Caloric Restriction Effect on MAPK and MMP-1 Levels in the Skin of Male Wistar Rats (*Rattus norvegicus*) Exposed to Ultraviolet B Radiation

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KEYWORDS

caloric restriction, skin aging, UVB, Mitogen-Activated Protein Kinase 1, Matrix Metallopro-teinase 1

ABSTRACT:

Introduction: Skin aging is greatly caused by prolonged exposure to ultraviolet (UV) radiation, reflected in the increased production of various molecular products, including mitogen-activated protein kinase (MAPK). This is followed by the activation of the activator protein-1 (AP-1) complex, leading to increased production of one type of collagenase matrix metalloproteinase (MMP) enzymes, MMP-1. MAPK and MMP-1 pathway contributes to the photo-aging process, manifests in formation of wrinkles and loss of skin elasticity. Caloric restriction is a dietary intervention that plays a role in suppressing growth pathways, leading to increased cellular maintenance and reparative activities. This research aimed to study the impact of caloric restriction in photo-aging inhibition through modulation of MAPK and MMP-1 levels in the skin exposed to UVB radiation.

Objectives: This study aimed to evaluate the role of caloric restriction in suppressing the increase of MAPK and MMP-1 levels in rat skin exposed to UVB radiation.

Methods: Experimental study was conducted on 30 Wistar rats, which were further divided into five groups: K1 (normal), K2 (UVB radiation + normal diet), P1 (UVB radiation + 20% caloric restriction), P2 (UVB radiation + 30% caloric restriction), and P3 (UVB radiation + 40% caloric restriction). Caloric restriction was given for 2 weeks before and throughout UVB exposure. UVB irradiation was conducted for 4 weeks with a total dose of 480 mJ/cm². ELISA analysis of MAPK and MMP-1 levels in skin tissue was done at 48 hours after last irradiation. Statistical analysis of Kruskal-Wallis was used for the MAPK variable and One-Way ANOVA for the MMP-1 variable.

Results: Statistical analysis test showed no significant differences in MAPK and MMP-1 levels among the groups ($p > 0.05$; 0.164 and 0.139, respectively).

Conclusions: Caloric restriction does not prevent the increase in MAPK and MMP-1 levels in the skin of Wistar rats exposed to UVB radiation.

1. Introduction

Skin can undergo photo-aging, a process of extrinsic aging caused by prolonged exposure to ultraviolet (UV) radiation (Cao *et al.*, 2020; Gromkowska-Kępka *et al.*, 2021). One of the main effects of UV radiation on the skin is the activation of growth factors, increased oxidative stress, and a pro-inflammatory response. These factors lead to the activation of the Mitogen-Activated Protein Kinase (MAPK) cascade, followed by the activation of the activator protein-1 (AP-1) complex (Oh *et al.*,

2020). This pathway leads to the production of matrix metalloproteinase (MMP) enzymes, which play a role in degrading extracellular matrix proteins such as collagen, fibronectin, elastin, and proteoglycans. Degradation of proteins caused by MMP-1, MMP-3, and MMP-9 contributes to photo-aging, leading to wrinkle formation and loss elasticity on skin. Among them, MMP-1 (collagenase type) is particularly activated by UVB exposure, playing a significant role in the photo-aging process (Gromkowska-Kępka *et al.*, 2021).

Caloric restriction (CR) is an intervention involving reducing energy intake from the diet while ensuring sufficient energy for metabolic homeostasis with high-quality micronutrients and fiber (Flanagan *et al.*, 2020). By suppressing the Insulin-like Growth Factor-1 (IGF-1) pathway, caloric restriction enhances proteostasis and autophagy in skin cells (Eckhart *et al.*, 2019) and inhibits cancer cell growth by preventing excessive activation of the mechanistic target of rapamycin (mTOR) complex (Hursting *et al.*, 2009). Additionally, caloric restriction reduces damage caused by inflammatory pathways and reactive oxygen species (ROS) accumulation in UVB-exposed skin (Tang *et al.*, 2022).

To date, few studies have explored the effects of caloric restriction on skin aging. Two previous studies have highlighted the role of caloric restriction in histological parameters (Bhattacharyya *et al.*, 2017) and acute inflammatory biomarkers (Tang *et al.*, 2022) in UVB-exposed rat skin.

