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Microbiome and Diet Impact in Scalp Disorder: The Example of Alopecia Areata

Pinto Daniela[1,2,3], Giammaria Giuliani[1,2], Sorbellini Elisabetta[2,3] and Rinaldi Fabio[1,2,3*]

[1] Giuliani S.p.A., Milan, Italy
[2] Human Advanced Microbiome Project-HMAP, Milan, Italy
[3] International Hair Research Foundation (IHRF), Milan, Italy

***Corresponding Author:** Rinaldi Fabio, Giuliani S.p.A, Human Advanced Microbiome Project-HMAP, Human Advanced Microbiome Project-HMAP, Milan, Italy E-mail: fabio.rinaldi@studiorinaldi.com; Tel.: ++39-2-76006089

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Abstract

Introduction: The impact of diet on hair growth disorder is well established as the influence of diet on the gut microbiome. Poor information is still available as regards the link between microbiome, especially scalp microbiome and hair diseases.

Aim: In the present work, we reported data on patients affected by Alopecia areata with the aim to study the impact of the diet on microbiome changing related to scalp disease.

Methodology: Data from the dietary survey, qRT-PCR on main bacterial strains inhabiting the scalp were matched and compared each other and with healthy population.

Results: Beyond the diet's well-known impact on general human health, our results highlighted the role of one's diet in modifying scalp microbiome, which in turn seems to have an impact on AA evolution.

Conclusions: Our results provide the first evidence of strict intercorrelation between microbial dysbiosis on the scalp of patients with AA and dietary habits.

Keywords: Alopecia Areata; Hair Disorders; Dietary Therapy; Microbiome; Dysbiosis

Alopecia Areata (AA) is a potentially reversible auto-immune disease affecting the scalp (Syed & Sandeep, 2013; D'Ovidio, 2014). Its typical manifestations occur in the form of non-scarring baldness on the scalp, which can be possibly extended to the entire body (Odom et al., 2006). When affected by non-scarring alopecia, a kind of disorder in hair follicle cycling has been observed (Paus, 1996), leading to the arrest of anagen phase, hair loss and, consequently, annular or patchy bald lesions (Tan et al., 2002; Camacho, 1997). In AA, in particular, this disorder has been reported to be strictly linked to immunity and inflammation (Brenner et al., 1979; Hordinsky et al., 2004; Trink et al., 2013). As the second most common type of hair loss disorder (incidence higher than 2%) (Dawber, 1989), AA has been extensively studied as regards causes (Syed & Sandeep, 2013) and clinical management options (Messenger et al., 2012). A novel innovative approach also includes the use of Platelet-rich plasma (PRP) as an inflammatory cytokine suppressor (Guo & Katta, 2017).

Hair follicle cells have a high turnover and a very active metabolism so they require a good intake of nutrients and energy from the diet. The impact of diet on hair growth disorder is well established, especially as regards nutritional deficiencies (Singh et al., 2017; Finner, 2017). Also, the influence of diet on shaping the gut microbiome and its implications for human health has been largely studied (Scott et al., 2013; Vaughn et al., 2017). Changing in diet regimen can induce large, reversible microbial alterations in less than one day (Scott et al., 2013). This is especially true when we speak about the gut but it is also true, for example, when talking about the skin (Bowe et al., 2010).

Poor information is still available as regards the link between microbiome and diseases and they are mostly related to gut microbiome (Rebello et al., 2017; Borde & Astrand, 2018). But poor knowledge is currently available about the impact of changing in scalp microbial communities in hair disorders (Clavaud et al., 2013; Xu et al., 2016). In a recently published work (Rinaldi et al., 2018) we reported, for the first time, evidence about a microbial shift in hair loss disorder, such as Alopecia androgenetica, AA and Lichen planopilaris. In the present work, we reported data on patients affected by Alopecia areata, with the aim to study the impact of the diet on microbiome changing related to scalp disease Thirty subjects affected by Alopecia Areata (20–60 years old; 30% male) were included in the study. We enrolled all subjects under dermatological control. Subjects have been previously diagnosed by means of clinical examination and classified according to the Severity of Alopecia Tool (SALT) (Olsen et al., 2004). The following exclusion criteria were used for both groups: a) pregnancy or lactation; b) affected by other dermatological diseases; c) anti-tumor, immunosuppressant or radiation therapy in the last 3 months; d) no topical or hormonal therapy on the scalp in the last 3 months; e) use of antibiotics in the last 30 days; f) probiotics in the last 15 days. Therefore, for scalp swab sampling, the last shampoo had to be performed at least 48h before.

Characteristics of the population studied are reported in Table 1.

Table 1 Characteristics of the population studied

	AA
Age (mean±SD)	41.66±13.04
BMI	23.20±2.38
SALT score	
S0	0%
S1	0%
S2	30%
S3	40%
S4	20%
S5	7%

Scalp surface has been sampled by mean of swab procedure according to previously reported methods (Grice et al., 2009; Gao et al., 2010), with minor modifications. Genomic DNA from scalp swabs was extracted by mean of QIAamp UCP Pathogen Mini Kit (Qiagen) according to manufacturer protocol, with minor modifications (Klindworth et al., 2013).

