

# Skin Protein Profile after Major Weight Loss and Its Role in Body Contouring Surgery

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**Background:** Chronic inflammation during morbid obesity significantly alters cutaneous tissue. Large weight loss achieved after bariatric surgery minimizes or halts damage caused by metabolic syndrome, but further deteriorates the clinical condition of skin. Postbariatric skin flaccidity produces major difficulties to plastic surgery. In this study, we analyzed differences in protein composition of the skin between patients with morbid obesity and those after large weight loss and established correlations between differentially expressed proteins and clinical characteristics of postbariatric skin tissue, to improve body contouring surgery techniques.

**Methods:** Skin fragments were removed from the abdomen of 32 patients, who were allocated into 3 groups: morbidly obese, large weight loss without surgery, and postbariatric surgery. Samples were subjected to proteomic analysis, and the protein profiles of the groups were compared. Six differentially expressed proteins of clinical interest were validated by immunohistochemistry and statistical analysis.

**Results:** Comparative analyses confirmed differences in protein profile of the skin between morbidly obese and large weight loss groups. A persistent increase in inflammatory markers such as haptoglobin was observed in all groups and decrease in the expression of collagen XIV, which regulates the physical properties of cutaneous tissue, was observed in the postbariatric group.

**Conclusions:** High expression of haptoglobin associated with the decrease of Collagen XIV, vinculin, and periplakin in the groups after major weight losses, mainly postbariatric, confirm that the inflammatory lesion remains active in the skin and causes changes in its structural organization, with serious repercussions on its clinical characteristics and physical properties. (*Plast Reconstr Surg Glob Open* 2019;7:e2339; doi: 10.1097/GOX.0000000000002339; Published online 19 August 2019.)

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Body contouring plastic surgery after large weight loss has become more important because of the increasing number of patients seeking effective treatments for the large skin flaccidity that occurs after bariatric surgery. In addition, recent studies have shown that body contouring procedures potentiate patients weight loss and may improve long-term body mass index (BMI) control.<sup>1-4</sup> Morbid obesity is recognized as a systemic inflammatory disease, in which the adipose tissue itself acts as a source of proinflammatory cytokines.<sup>5-7</sup> The knowledge of the mechanisms responsible for diseases associated with the metabolic syndrome<sup>8</sup> allowed the development of new therapeutic strategies for this serious global public health problem. In this same way, studies of the phenomena affecting the skin of morbidly obesity patients and those who achieved great weight loss will provide information capable to improve treatments, including the body contouring surgery results.

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Large weight loss and complete remission or improvement of clinical and laboratory parameters of the main comorbidities associated with metabolic syndrome have made bariatric surgery the most effective therapeutic modality for morbid obesity.<sup>9</sup> However, in contrast to typical metabolic diseases,<sup>10</sup> postbariatric surgery skin shows clear signs of clinical deterioration, such as reduced elasticity and turgor proportional to the progression of weight loss.

Previous studies have examined the clinical characteristics, histological organization, and molecular composition of skin<sup>11–15</sup>. They revealed important differences between normal cutaneous tissue, in obesity and great weight loss; however, the characteristics of postbariatric skin<sup>16</sup> requires further analysis.

Knowledge about dynamic behavior of collagen and other proteins in the cutaneous tissue, capable of changing its structure, interactions with other proteins, and biological functions in response to changes in local and systemic conditions of the organism,<sup>17</sup> may reveal the causes of clinical differences in skin among these groups of patients. Proteomic analysis<sup>18–20</sup> and immunohistochemistry (IHC)<sup>21</sup> enable determination of the protein composition of the skin and allow for comparisons between groups of patients.

The differences observed between the flaccidity of postbariatric surgery skin and the skin after major weight loss without surgery<sup>22</sup> are not well understood at the molecular level. Clinical observation revealed significantly different characteristics and behaviors over time, generating important responses in the results of body contouring surgeries practiced in these 2 groups of patients.

In this study, we examined the differences in the protein profiles of the skin in 3 groups of patients: morbid obesity, after major weight loss without surgery and postbariatric, and established correlations between differentially expressed proteins and the clinical characteristics of cutaneous tissue after bariatric surgery. The understanding of the protein alterations that occur in the postbariatric skin can generate adjuvant clinical treatments able to improve the quality of the skin before and after the body contouring surgeries, with consequent improvement of its results.

## PATIENTS AND METHODS

Thirty-two female white patients, aged 18–53 years (average, 34 years) and diagnosed with morbid obesity (BMI  $\geq 40$  kg/m<sup>2</sup>), were selected.<sup>23</sup> All patients had at least one year of weight stabilization to undergo body contouring surgery.