2. Objectives

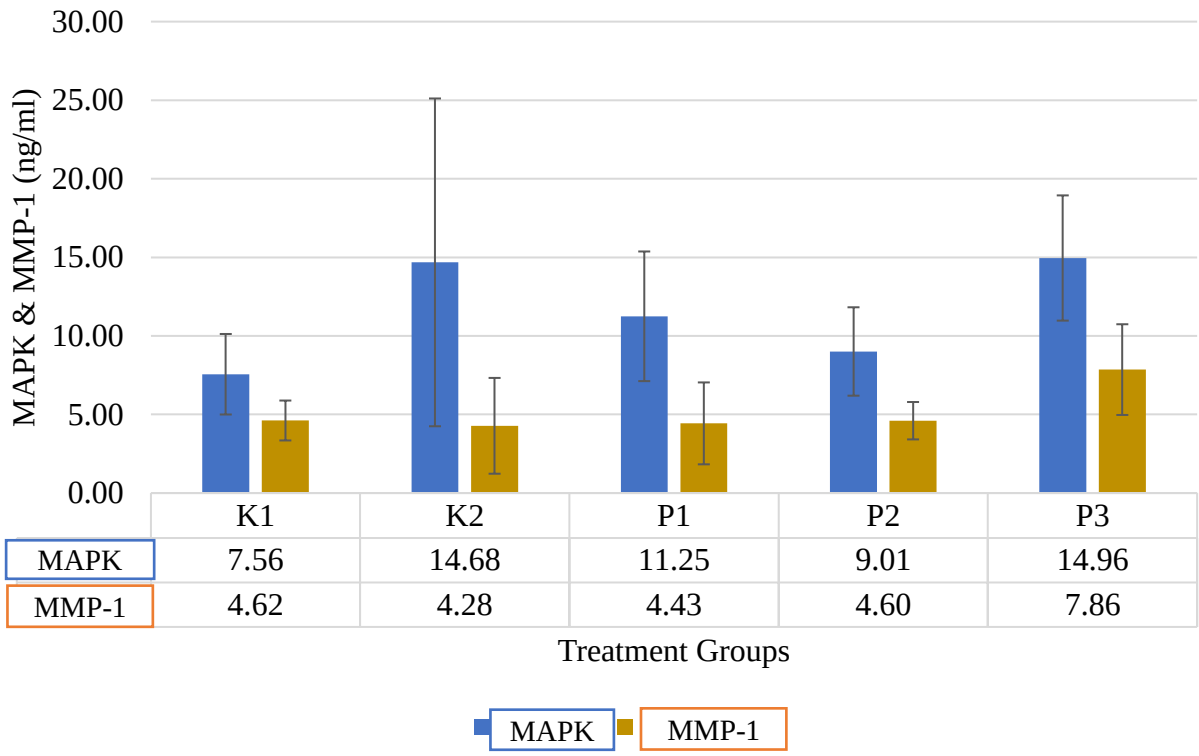
This study aims to describe the effects of caloric restriction (20%, 30%, 40%) in suppressing MAPK and MMP-1 levels as markers of the skin aging process in UVB-exposed rats.

3. Methods

This post-test only control group designed experimental study was conducted under the approval of Faculty of Medicine Ethics Committee, Universitas Udayana, Bali, Indonesia. A number of 30 male Wistar rats (*Rattus norvegicus*) were divided into five groups: K1 (given normal diet and no UVB radiation), K2 (given normal diet and UVB radiation), P1 (20% caloric restricted and UVB irradiated), P2 (30% caloric restricted and UVB irradiated), and P3 (40% caloric restricted and UVB irradiated). During first week of acclimatization period, average daily intake of each rat was calculated and used as a measure of caloric restriction. After one week of acclimatization, caloric restriction was given for 2 weeks before and during UVB exposure. UVB irradiation was conducted for 4 weeks with a total dose of 480 mJ/cm², given 3 times weekly from 50 mJ/cm² in the first week, 70 mJ/cm² in the second, and 80 mJ/cm² in the third and fourth. After 48 hours of the last UVB radiation, rats were euthanized and skin was excised for ELISA analysis of MAPK and MMP-1 levels. Additional histopathology examination of rat's skin was done to assess caloric restriction effect on adipose tissue. Image was captured using Olympus Epi 50 and Qupath application was used to quantify adipose tissue area percentage at 100x magnification.

