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Protein Malnutrition Disrupts Skin Histomorphometry and Dermal Fibrocollagen Deposition in Wistar Rats

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ABSTRACT: Protein malnutrition compromises tissue growth and repair, but quantitative evidence describing its effects across multiple structural compartments of the skin remains limited. This study evaluated skin histomorphometry and dermal fibrocollagen deposition in male Wistar rats exposed to a 5% protein diet for eight weeks. Ten rats were allocated to a normal control group and a protein-malnutrition group (n = 5 each). Skin sections were stained with hematoxylin and eosin to measure epidermal, dermal, and subcutaneous fat thickness and to count hair-follicle profiles per unit sectional area. Sirius Red staining and ImageJ analysis were used to quantify the proportion of dermal fibrocollagen. Protein malnutrition significantly reduced epidermal thickness from 25.04 ± 3.17 to 15.00 ± 2.76 μm (40.1%; $p = 0.0007$), dermal thickness from 624.16 ± 50.66 to 470.29 ± 50.26 μm (24.7%; $p = 0.0013$), subcutaneous fat thickness from 193.98 ± 25.53 to 47.46 ± 16.53 μm (75.5%; $p < 0.0001$), and hair-follicle profiles from 66.40 ± 8.17 to 38.80 ± 10.55 per unit area (41.6%; $p = 0.0018$). Dermal fibrocollagen area decreased from 56.88 ± 2.45% to 39.69 ± 5.24% (30.23%; $p < 0.001$). Histological observations corroborated thinning of the epidermis and dermis, loss of subcutaneous adipose tissue, reduced follicular profiles, and sparse, disorganized collagen bundles. These findings demonstrate that prolonged protein restriction disrupts the integrated architecture of the skin, with the largest proportional loss occurring in subcutaneous fat, and support skin histomorphometry as a sensitive indicator of protein-malnutrition-related tissue deterioration.

1. INTRODUCTION

Protein-energy malnutrition remains a major biological and clinical problem because insufficient protein intake impairs growth, immune competence, tissue maintenance, and recovery from injury. Although its systemic manifestations are well recognized, the skin is sometimes treated only as an external clinical marker rather than as a metabolically active organ that responds directly to nutritional deprivation. Cutaneous signs of nutrient deficiency include xerosis, altered pigmentation, dermatitis, hair abnormalities, and delayed tissue repair [1, 2]. These manifestations indicate that inadequate nutrition can affect the epidermal barrier, dermal extracellular matrix, skin appendages, and underlying adipose tissue simultaneously.

The epidermis depends on continuous keratinocyte proliferation and differentiation to maintain its barrier function. These processes require adequate energy, amino acids, micronutrients, and growth-factor signaling. Protein restriction may therefore reduce epidermal renewal and structural protein synthesis. The dermis is similarly vulnerable because fibroblasts require amino acids, particularly glycine, proline, and lysine, to synthesize procollagen and organize mature collagen fibers. Collagen is the principal load-bearing component of the dermal extracellular matrix and is essential for tensile strength, elasticity, cell migration,

and tissue repair [3, 4]. Disturbance of fibroblast function or extracellular-matrix turnover can consequently weaken the entire skin structure.

Subcutaneous and dermal adipose tissues also contribute to skin homeostasis. In addition to storing energy and providing thermal and mechanical protection, cutaneous adipocytes release paracrine mediators that influence fibroblasts, vascular cells, immune defense, and hair-follicle cycling [5, 6]. During prolonged nutritional deprivation, enhanced lipolysis may markedly reduce this adipose compartment. Hair follicles are likewise metabolically demanding mini-organs. Their rapidly proliferating cells, particularly during anagen, are sensitive to deficiencies of protein, energy, vitamins, and minerals, which may produce follicular miniaturization or premature transition from active growth [7].

Previous studies have largely emphasized wound healing or visible dermatological manifestations of malnutrition. Fewer experimental studies have quantified, within the same model, changes in epidermal thickness, dermal thickness, subcutaneous fat, hair-follicle profiles, and dermal fibrocollagen deposition. Integrating these endpoints is important because it distinguishes generalized tissue shrinkage from specific loss of extracellular matrix and skin appendages. Previous work by Maliza et al. documented systemic endocrine and inflammatory changes in malnourished rats and nutritional recovery of growth and cognitive outcomes [8, 9]. These studies provide a broader context for examining the skin as another potentially vulnerable tissue, but do not establish the cutaneous effects investigated here.