Patients were allocated to 3 groups according to clinical criteria (Table 1). In the large weight loss groups (B and C), only patients who lost at least 60% of excess weight—considering the excess weight as the difference between the maximum weight recorded and the ideal weight with BMI fixed at 25 kg/m<sup>2</sup>—were included.<sup>24,25</sup> Smokers, patients with diabetes mellitus, thyroid diseases, and patients with previous abdominal plastic surgeries were excluded.

Skin samples containing the epidermis and dermis were obtained from the anterior region of the abdomen

**Table 1. Composition of Study Groups**

Group	No. Patients	Proposed Surgery
Group A (morbidly obese)	12	RYGB <sup>26,27</sup>
Group B (large weight loss without surgery)	8	Classic abdominoplasty <sup>28</sup> or modified vertical abdominoplasty <sup>29</sup>
Group C (large weight loss postbariatric surgery)	12	Modified vertical abdominoplasty

RYGB indicates Roux-en-Y gastric bypass.

during Roux-en-Y gastric bypass procedures<sup>26,27</sup> or abdominoplasty,<sup>28,29</sup> immediately frozen in liquid nitrogen, and stored in a freezer at  $-80^{\circ}\text{C}$ . Each sample was divided into 2 fragments, one for proteomic analysis and the other reserved for IHC validation tests.

### Proteomic Analysis

Frozen skin fragments were solubilized and the tissue proteins extracted and then quantified by the Bradford method.<sup>30</sup> To minimize individual differences, the proteins were grouped into 5 sample pools with equivalent amount of proteins extracted from each skin fragment (Table 2).

The 5 protein pools were analyzed by 1-dimensional electrophoresis (1DE) and 2-dimensional electrophoresis (2DE). All 1DE gel slices and spots of interest were removed from the 2DE gels, subjected to trypsin digestion, and the resulting peptides were identified by mass spectrometry (MS) using electrospray ionization/quadrupole time-of-flight MS. MS data analysis was performed by bioinformatics searches with the Mascot Database (<http://www.matrixscience.com>) tool in human databases. Comparative analyses of the proteins identified by 1DE were performed using Scaffold 3.3.3 software (Proteome Software, Inc., Portland, Ore.).

After comparing the pools of each group, qualitative and quantitative differences in the expression of proteins with clinically relevant biological roles in this study (<http://www.ncbi.nlm.nih.gov/pubmed/>, <http://www.ncbi.nlm.nih.gov/pubmed/gene>, <http://www.uniprot.org>) were used to select 6 proteins for IHC validation.

### Validation by IHC

The skin fragments reserved for IHC were fixed in formalin and embedded in paraffin, after gradual thawing. Twenty histological sections (3  $\mu\text{m}$ ) for each sample were used for slide preparation.

For antigen-antibody reactions, deparaffinization, rehydration, and endogenous peroxidase blocking were

**Table 2. Composition and Characteristics of Protein Pools of the Samples**

Pool Number	No. Samples	Protein Mass	Clinical Characteristics of the Sample	Study Group
I	6	3,000 $\mu\text{g}$	Morbidly obese for $<35$ years	A
II	6	3,000 $\mu\text{g}$	Morbidly obese for $>35$ years	A
III	8	3,000 $\mu\text{g}$	Large weight loss without surgery	B
IV	6	3,000 $\mu\text{g}$	Postbariatric surgery for $>35$ years	C
V	6	3,000 $\mu\text{g}$	Postbariatric surgery for $<35$ years	C

**Table 3. Information Regarding Primary Antibodies (AB) Used for IHC Validation**

Antibody	Specificity	Dilution	Buffer	Positive Control	Primary AB Brand
Anticollagen XIV (Anti-COLXIV A1)	Polyclonal (rabbit)	1:25	Citrate (pH 6)	Human cardiac tissue	Lifespan (LS C119470)
Antivinculin	Monoclonal (mouse)	1:2,000	Citrate (pH 6)	Fibroblasts	ABCAM (ab 1194)
Antiperiplakin	Monoclonal (rabbit)	1:900	Citrate (pH 6)	Human skin	ABCAM (ab 131269)
Antiannexin V	Polyclonal (rabbit)	1:400	Citrate (pH 6)	Cutaneous melanoma	ABCAM (ab 14196)
Antihaaptoglobin	Monoclonal (mouse)	1:100	Citrate (pH 6)	Lung cancer	ABCAM (ab 13429)
Anti-alpha-1 anti-trypsin	Polyclonal (rabbit)	1:2,000	Citrate (pH 6)	Human tonsil	ABCAM (ab 49088)

used to prepare the samples. Antigenic recovery was conducted according to the procedures in the primary antibody (AB) datasheets (Table 3).