Main bacterial species (Propionibacterium acnes, Staphylococcus epidermidis and Staphylococcus aureus) on the scalp were quantified by real-time quantitative PCR (RT qPCR), using Microbial PCR assay kit (Qiagen). Samples were mixed with 12.5µL of Microbial qPCR Mastermix, 1 µL of Microbial DNA qPCR Assay, 5ng of genomic DNA sample and Microbial-DNA-free water up to a final volume of 25 µL. Positive PCR Control, No Template Control, and Microbial DNA Positive Control were also included. Pan-bacteria assays are also included as positive controls for the presence of bacterial DNA, as human GAPDH and HBB1 for the determination of proper sample collection. The following thermal cycling conditions were used: 95°C for 10 min, 40 cycles of 95°C for 15 sec, 60°C for 2 min. Each PCR reaction was performed in duplicate using an MX3000p PCR machine (Stratagene, La Jolla, CA). Relative abundance in the expression of each strain was calculated using the $\Delta\Delta C^t$ method (Vigetti et al., 2008), normalizing fold-change against PanBacteria, using MX3000p software (v.3; Stratagene). Enrolled subjects were also asked to fill out a 7-day dietary survey at the time of enrollment, following being instructed by a dietician on how to record the food and beverages consumed. The food surveys were analyzed by Winford software (Winfood 2.7 Medimatica Srl, Colonnella, Italy) in order to estimate the energy intake and the percentage of macronutrients and micronutrients. The data collected was compared to the tables of food consumption and recommended dietary intakes of the Italian National Institute of Nutrition and Food Composition Database in Italy. The data is expressed as Relative abundance % ± SEM for qRT-PCR analysis. The results were checked for normal distribution using the D'Agostino & Pearson normality test before further analysis. Statistically significant differences in the bacterial community were determined by Student's t with Welch's correction. The analysis was performed with GraphPad Prism 7.0 (GraphPad Software, Inc., San Diego, CA). P–values equal to or less than 0.05 were considered significant.

Recent evidence suggests a strong correlation between skin microbiome, including that of the scalp, and many dermatological conditions (Cogen et al., 2009; Zeeuwen et al., 2013; Belkaid & Hand, 2014). Poor information is currently available as regards the microbial community inhabiting the scalp and hair growth disorders. In a previous work we highlighted, for the first time, the presence of a bacterial unbalance in subjects affected by Alopecia (Rinaldi et al., 2018).

Figure 1 reports the % of the distribution of main bacterial strains on the scalp in analyzed subjects, grouped as regard SALT score. Data are expressed as % of fold induction versus healthy subjects in our database. All analyzed groups showed an increase in the P. acnes population and no significant (p<0,005) differences were reported between groups. All groups also showed a significant (p<0.005) decrease of S. epidermidis species compared to baseline (healthy subjects) (Fig. 1). No significant (p<0,005) differences were found as regard S. aureus species in group S2, S3 and S4 (Fig. 1). On the contrary, a significant (p<0.005) decrease was reported in the S5 group (Fig. 1).

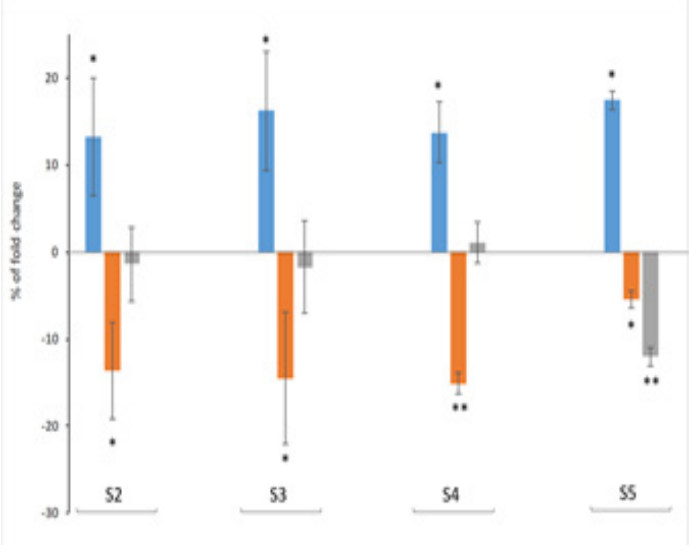
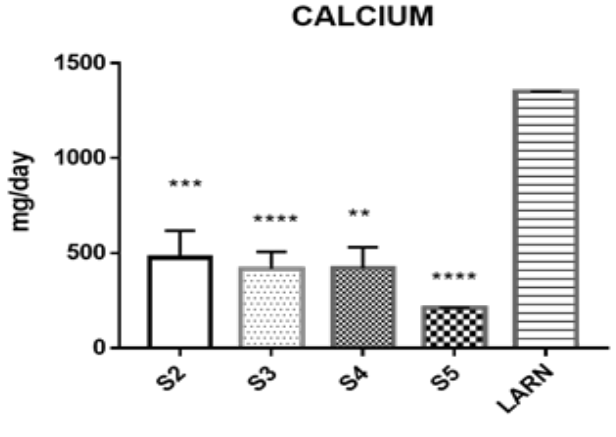


Figure 1: RT-qPCR quantification (% of fold change) of main bacterial species (P. acnes, S. epidermidis, and S.aureus) inhabiting the scalp in subjects affected by alopecia areata (AA) (N=15). S2: 25%-49% hair loss; S3: 50%-74% hair loss; S4: 75%-99% hair loss; S5: 100% hair loss. * p<0.05; **p<0.01.