This study therefore compared skin histomorphometry and Sirius Red-positive dermal fibrocollagen between normally nourished and protein-malnourished Wistar rats. We hypothesized that eight weeks of a 5% protein diet would reduce epidermal, dermal, and subcutaneous thickness, decrease the number of hair-follicle profiles, and diminish dermal fibrocollagen deposition.

2. MATERIALS AND METHODS

2.1 Study Design and Animals

This experimental study used ten male Wistar rats (*Rattus norvegicus*), aged 4-5 weeks and weighing 60-80 g. The animals were obtained from an accredited laboratory animal facility and acclimatized for seven days before the experiment. They were maintained under controlled laboratory conditions at 22 ± 2 °C, 50-60% relative humidity, and a 12 h light-dark cycle. The rats were housed in standard laboratory cages with free access to food and drinking water. After acclimatization, the animals were randomly allocated to two groups (n = 5 per group): a normal control group receiving Ratbio and a protein-malnutrition group receiving Prosphet for eight consecutive weeks. These diets had nominal protein contents of 21% and 5%, respectively; their laboratory-analyzed nutrient composition is reported in Section 2.3.

2.2 Ethical Approval

All animal procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals. Ethical approval was granted by Faculty of Medicine, Universitas Andalas, Indonesia under approval number 88/UN16.10.D.KEPK-FF/2025. All efforts were made to minimize animal discomfort and the number of animals used.

2.3 Induction of protein malnutrition

Protein malnutrition was induced by feeding the malnutrition group a formulated diet containing 5% protein for eight consecutive weeks. The normal control group received a nutritionally adequate standard diet containing 20 % protein. Both diets were provided *ad libitum*, and drinking water was available throughout the experimental period. Body weight and food intake were recorded weekly to monitor nutritional status and growth.

2.4 Skin Sampling and Tissue Processing

At the end of the eight-week intervention, the animals were anesthetized by intraperitoneal administration of ketamine hydrochloride (80 mg/kg body weight) and xylazine (10 mg/kg body weight). Following confirmation of deep anesthesia, the animals were euthanized by exsanguination. Full-thickness skin specimens were collected from the dorsal region using the same anatomical location and orientation for all animals. Adherent connective tissue was carefully removed without damaging the dermis or subcutaneous layer. The skin samples were immediately fixed in 10% neutral-buffered formalin for 24 h. Subsequently, the tissues were dehydrated through graded ethanol concentrations, cleared in xylene, and embedded in paraffin.

Paraffin blocks were sectioned at a thickness of 5 μm using a rotary microtome. Consecutive sections were mounted on glass slides and prepared for hematoxylin and eosin and Sirius Red staining.

2.5 Hematoxylin and Eosin Staining and Histomorphometry

Skin sections were deparaffinized in xylene, rehydrated through descending ethanol concentrations, and stained with hematoxylin and eosin according to a standard histological procedure. The stained sections were examined using an Olympus CX33 light microscope equipped with a 3.1 MP Sony Exmor digital camera. One H&E-stained preparation per animal was evaluated. Epidermal thickness, dermal thickness, and subcutaneous fat thickness were measured in ten non-overlapping fields at different locations within each preparation at 100× total magnification using a 10× objective lens. Measurements were performed using calibrated ImageJ software (National Institutes of Health, Bethesda, MD, USA) [12]. The epidermal and dermal compartments were identified based on their histological morphology. Epidermal thickness was measured from the surface of the stratum corneum to the dermal–epidermal junction, whereas dermal thickness was measured from the dermal–epidermal junction to the boundary between the dermis and subcutaneous tissue. Subcutaneous fat thickness was measured from the lower dermal boundary to the panniculus carnosus muscle layer. Hair-follicle profiles visible within the predefined sectional area were counted and expressed as the number of follicular profiles per unit area. The term "hair-follicle profiles" was used because the measurements were obtained from two-dimensional histological sections rather than by stereological estimation. All groups were evaluated using identical anatomical sampling locations, tissue orientations, magnifications, image calibrations, and field-selection criteria. Histological preparations were labeled with codes that did not disclose group allocation, and histomorphometric assessment was performed with the assessor blinded to the experimental groups.