Samples were analyzed under a light microscope with 10× and 40× objectives. Analysis and scoring were conducted by the same pathologist (Table 4).

### Statistical Analysis

Statistical tests were performed to compare groups B (large weight loss without surgery) and C (postbariatric surgery). The hypothesis of independence ( $H_0$ ) or some dependency ( $H_1$ ) was tested between the method of weight loss and expression of the protein of interest in the skin.

The sample size of this study was small; additionally, clinical data were converted into numerical values. Thus, Bayesian method of comparison of models was conducted; this is based on Bayes factor ( $BF_{10}$ )<sup>31,32</sup> and is analogous to the likelihood ratio test calculations, which were found to be inadequate for statistical analysis in this study (Table 5).<sup>32</sup>

The IHC results for groups B and C were organized into tables with 2 variables: method of weight loss (X) and score of the protein of interest (Y).

In this study, the hypothesis (H1) tested, or model M1, was that the presence or absence of bariatric surgery in patients after large weight loss is related to the expression of proteins of interest in the skin samples in these 2 groups of patients.

The package LearnBayes in software R (R Core Team, 2019, Austria)<sup>33</sup> was used to calculate  $BF_{10}$ . Statistical analyses were restricted to comparisons between groups B and C because of the specific clinical interest of these groups for plastic surgery.

**Table 4. Analysis of IHC Validation**

Staining Pattern Observed	Score (in Crosses)	Numerical Value
Absence of staining	No score	0
Weak or focally positive staining	+	1
Clear positive and distributed staining	++	2
Strongly positive and diffuse staining	+++	3

**Table 5. Values for Interpretation of  $BF_{10}$** <sup>32</sup>

$BF_{10}$	Interpretation
<1	In favor of $M_0$
Between 1 and 3	Weakly favorable to $M_1$
Between 3 and 20	In favor of $M_1$
Between 20 and 150	Strong evidence in favor of $M_1$
>150	Strong evidence in favor of $M_1$

## RESULTS

### Proteomic Analysis

MS was analyzed using the Mascot Distiller and Daemon (Mascot Software, Boston, MA, USA) and Scaffold 3.3.3 softwares, which identified 113 proteins in 1DE and 100 proteins in 2DE. Based on these results, the skin profile of each group was established. Figures 1 and 2 show the results of the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and Figure 3 shows the results of the Scaffold featured 113 identified proteins in 1DE.

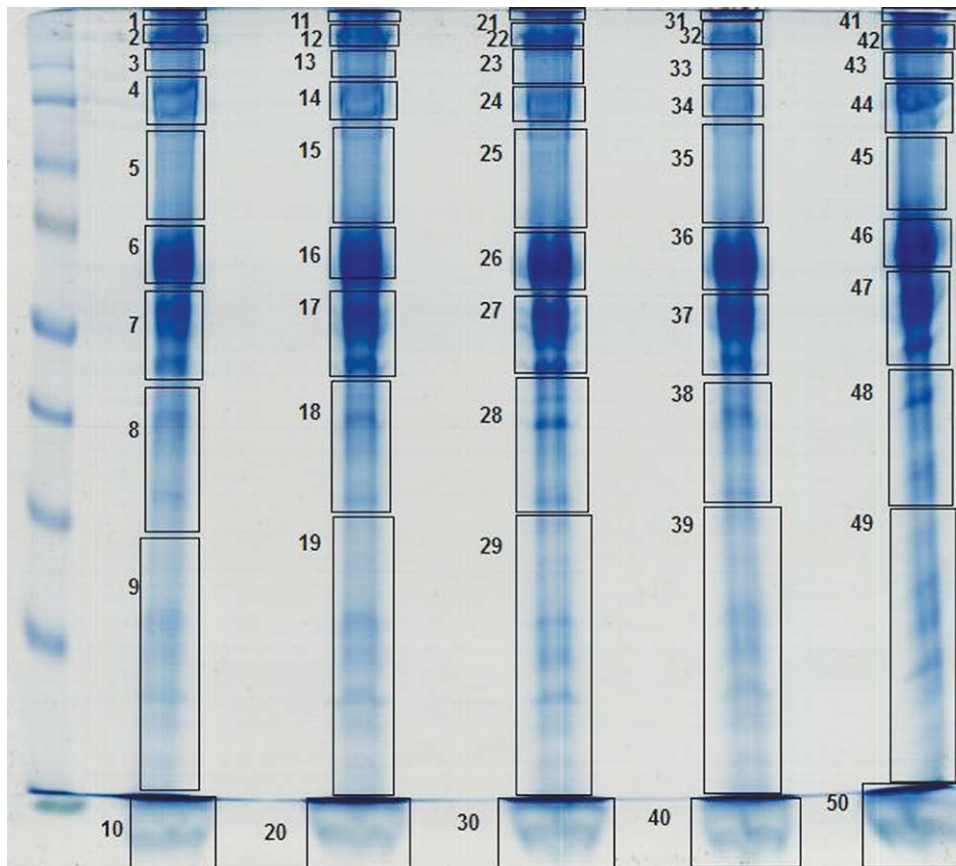
Among the proteins identified in 1DE, analyses with Scaffold 3.3.3 using predetermined parameters, protein threshold  $\geq 95\%$  and fold-change  $>2.0$  or  $<0.4$ , revealed 28 differentially expressed proteins in the skin samples of group A (morbidly obese) when compared with patients with large weight loss without bariatric surgery (group B). Comparison of groups A and C (postbariatric) revealed 23 proteins with differential expression. Comparison of patients' skin protein profiles after large nonsurgical and postbariatric weight loss (groups B and C) demonstrated that 23 proteins were differentially expressed. The main differences in skin protein composition between groups are presented in Tables 6 and 7.

