin than the more distant regions of the globe [20].

The disparity in skin elasticity between photoexposed and photoprotected regions tends to diminish with age, as photoprotected skin progressively loses elasticity [21]. The dermis, primarily composed of an extracellular matrix rich in collagen, provides strength, resilience, and elasticity to the skin. These qualities deteriorate with both natural aging and photoaging, with more severe effects observed in photoexposed skin [16,21]. Collagen, the main component of the dermis, organizes into fibrils that form complex networks with other matrix proteins, such as elastin fibers and non-fibrillar elements. Aging induces microstructural changes in the dermis, affecting collagen fibers and other extracellular matrix components, resulting in a gradual reduction of collagen, elastin, and proteoglycans, an increase in glycation cross-links, and fragmentation of matrix proteins, leading to a loss of organized structure [22].

Photoexposed skin also presents deep wrinkles, sagging, and a rough texture, reflecting accumulated sun damage. These changes are less pronounced in photoprotected regions, where the skin maintains a more intact and functional structure, resulting in lower apparent elasticity in comparative measurements. Although solar elastosis is absent in chronologically aged skin, the elastin network still deteriorates with age in photoprotected skin. In young skin, elastic fibers are thin and single-stranded, but they become beaded and lose terminal fibrils as the skin ages. While elastin proteins are produced throughout life, their assembly into functional fibers decreases with natural aging [16].

Comparative studies using different methodologies, such as High-Frequency Ultrasound and Reflectance Confocal Microscopy, provide further insights into these aging processes [21] and align with the results obtained in this study. Ultrasound imaging revealed that photoaged skin tends to be thinner and less dense due to the breakdown of structural proteins [3,21] and Reflectance Confocal Microscopy has shown that photoaged skin exhibits more disarrayed cell patterns, polycyclic papillae, irregular and coarse collagen fibers and clusters, and increased elastosis compared to chronologically aged skin [23-25].

Our findings also highlight significant differences in skin aging between men and women in photoexposed areas, which are mostly influenced by hormonal constitution and changes throughout the years. In women, the abrupt decline in estrogen levels during menopause [26], which occurs around the fourth or fifth decade of life, accelerates skin aging, leading to decreased collagen production, reduced skin elasticity, and increased dryness. Conversely, men experience a more gradual decline in testosterone levels, which also affects skin structure but at a slower and continuous rate. This hormonal disparity explains why women often exhibit more pronounced signs of aging than men [3,26].

This study was conducted with a diverse group of subjects and under controlled conditions, so despite the relatively small sample size and the limited lifestyle information, the findings provide valuable insights into the biophysical characteristics of chronologically aged and photoaged skin. These findings underscore the importance of considering both intrinsic and extrinsic factors in the study of skin aging. They also highlight the need for tailored anti-aging treatments that address the specific needs of different skin types and conditions.

5. Conclusion

Sun exposure leads to a faster aging than expected by chronological aging, causing a disfunctional skin barrier and malleability, being more important than age for elastic properties. Moreover, females can be more affected than males in the loss of skin elasticity. Our study highlights the significant differences between photoprotected and photoexposed skin, emphasizing the distinct impacts of intrinsic and extrinsic aging factors. By identifying specific changes in skin firmness, elasticity, hydration, and barrier function, it is necessary to reinforce the importance of sun protection factor products, but also tailor anti-aging treatments to address these unique needs effectively. Understanding which skin properties change with photoaging and how will allow the development of cosmetic products to be more assertive and innovative, as it will be possible to consider the particularities of each region of the skin and type of aging present. Future studies should continue to explore the interplay between hormonal changes, UV exposure, and other environmental factors to develop comprehensive strategies for skin care. By integrating these findings into the formulation of cosmetic products, we can enhance their efficacy and provide more personalized solutions for maintaining healthy, youthful skin.

6. References

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