4. Results

Figure 1. Average MAPK and MMP-1 Levels Between Groups



In **Figure 1**, it was found that among the groups exposed to UVB, there was a tendency for a decrease in the mean MAPK levels in groups P1 and P2, namely 11.25 ng/mL and 9.01 ng/mL respectively, compared to their control group, K2 (mean MAPK = 14.68 ng/mL). There was no significant difference of MMP-1 levels between the treatment groups P1 and P2 and their control. However, in group P3 where rats were given highest level of calorie restriction, mean MAPK and MMP-1 levels were found to be higher than in all other groups, at 14.96 ng/mL and 7.86 ng/mL, respectively.

Table 1. Statisctical Analysis on MAPK Levels Between Groups

Variable	Group	n	Mean MAPK	p value
MAPK	K1	6	7.56 ± 2.56	
	K2	5	14.68 ± 10.43	
	P1	6	11.25 ± 4.12	0.164
	P2	6	9.01 ± 2.81	
	P3	5	14.96 ± 3.98	

Note: MAPK calculated in ng/mL
(Source: Juslim, G; Harimawan, A; Wihandani, D; Sumadi, I; Damayanti, P; Primayanti, I.)

Statistical analysis for MAPK variable was conducted using Kruskal-Wallis test as data was not normally distributed (Shapiro-Wilk's test, $p<0.05$) and data variance was not homogenous (Levene's test, $p<0.05$). **Table 1**. shows non-parametric Kruskal-Wallis test on MAPK levels for each

group. Calculated p-value was 0.164 (p>0.05), therefore it was concluded that there was no significant difference in the mean MAPK levels among the groups.

Table 2. Statisctical Analysis on MMP-1 Levels Between Groups

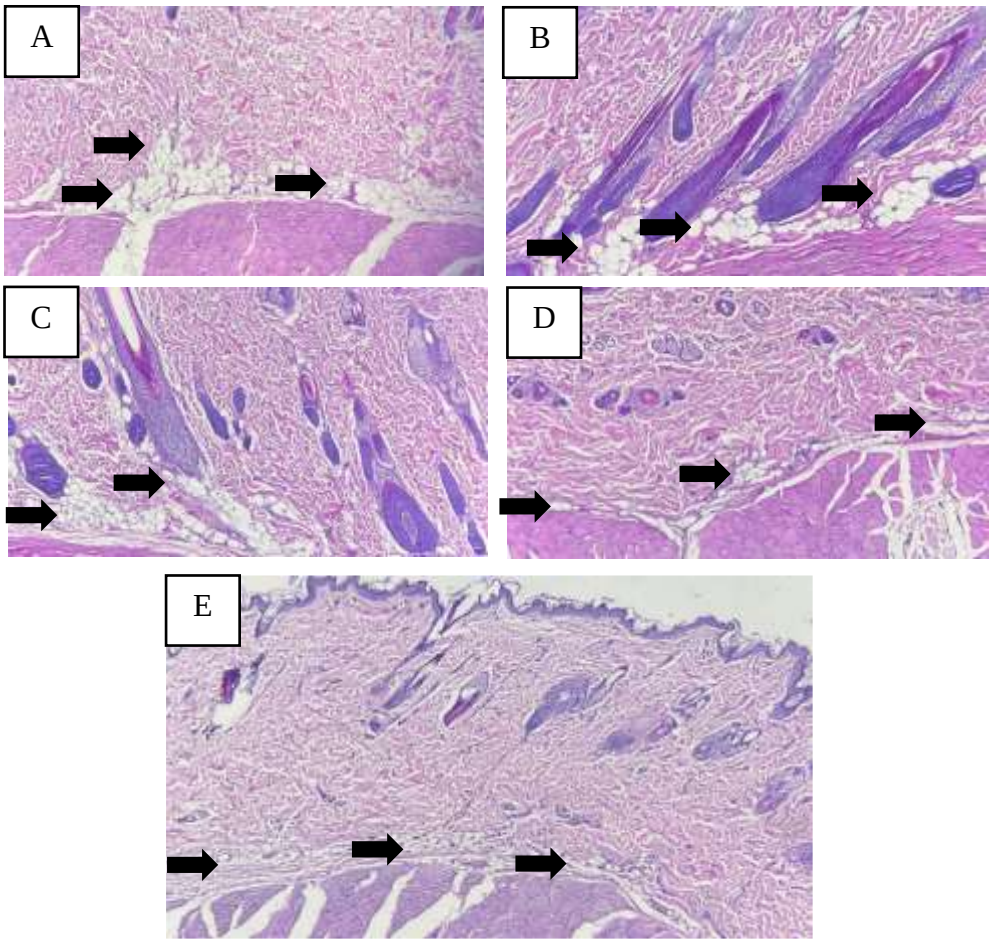
Variable	Group	n	Mean MMP-1	p value
MMP-1	K1	6	4.62 ± 1.27	
	K2	5	4.28 ± 3.05	
	P1	6	4.43 ± 2.61	0.139
	P2	6	4.60 ± 1.19	
	P3	5	7.86 ± 2.89	