Collagen XIV (COL14A1), haptoglobin (HP), periplakin (PPL), vinculin (VCL), annexin A5, and alpha-1-antitrypsin were selected for IHC validation tests because they showed differences in expression between groups and because of the high clinical relevance of their biological functions (<http://www.ncbi.nlm.nih.gov/pubmed/>), (<http://www.ncbi.nlm.nih.gov/pubmed/gene>, <http://www.uniprot.org>; Table 8).

### Validation by IHC and Statistical Analysis

The sample staining score for COL14A1 confirmed the proteomic analysis data, showing higher expression in the skin of patients with large weight loss without surgery (group B), compared with the postbariatric group (group C) (Figs. 4, 5). The  $BF_{10}$  of 3.83 supports the model of dependence (M1) between the weight loss method and expression of COL14A1 in patient skin; there was a significant difference in the levels of this protein in the tissue between groups B and C.

Most samples stained for PPL showed low or zero scores; however, the highest expression of this protein was observed in samples from group B, and not in those from group C, validating the proteomics data. A  $BF_{10}$  of 1.32 indicated weak support for the model of dependence and revealed the differential expression of PPL in the skin of large weight loss patients in this study.



**Fig. 1.** One-dimensional electrophoresis. Scanned image of 12.5%, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) GE stained with Coomassie blue. Highlighted, 50 slices, 10 from each pool, digested by trypsin and subjected to protein identification.

All samples exhibited staining for annexin A5, reflecting the widespread presence of this protein in cutaneous tissue. The analysis revealed higher scores in the group of large weight loss patients without surgery compared with the postbariatric surgery group, confirming the proteomics data. These differences were significant with a  $BF_{10}$  of 2.74, indicating weak support for the dependence model tested.

The slides with samples marked for HP and alpha-1-anti-trypsin displayed weak or null staining, but positive samples were detected in all groups. No significant differences were observed in the comparisons between groups.

The IHC results for VCL were unlike those obtained with the proteomic analysis, displaying greater staining in samples from large weight loss groups when compared with the group of patients with morbid obesity. Statistical analysis supported the independence model with a  $BF_{10}$  of 0.74.

## DISCUSSION

The increase in the number of patients with large weight loss has generated demand for plastic surgery. Technical innovations have been proposed to achieve better results in the redefinition of body contour<sup>29,42–46</sup>, however, the clinical characteristics of postbariatric skin, unlike nor-