2.6 Sirius Red Staining and Fibrocollagen Quantification

Sirius Red staining was used to visualize collagen-rich structures within the dermal extracellular matrix. Deparaffinized and rehydrated skin sections were stained with 0.1% Sirius Red in saturated picric acid, rinsed in acidified water, dehydrated through graded ethanol, cleared in xylene, and mounted with a resinous mounting medium. The use and interpretation of picrosirius staining were informed by established methodological literature [10, 11].

Five non-overlapping dermal fields at different locations within the stained preparation from each animal were captured at 400× total magnification using identical illumination and camera settings. Digital images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) [12]. Before analysis, image calibration and color-threshold settings were standardized and applied consistently to all images. The red-stained collagen area was selected using a fixed color threshold. Fibrocollagen deposition was calculated using the following equation:

$$\text{Fibrocollagen area } (\%) = \frac{\text{Sirius Red-positive area}}{\text{Total dermal area}} \times 100$$

The mean percentage obtained from five microscopic fields was calculated for each animal. The individual animal, rather than each microscopic field, was considered the experimental unit. Image analysis was performed using coded preparations, with the assessor blinded to group allocation.

2.7 Statistical Analysis

Data are presented as mean ± standard deviation. The normality of the data distribution was assessed using the Shapiro-Wilk test, while homogeneity of variance was evaluated using Levene's test. Comparisons between the normal control and protein-malnutrition groups were performed using an independent-samples *t*-test. Welch's correction was applied when the assumption of equal variance was not met. Statistical significance was set at $p < 0.05$. Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Measurements from ten H&E fields and five Sirius Red fields were averaged separately for each animal before statistical analysis; animals, rather than individual fields, were the independent experimental units (n = 5 per group).

3. RESULTS AND DISCUSSION

3.1 Protein Malnutrition Alters Skin Histomorphometry

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Table 1. Skin Histomorphometry in Normal Control and Protein-Malnutrition Rats

Parameter	Normal control (n = 5)	Malnutrition (n = 5)	Change	p value
Epidermal thickness (μm)	25.04 ± 3.17	15.00 ± 2.76	−40.1%	0.0007
Dermal thickness (μm)	624.16 ± 50.66	470.29 ± 50.26	−24.7%	0.0013
Subcutaneous fat thickness (μm)	193.98 ± 25.53	47.46 ± 16.53	−75.5%	<0.0001
Hair-follicle profiles (n/unit area)	66.40 ± 8.17	38.80 ± 10.55	−41.6%	0.0018

Note: Values are mean ± standard deviation. Negative values indicate reductions relative to the normal control group.

Protein malnutrition reduced every measured structural compartment of the skin (Table 1). Epidermal thickness decreased by 40.1%, from 25.04 ± 3.17 μm in normal controls to 15.00 ± 2.76 μm in malnourished rats ($p = 0.0007$). Dermal thickness decreased by 24.7%, from 624.16 ± 50.66 to 470.29 ± 50.26 μm ($p = 0.0013$). The largest proportional change occurred in subcutaneous fat, which declined by 75.5%, from 193.98 ± 25.53 to 47.46 ± 16.53 μm ($p < 0.0001$). Hair-follicle profiles decreased by 41.6%, from 66.40 ± 8.17 to 38.80 ± 10.55 per unit sectional area ($p = 0.0018$). Histological sections corroborated these measurements, showing a thinner epidermis and dermis, marked depletion of subcutaneous adipose tissue, reduced follicular profiles, and a less compact dermal matrix in the malnutrition group.