Note: MMP-1 calculated in ng/mL.
(Source: Juslim, G; Harimawan, A; Wihandani, D; Sumadi, I; Damayanti, P; Primayanti, I.)

Statistical analysis for MMP-1 variable was conducted using parametric test, One Way ANOVA, as data was normally distributed (Shapiro-Wilk's test, p>0.05) and data variance was homogenous (Levene's test, p>0.05). **Table 2.** shows One Way ANOVA test on MMP-1 levels for each group. Calculated p-value for MMP-1 levels between group was p = 0.139 (p>0.05). It was also concluded that there was no significant difference in the mean MMP-1 levels among the groups.

Additional histopathological analysis of skin tissue was conducted to assess the effect of caloric restriction on adiposity. **Figure 2** shows that in the hypodermis layer, there is a difference of adipocytes in caloric restricted vs. control groups. Smaller adipocytes found in the treatment groups P1, P2, and P3 compared to control groups (K1 and K2). A parametric One-Way ANOVA test was also performed to examine the differences in the mean percentage of adipose tissue between groups. The results indicated a significant difference between the groups (p<0.001), as shown in **Table 3**.

Figure 2. Histopathology of Adipocytes in Hematoxylin and Eosin (H&E) Staining



Note: 400x magnification of adipocytes in (A) K1 group, (B) K2 group, (C) P1 group, (D) P2 group, and 100x magnification for (E) P3 group.

Table 3. Statisctical Analysis on Adipose Tissue Area Percentage Between Groups

Variable	Group	n	Adipose Tissue	p value
Adipose Tissue Area Percentage	K1	6	39,40	
	K2	5	34,20	
	P1	6	19,00	<0,001
	P2	6	19,33	
	P3	5	28,60	

Note: Adipose tissue area percentage (%) calculated by dividing adipose tissue area by total area within a single field of view.
(Source: Juslim, G; Harimawan, A; Wihandani, D; Sumadi, I; Damayanti, P; Primayanti, I.)

5. Discussion

Currently, there are no studies that demonstrate the impact of calorie restriction on MAPK levels in skin exposed to UVB radiation. In this study, it was found that calorie restriction at doses of 20%, 30%, and 40% statistically did not inhibit the increase in MAPK levels in the skin of male Wistar rats exposed to UVB. However, there was a tendency for a decrease in mean MAPK levels in the

groups receiving 20% and 30% calorie restriction compared to the control group in the descriptive analysis. Previous study using 20% calorie restriction for 10 weeks to SENCAR rats resulted in a significant decrease in h-Ras levels of the skin, followed by a decrease in p42/p44-MAPK levels and p110 PI3K9 protein (Xie *et al.*, 2007). Other studies also mention that 20% calorie restriction for 10 weeks can reduce gene expression associated with the PI3K-Akt and Ras-MAPK signaling pathways (Standard *et al.*, 2013). The explanation behind these findings is that calorie restriction plays a role in reducing cellular damage due to oxidative stress while simultaneously enhancing the antioxidant defense system (López-Lluch & Navas, 2016). Since MAPK activation is closely influenced by the presence of free radicals (Son *et al.*, 2011), the antioxidant effect of calorie restriction has the potential to suppress MAPK activation.