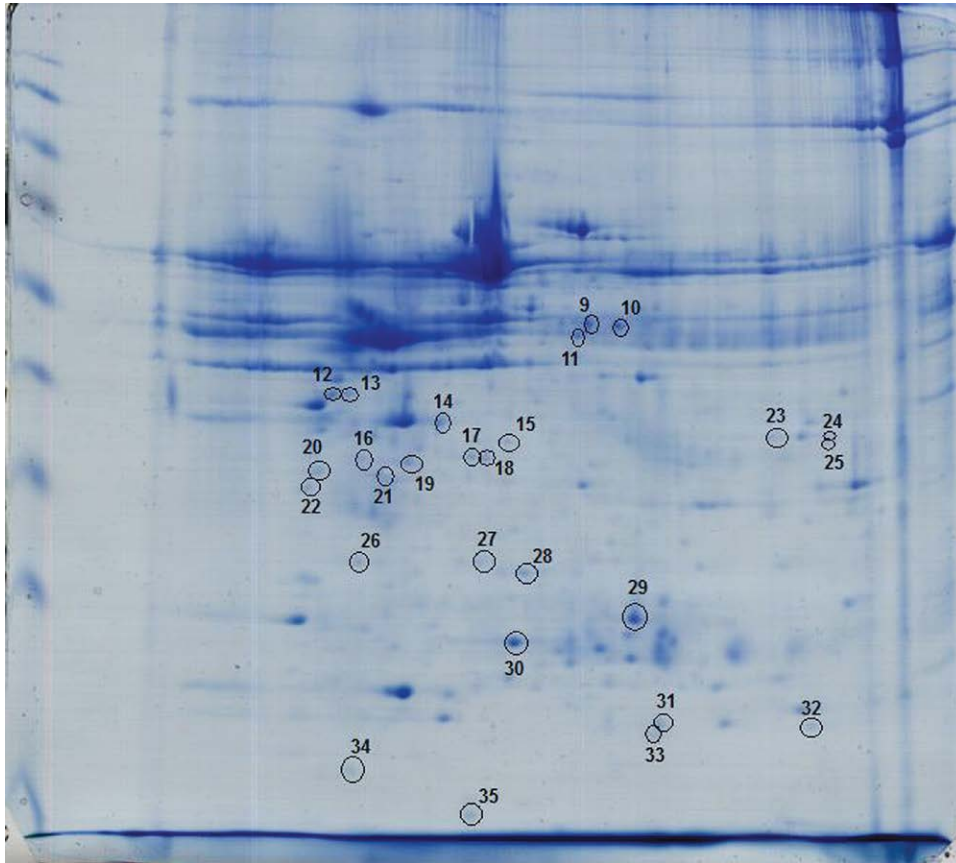
mal cutaneous tissue, represent major challenges for the development of these new surgical procedures.

The anatomy, histology, and molecular composition of normal skin<sup>47–49</sup> as well as changes in cutaneous tissue in individuals with obesity have been described and reviewed in detail.<sup>11,12</sup> However, knowledge of processes occurring in the skin after major weight loss remains incomplete.

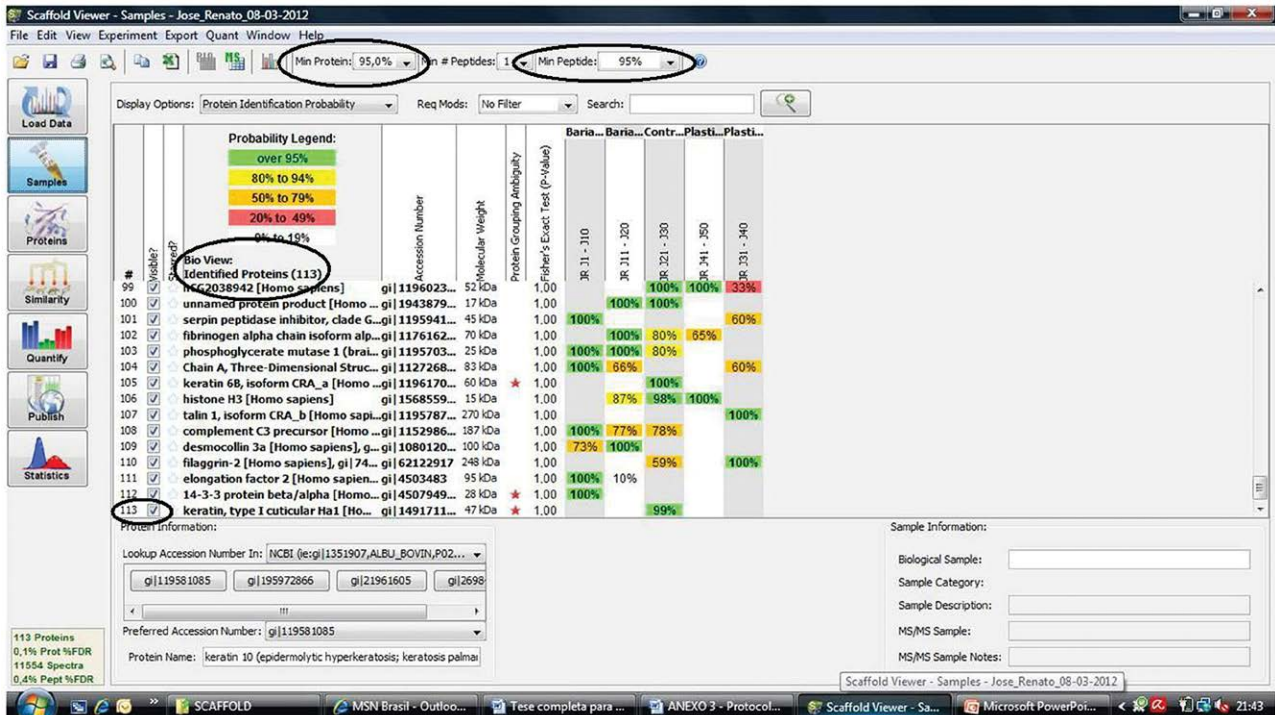
The use of proteomics<sup>19</sup> and IHC<sup>21</sup> enabled comparative analysis of skin protein composition in the morbid obesity and after massive weight loss groups. The differences between the protein profiles in the cutaneous tissue of patients with large weight loss without surgery (group B) compared with the postbariatric surgery group (group C) confirmed the need for further analysis of undesired consequences after bariatric surgery and limitations of body contour techniques.