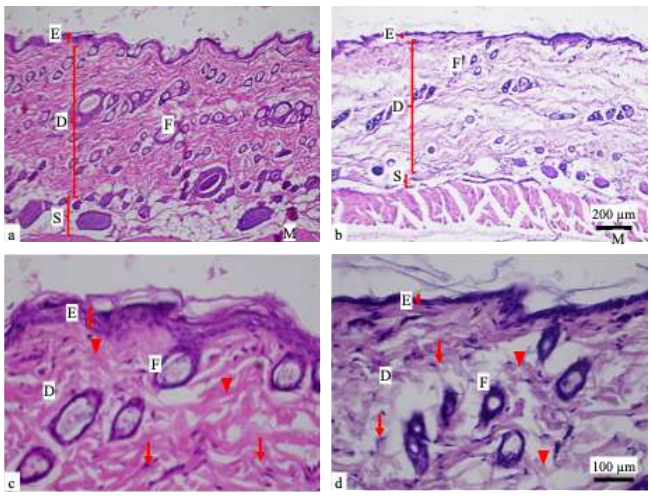


Figure 1. Histological features of the skin in normal control and protein-malnourished rats. The left panels (a and c) represent normal control rats, whereas the right panels (b and d) represent rats fed a 5% protein diet for eight weeks. The micrographs show the epidermis (E), dermis (D), subcutaneous tissue (S), hair follicles (F), and *panniculus carnosus* muscle layer (M). Collagen fibers and fibroblasts are indicated by arrowheads (▼) and arrows (↓), respectively. Compared with normal controls, protein-malnourished rats exhibited thinning of the epidermis and dermis, reduced density and disorganization of the dermal fibrocollagen matrix, depletion of subcutaneous adipose tissue, fewer hair-follicle profiles, and reduced size of some follicles. Hematoxylin and eosin staining. Upper panels (a and b): 4× objective, scale bar = 200 μm; lower panels (c and d): 40× objective, scale bar = 100 μm.

3.2 Protein Malnutrition Reduces Dermal Fibrocollagen Deposition

Table 2. Dermal Fibrocollagen Area in Normal Control and Protein-Malnourished Rats

Group	n	Fibrocollagen area (%)	Change	p value
Normal control	5	56.88 ± 2.45	–	–
Malnutrition	5	39.69 ± 5.24	−30.23%	<0.001

Note: Values are mean ± standard deviation. Fibrocollagen area was quantified from Sirius Red-stained sections using ImageJ.

Sirius Red staining revealed dense, relatively organized collagen bundles throughout the dermis of normal rats. In malnourished rats, the stained bundles appeared sparse and irregular, with wider matrix spaces. Quantitative image analysis showed that dermal fibrocollagen area decreased from 56.88 ± 2.45% in normal controls to 39.69 ± 5.24% in malnourished rats, representing a 30.23% reduction ($p < 0.001$; Table 2). The lower stained area fraction indicates that dermal thinning was accompanied by a lower relative area occupied by collagen-rich matrix; it does not independently quantify total collagen mass or exclude tissue-processing effects.

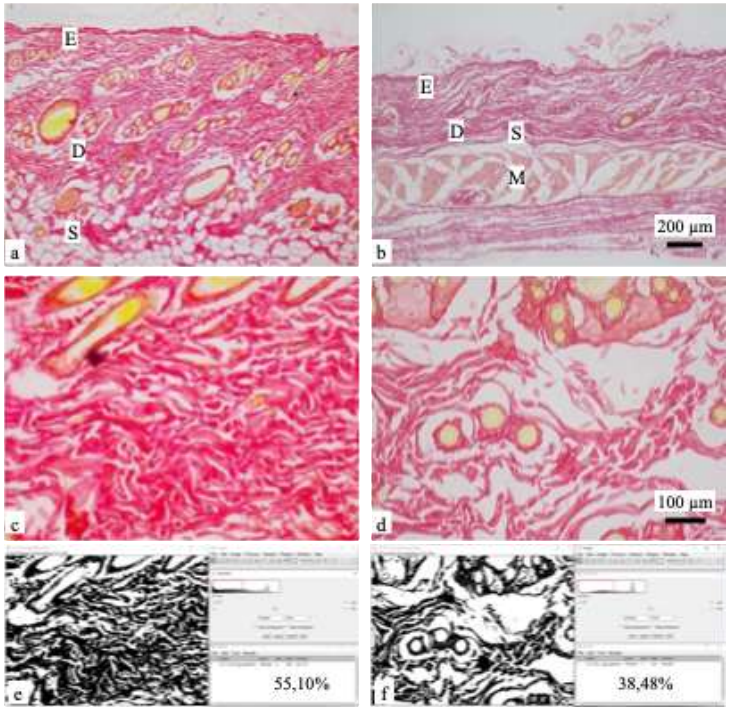


Figure 2. Dermal fibrocollagen matrix deposition in normal control and protein-malnourished rats. The left panels (a, c, and e) represent normal control rats, whereas the right panels (b, d, and f) represent rats fed a 5% protein diet for eight weeks. Normal controls exhibited a denser and more regularly organized fibrocollagen matrix than protein-malnourished rats. The malnutrition group showed reduced collagen-fiber density, disorganized collagen bundles, and wider intermatrix spaces. E: epidermis; D: dermis; S: subcutaneous tissue; M: *panniculus carnosus* muscle. Sirius Red staining; scale bars = 200 µm in the upper panels (a and b) and 100 µm in the middle panels (c and d). ImageJ analysis (e and f) showed representative fibrocollagen area proportions of 55.10% in the normal control group and 38.48% in the malnutrition group.