However, the statistical analysis showing no significant difference in MAPK levels between the groups encourages the need for a deeper exploration of the role of calorie restriction in the aging process. MAPK cascade consists of three main pathways: p38, JNK, and ERK. The MAPK levels assessed in this study were MAPK1, which represents the ERK 1/2 pathway (NCBI, 2025). This means that MAPK signaling through the p38 and JNK pathways could not be detected in this study. In previous studies, caloric restriction supressed phosphorylation of ERK1/2 MAPK (Liu *et al.*, 2001; Kim *et al.*, 2002), but other showed that it could also affect JNK pathway (Chen *et al.*, 2023). Different study on myocardium showed that caloric restriction did not modulate ERK1/2 and p38 MAPK, but gave significant result in AKT and AMPK pathway (Noyan *et al.*, 2015). Additionally, in skin cells with a high protein turnover rate, the short-term effects of calorie restriction that can be observed through the suppression of the p38/MAPK proliferation pathway (Bury *et al.*, 2020; Hsieh *et al.*, 2005). It is possible that calorie restriction in this study first suppressed the activation of the p38/MAPK pathway before affecting the ERK1/2 MAPK pathway as observed in this study.

Caloric restriction effect on aging is observed mainly through three nutrient sensing pathways: IGF-1, AMPK, and sirtuin (Ferreira-Marques *et al.*, 2021; Li *et al.*, 2017; Yuan *et al.*, 2020). One study demonstrated the effect of calorie restriction on the expression of p-AMPK protein, Sirtuin 1 protein and mRNA, and PGC-1α mRNA, showing its protective effect on the myocardium under ischemia (Ma *et al.*, 2020). Another study confirmed the involvement of the AMPK/sirtuin pathway in the effects of calorie restriction on glucose tolerance, as both are key sensors and early initiators of the adaptive process in skeletal muscle (Silvestre *et al.*, 2014). The neuroprotective effects of calorie restriction were also demonstrated through its role in the activation of sirtuin 1 and the suppression of mTOR (Ma *et al.*, 2015). It is possible that caloric restriction in this study may affect upstream pathways such as IGF-1/mTOR and AMPK/Sirtuin signaling (Muthusamy & Piva, 2010) before giving significant change to MAPK levels.

There is variability in the duration of calorie restriction applied in previous studies. Short-term studies have reported that a 30% calorie restriction for 4–6 weeks can improve glucose tolerance and insulin sensitivity (Park *et al.*, 2006). Another study found that a 50% calorie restriction for 4 weeks could suppress inflammatory pathways in neurons by reducing the expression of pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and MCP-1 (Chiba & Ezaki, 2010). Caloric restriction for 2–4 weeks has been shown to enhance antioxidant defense and reduce inflammatory biomarkers as well as IGF-1 signaling, thereby improving kidney protection against ischemia (Mitchell *et al.*, 2010). However, in this study, a 6-week calorie restriction did not result in significant changes in MAPK levels. This may be due to the sensitivity of skeletal muscle, nerves, and kidneys to energy imbalances triggered by calorie restriction (Casanova *et al.*, 2019; Tran *et al.*, 2022). In organs with high metabolic rates,

such as the heart, brain, liver, and skeletal muscle (Wang *et al.*, 2010), energy utilization is more sensitive to mitochondrial changes induced by calorie restriction (Most & Redman, 2020). The potential effects of calorie restriction observed in short-term studies may not always be applicable to studies involving the skin, which has a slower metabolic rate (Wang *et al.*, 2010).