Identification and analysis of the biological roles of proteins such as HP<sup>50–54</sup> (acute phase protein) and structural proteins such as Collagen XIV<sup>34</sup> and VCL<sup>41</sup> may pave the way for novel treatment protocols that seek to prevent the progression of inflammatory skin lesions and may promote replacement of essential elements to the maintenance of the physical and mechanical characteristics of the skin after major weight losses.

The profile of HP, a biomarker of inflammation and oxidative stress,<sup>50,51</sup> indicated that systemic inflammatory



**Fig. 2.** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) GE (12.5%), stained with Coomassie blue, in 2DE of pool III (patients after major weight loss without surgery). Highlighted, spots selected for digestion with trypsin and protein identification.



**Fig. 3.** Parameters used in Scaffold 3.3.3 software to identify 1DE proteins from databases. The minimum percentage of applied reliability (min protein = 95%) and 113 proteins identified are highlighted.

**Table 6. Main Proteins Differentially Expressed in the Skin, with Comparisons Between Morbidly Obese (A) and Large Weight Loss Groups (B and C)**

Universal Protein Database (UNIPROT)/Gene Accession Identification (ID)	Protein Name	Gene symbol	Groups Compared	Fold-Change (Greater Among the Pools)
P00738/3240	Haptoglobin	HP	A × B	70.0
P00738/3240	Haptoglobin	HP	A × C	6.0
P18206/7414	Vinculin	VCL	A × B	30.0
P18206/7414	Vinculin	VCL	A × C	3.0
P21333/2316	Filamin A	FLNA	A × B	6.0
P21333/2316	Filamin A	FLNA	A × C	6.0
P02458/1280	Collagen II- $\alpha$ -1	COL2A1	A × B	14.0
P02458/1280	Collagen II- $\alpha$ -1	COL2A1	A × C	2.8
Q07507/1805	Dermatopontin	DPT	A × B	4.0
P16422/4072	Epithelial cell adhesion molecule	EPCAM	A × C	3.8

**Table 7. Main Proteins Differentially Expressed in the Skin, with Comparisons of Large Weight Loss Without Surgery (B) and Postbariatric Surgery (C) Groups**

UNIPROT/ Gene Accession ID	Protein Name	Gene Symbol	Groups Compared	Fold-Change (Greater Among the Pools)
Q05707/7373	Collagen alpha-1 (XIV) chain	COL14A1	B × C	7.0
P02458/1280	Collagen alpha-1 (II) chain	COL2A1	B × C	0.2
O60437/5493	Periplakin	PPL	B × C	3.0
P07951/7169	Tropomyosin beta chain	TPM2	B × C	4.0
P35579/4627	Myosin-9	MYH9	B × C	8.0
P58107/83481	Epiplakin	EPPK1	B × C	0.2

UNIPROT, Universal Protein Database; ID, identification.