3.3 Discussion

The concurrent reductions in epidermal thickness, dermal thickness, adipose thickness, and Sirius Red-positive area indicate that the low-protein dietary intervention affected several structural compartments of the skin. The contribution of this study is the combined assessment of these compartments in skin without an experimentally induced wound. This distinction matters because much of the experimental literature evaluates repair after injury, whereas the present measurements describe tissue architecture at the end of dietary exposure. The findings are compatible with impaired skin growth or maintenance, but the single endpoint cannot distinguish reduced tissue development from loss of previously formed tissue.

As shown in Table 1, the 40.1% reduction in epidermal thickness is consistent with the sensitivity of the epidermis to inadequate nutrition [1, 2]. Reduced amino acid availability could constrain the synthesis of structural proteins needed for epidermal renewal. However, epidermal thickness alone cannot demonstrate reduced keratinocyte proliferation, altered differentiation, or barrier failure. These explanations remain hypotheses because proliferation markers, differentiation proteins, and transepidermal water loss were not assessed. Nutrition also affects repair through multiple interacting processes, including substrate availability and cellular responses [13]. Consequently, the present result supports a structural effect on the epidermis without identifying a specific cellular pathway.

Dermal thickness decreased by 24.7%, while the Sirius Red-positive dermal area fraction decreased by 30.23%. Together, these findings support a reduction in the relative representation of collagen-rich matrix within a thinner dermis. Fibroblast–matrix interactions are central to dermal organization, and distinct fibroblast lineages contribute to different dermal compartments [14, 15]. Collagen assembly depends on the synthesis and processing of its polypeptide chains [16]. Limited protein availability is therefore a plausible contributor to the observed matrix changes, although collagen synthesis, degradation, and cross-linking were not measured directly.

The collagen findings agree in direction with experimental studies of nutritionally compromised wound repair. Sampaio et al. found that protein malnutrition impaired fibroblast proliferation and collagen accumulation in wounded rat skin [17]. Yamane et al. reported lower collagen deposition and increased MMP2 activity in rats receiving a low-quality protein diet; lysine supplementation improved the impaired healing response [18]. These studies support a relationship between protein nutrition and matrix maintenance, but their wound models, dietary compositions, and observation periods differ from those used here. Their findings therefore provide biological support rather than a direct quantitative benchmark for the magnitude of collagen reduction in intact skin.

Altered extracellular-matrix turnover is another possible explanation for the collagen findings. Matrix metalloproteinases participate in regulated matrix remodeling, whereas dysregulated proteolysis can compromise tissue repair [19]. Matrix organization also depends on interactions between fibroblasts and the surrounding extracellular matrix [14]. Although increased degradation or altered fibroblast signaling could contribute to the present findings, neither MMP activity nor TGF-β signaling was assessed. Lower Sirius Red-positive area must therefore not be interpreted as direct evidence of MMP activation, suppressed TGF-β signaling, or a specific collagen synthesis defect.

The 75.5% reduction in adipose thickness was the largest proportional difference among the measured endpoints. This finding suggests particular vulnerability of the measured cutaneous fat compartment during prolonged dietary protein restriction. It could reflect reduced lipid storage, impaired adipose development, increased lipid mobilization, or a combination of these processes. The study did not measure adipocyte size, lipid flux, or whole-body fat mass, so the underlying process cannot be resolved. Moreover, literature distinguishes dermal white adipose tissue from deeper subcutaneous adipose depots [5, 6]. The anatomical compartment measured between the dermis and panniculus carnosus should therefore be identified consistently, and functional findings from one adipose depot should not automatically be assigned to another.

The loss of cutaneous fat may have implications beyond energy storage. In mice, adipocyte lineage cells can regulate follicular stem-cell activation [20], and dermal adipocytes contribute to antimicrobial defense through cathelicidin production [21]. These experimental observations establish that local adipose tissue can influence neighboring structures and host defense. They make interactions between adipose depletion, follicular changes, and dermal remodeling biologically plausible. However, the present study measured neither adipose-derived signals nor antimicrobial function, and the mouse findings cannot establish these mechanisms in the malnourished Wistar rats examined here.