The lack of a significant difference in MMP-1 levels among the treatment groups in this study is consistent with the findings for MAPK, which also showed no significant difference. MAPK signaling is closely related to the expression of MMP-1 through its role in the activation of the AP-1 complex (Park, 2023). It has previously been known that calorie restriction plays a role in intrinsic skin aging. Calorie restriction can reduce collagen glycation products (Cefalu *et al.*, 1995) and increase the percentage of collagen and elastin fiber areas (Zidan & Abd El-Haleem, 2011). Currently, there are few studies that report the effects of calorie restriction on extrinsic skin aging which is mainly caused by UVB exposure. Caloric restriction as much as 30% was found to reduce myeloid infiltration and inflammatory cytokines such as IL-1β, IL-6, IL-10, and interferon-γ in the skin of rats exposed to UVB radiation (Tang *et al.*, 2022), but its effect on DNA damage was not conclusive. Another study involving 40% calorie restriction produced varied results in histological parameters. This 40% calorie restriction influenced epidermal thickness, fibroblast count, mast cell count, and dermal cellularity. However, its role in modulating in Rz values (wrinkles), collagen percentage, and elastic fibers was only observed in the comparison of groups that did not receive UVB exposure (Bhattacharyya *et al.*, 2017). Caloric restriction effect on photo-aging of skin may differ from intrinsic aging and further comparison studies may be needed.

In previous study of caloric restriction effect on photo-aging, it significantly reduced cytokines and myeloid infiltration (Tang *et al.*, 2022), indicating its positive effect on inflammatory pathway. UV radiation causes DNA damage and ECM homeostasis disruption, activating an inflammatory response in the skin induced by the NF-κB and p38MAPK pathways. DNA damage also leads to the activation of the NLRP3 inflammasome, causing an increase in the secretion of inflammatory cytokines such as IL-1β, IL-1α, IL-6, and TNF-α (Hasegawa *et al.*, 2016). Cytokine TNF-α plays an important role in dermal collagen degradation through its action on MMP-1 and MMP-3. Conversely, MMP-3 can activate TNF-α, which then induces an increase in MMP-9 expression (Feng *et al.*, 2024). Additionally, the presence of pro-inflammatory cytokines and ROS from UV radiation also causes fibroblast cellular aging (Salminen *et al.*, 2022). Photo-aging in the dermis due to UV exposure involves complex inter-molecular signalling. Although the effect of calorie restriction on MMP-1 levels in this study showed no significant results, calorie restriction may play better role in the skin aging process through inflammatory pathway such as NF-κB.

Histopathology examination showed a decrease in fat area percentage in the groups subjected to caloric restriction. This indicates that the caloric restriction in this study has led to a metabolic adaptation. Following initial phase of restriction which is characterized by weight loss and reduction in fat-free mass (FFM) due to glycogen breakdown and loss of intracellular fluids and electrolytes, settling phase is marked by a reduction in fat mass (FM) which occurs due to continuous fat oxidation. These phases are termed cellular adaptation as an evolutionary mechanism in response to weight loss (Most & Redman, 2020). Excessive calorie consumption is also strongly linked to increased adiposity and adipose tissue inflammation (Lee *et al.*, 2011). As previously mentioned, caloric restriction has been shown to suppress the production of pro-inflammatory cytokines and biomarkers of myeloid and neutrophil infiltration in the skin exposed to acute UVB radiation (Tang *et al.*, 2022). The histopathological findings of a lower adipose tissue percentage in the caloric restriction group in this

study may provide new insights into the effects of caloric restriction on inflammatory pathways in the photo-aging process.

Several limitations were found in this study. Assessment of MAPK and MMP-1 levels conducted at only one time point may not fully reflect the fluctuations in MAPK and MMP-1 levels throughout the UVB exposure period. Furthermore, this study focused only on the assessment of MAPK and MMP-1 levels, without evaluating other biomarkers involved in upstream signalling pathways, such as nutrient sensing pathways IGF-1, AMPK, sirtuin, and the NF-κB inflammatory pathway, all of which can be influenced by calorie restriction and are closely interconnected. Moreover, this study only conducted biochemical examinations of the MAPK and MMP-1 levels without additional histological assessments, such as collagen, elastin, and dermal cellularity, which could help substantiate the role of calorie restriction in the phenotype of photo-aging.

This study indicates that the role of calorie restriction in the skin aging process induced by UVB exposure cannot yet be explained through the MAPK and MMP-1 pathways. However, caloric restriction may affect other pathways such as IGF-1, AMPK, sirtuin, and NF-κB, in mitigating damage caused by UVB exposure. Further studies of caloric restriction effect on skin photo-aging may focus on these other pathways and exploring other phenotype of photo-aging to better evaluate the connection between biochemical and histological examination.

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