**Table 8. List of 6 Proteins Selected for Validation Testing by IHC**

UNIPROT/ Gene Accession ID	Protein Name	Gene Symbol	Groups Compared	Fold-Change	Main Function in Skin
Q05707/7373	Collagen alpha-1 (XIV) chain	COL14A1	B × C	7.0	Regulation of intracellular fibrillogenesis <sup>34</sup>
P00738/3240	Haptoglobin	HP	A × B and A × C	70.0 and 6.0	Inflammation marker (APP) <sup>35</sup>
P01009/5265	Alpha-1-anti-trypsin	SERPINA1	A × B	0.2	Inflammation marker (APP), Inhibition of neutrophil elastase <sup>36</sup>
O60437/5493	Periplakin	PPL	B × C	3.0	Cytoskeletal connections (intermediate filaments, junctional complexes and desmosomes) <sup>37,38</sup>
P08758/308	Annexin A5	ANXA5	B × C	B > C (2DE)	Regulation of apoptosis (PCD) <sup>39</sup>
P18206/7414	Vinculin	VCL	A × B	30.0	Cell-ECM (focal adhesions) and intercellular connections (junctional complexes) <sup>40,41</sup>

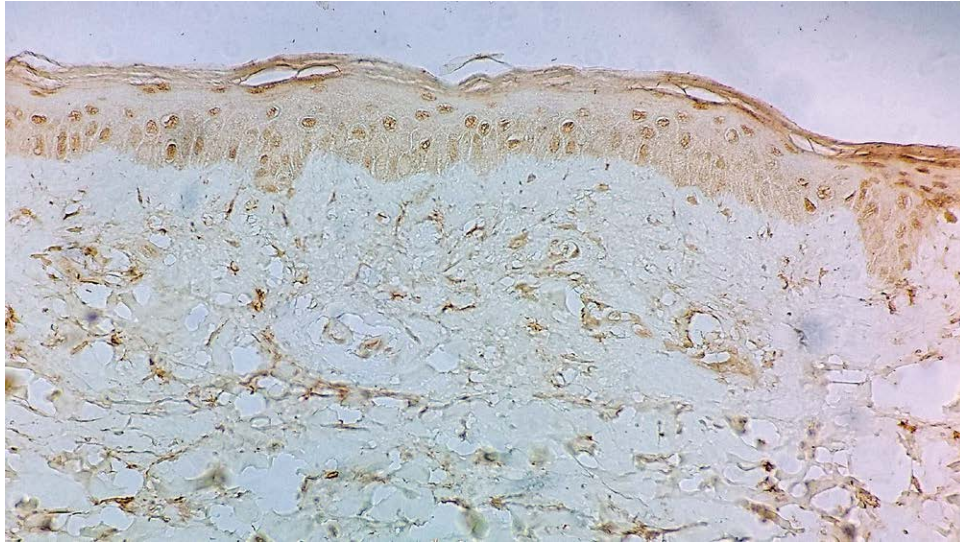
UNIPROT, Universal Protein Database; ID, identification; AAP, acute phase protein; PCD, programmed cell death.

injury extends to the cutaneous tissue of patients with morbid obesity (group A) and remains active even after large weight loss (groups B and C). Thus, HP is one of the factors involved in the progressive worsening of clinical conditions and histological changes observed in the cutaneous tissue of postbariatric patients.<sup>13,14,52-54</sup> Treatments capable of combating the inflammatory process on the skin of patients undergoing weight loss can reduce the deleterious effects of HP on collagenases and elastases, reducing foci of tissue fibrosis,<sup>52-54</sup> preserving skin elasticity,<sup>22</sup> and improving the clinical conditions of body contouring surgeries.

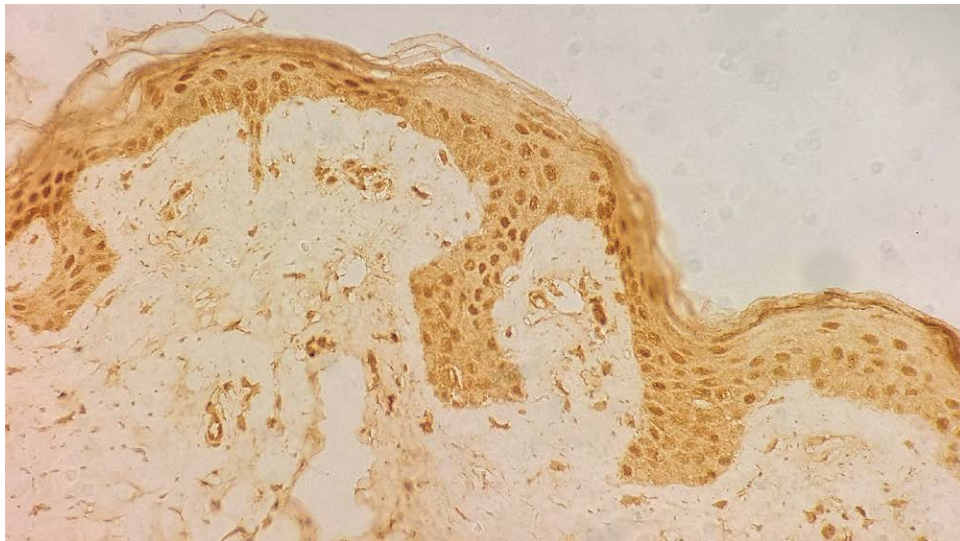
Changes in the metabolism of collagen, initiated by obesity,<sup>11,55</sup> remain present after weight loss and are key to understanding the processing occurring in the skin of patients with weight loss after bariatric surgery. COL14A1, which plays a central role in regulating the biomechanical properties of the skin,<sup>34,56</sup> showed lower expression in