The 41.6% reduction in hair-follicle profiles should be interpreted cautiously. Nutritional disorders are associated with hair changes [1, 2], and micronutrient literature further illustrates the nutritional sensitivity of hair growth [7]. Nevertheless, evidence concerning vitamin or mineral deficiency does not specifically establish the consequences of protein restriction. In addition, follicle morphology and depth vary across the hair cycle, as demonstrated in murine staging studies [22]. Section orientation, sampling depth, and cycle distribution can therefore alter two-dimensional profile counts. The result indicates fewer visible follicular profiles in the sampled sections; it does not establish permanent follicle loss, reduced total follicle number, or clinical alopecia.

These cutaneous findings also fit within the broader systemic consequences of malnutrition described by Maliza et al. In a separate rat study, malnutrition was associated with changes in growth, circulating protein measures, and pituitary–liver-axis regulation, with improvement following lima bean supplementation [8]. Another study reported recovery of growth and cognitive outcomes alongside improvements in brain histopathology after dietary lima bean flour supplementation [9]. In a separate experiment, six weeks of a 12% protein diet was associated with altered brain histopathology and cognitive function relative to a 20% protein diet [23]. More recently, Maliza et al. reported intestinal structural injury, oxidative imbalance, and hematological alterations in protein-malnourished rats, with improvement following dietary lima bean flour supplementation [24]. Together, these publications support the relevance of nutritional status to multiple organ systems, although differences in

protein concentration, intervention duration, and target organs preclude direct comparison of effect sizes. They do not show that the same endocrine or inflammatory changes occurred in the current animals, nor that lima bean supplementation would reverse the skin changes reported here. Such recovery would require a dedicated intervention study.

The histological approach also determines what can be concluded about collagen. Picrosirius staining is useful for visualizing collagen networks [10, 11], and ImageJ enables quantitative analysis of digital images [12]. However, a stained area fraction represents the proportion of the selected region occupied by the segmented signal, not collagen mass, synthesis rate, or mechanical strength. Bright-field analysis does not identify collagen types I and III, and polarization colors alone should not be treated as definitive collagen typing [11]. Biochemical collagen measurement, collagen-specific immunostaining, and mechanical testing would provide complementary evidence. The representative percentages in Figure 2 describe individual images and should be distinguished from the animal-level group means in Table 2.

This study was limited by the small sample size (n = 5 per group), a single assessment time point, and the specific low-protein dietary model in young male rats. Microscopic fields were within-animal subsamples, and multiple outcomes were tested without multiplicity adjustment. Differences in dietary composition, energy intake, and body-weight changes may have contributed to the findings. Biochemical and functional measurements were unavailable, limiting mechanistic and functional interpretation. Larger independent studies are needed before extrapolating these findings to human malnutrition or clinical skin outcomes. Future studies should assess epidermal proliferation and barrier function, collagen content and turnover, and hair-cycle stage, together with systemic nutritional measures. A nutritional rehabilitation group would determine whether the tissue changes recover after restoration of adequate protein intake. Future experiments should also report sample-size rationale, randomization, blinding, and animal-level analyses transparently. Overall, the present data identify a coordinated structural response of the skin to a low-protein dietary intervention and provide a basis for testing the mechanisms and reversibility of that response.

4. CONCLUSION

Eight weeks of a 5% protein diet were associated with reduced epidermal and dermal thickness, marked depletion of the measured cutaneous adipose layer, fewer hair-follicle profiles, and a lower Sirius Red-positive dermal area fraction in young male Wistar rats. Adipose thickness showed the largest proportional reduction. These findings indicate that the low-protein dietary intervention affected multiple skin compartments. Further studies are needed to establish the underlying mechanisms, functional consequences, and reversibility during nutritional rehabilitation.

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AUTHOR CONTRIBUTIONS

Alimuddin Tofrizal: Conceptualization, Methodology, Investigation-histological assessment and interpretation.

Rita Maliza: Conceptualization, Methodology, Investigation-*in vivo* experiments, Supervision.

Samudera Mukhalid: Investigation-animal handling and experimental assistance.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS STATEMENT

All animal procedures were approved by the ethics committee of the Faculty of Medicine, Universitas Andalas, Indonesia ((Approval No. 88/UN16.10.D.KEPK-FF/2025), as stated in the Materials and Methods.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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