the skin of postbariatric surgery patients (group C) compared with group B (large weight loss without surgery), suggesting acquired deficiency of this collagen after bariatric surgery. COL14A1, a fibril-associated collagen with an interrupted triple helix, regulates fibrillogenesis and coordinates the structural organization of collagen types I, III, and V. This protein controls the associations between extracellular matrix (ECM) fibers and keratinocytes and skin fibroblasts.<sup>34,56-58</sup> Therefore, the structural composition and molecular organization of collagen in cutaneous tissue postbariatric surgery patients become disordered, which differs from the processes in the skin of large weight loss patients without surgery. Thus, the challenges of postbariatric body contouring surgery extend beyond the technical development required for removing large quantities of excess skin.

In this context, PPL<sup>37,38,59</sup> and VCL,<sup>40,41,60-63</sup> 2 other proteins that function in tissue architecture and regulate the



**Fig. 4.** Photomicrography of immunohistochemistry staining of collagen XIV (COL14A1) in skin sample of large weight loss postbariatric surgery group (group C). The dermal area shows a weak staining (light brown) scored with 1 cross by the pathologist's analysis. (IHC; original magnification, x40.)



**Fig. 5.** Photomicrography of immunohistochemistry staining of collagen XIV (COL14A1) in skin sample of large weight loss without surgery group (group B). The dermal area shows a strongly positive and diffuse staining (dark brown) scored with 3 crosses by the pathologist's analysis. (IHC; original magnification, x40.)

mechanical properties of cutaneous tissue and cell adhesion, also displayed lower expression in the skin of patients after bariatric surgery (group C). PPL is responsible for associations between elements of the cellular skeleton, particularly the cytoplasmic intermediate filaments and junctional complexes (JCs) of the plasma membrane. As a structural component of the corneal envelope and desmosomes in keratinocytes, PPL plays a key role in the protection and resistance of cutaneous tissue.<sup>37,38</sup> VCL is an essential protein component in the structure and function of focal adhesions (FAs) and JC.<sup>40,41</sup> FAs transmit stimuli between cytoskeleton actin and ECM proteins, including collagen, to modulate the physical properties of cutaneous

tissue.<sup>60,64</sup> The JCs function as intercellular adhesion structures.<sup>40</sup> The low expression of VCL in the skin after major weight loss reduced FA and JC levels, worsening flaccidity. The lower tissue availability of PPL and VCL severely impaired the regulation of rigidity, both in the static stabilization force and transmission of intercellular contractile forces, and between cells and the ECM.<sup>37,59,60,62,63,65</sup>

Taken together, differences in the expression of COL14A1, PPL, and VCL, which are proteins directly related to the structural composition and mechanical properties of cutaneous tissue, make up de set of molecular alterations responsible for the distinct clinical characteristics observed between skin flaccidity in postbariatric

patients and after major weight loss without surgery. Thus, this group of patients requires different and broader therapeutic planning, including managing expectations related to the final aesthetic result based on the specific clinical characteristics of the skin, protocols of preoperative protein supplementation capable of improving the clinical conditions of the skin, development and application of techniques that allow greater withdrawal of excess skin, and understanding of changes in the skin's protein profile.

## CONCLUSIONS

The high expression of HP associated with the decrease of Collagen XIV, VCL, and PPL in the groups after major weight losses, mainly postbariatric, confirms that the inflammatory lesion remains active in the skin and causes changes in its structural organization, with serious repercussions on its clinical characteristics and physical properties. Therapeutic protocols capable of combining anti-inflammatory actions and specific protein supplementation may improve the clinical conditions of the skin after major weight loss, impacting the results of the body contouring surgery.

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