Figure 3 show the intake of macronutrients and micronutrients in the analyzed group, compared to Recommended (LARN) values in Italy.



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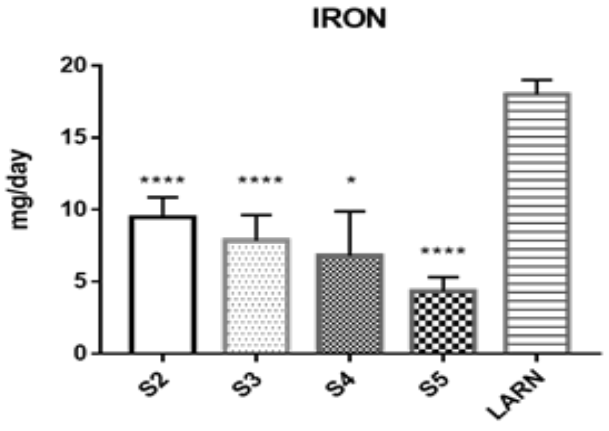

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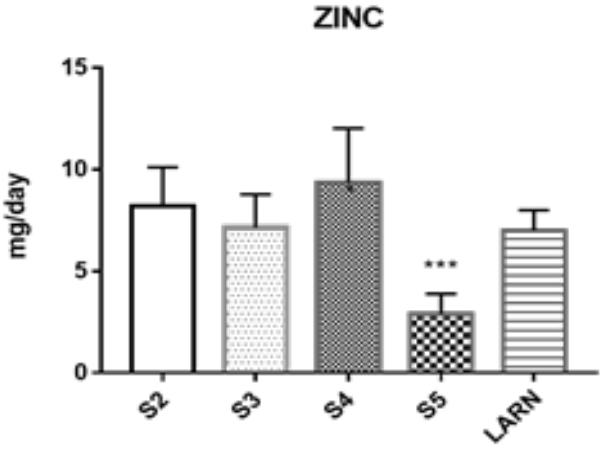

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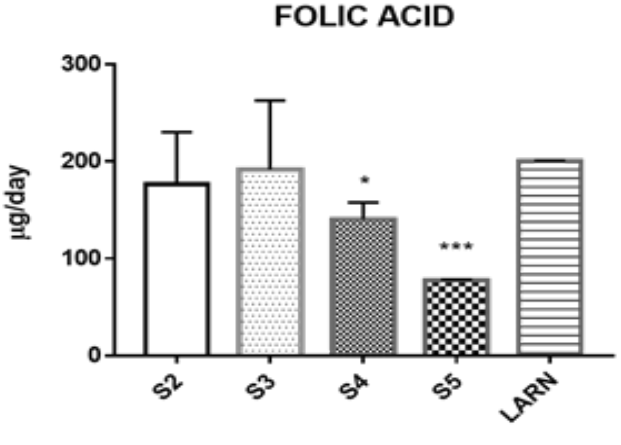

Figure: 3d

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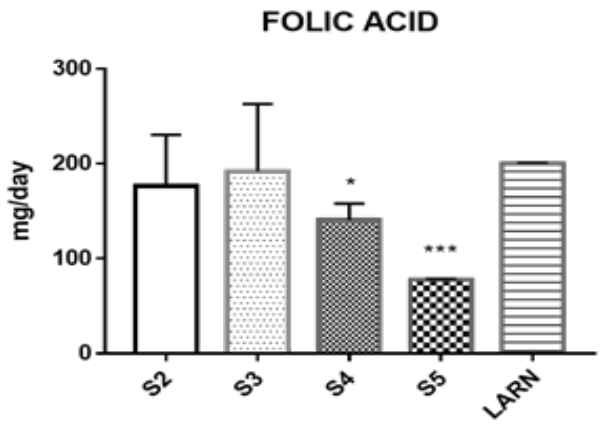

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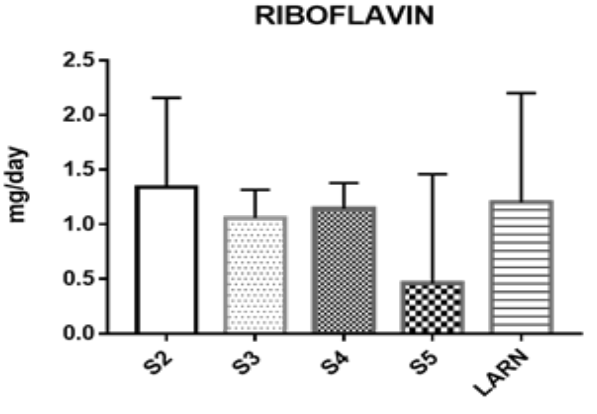

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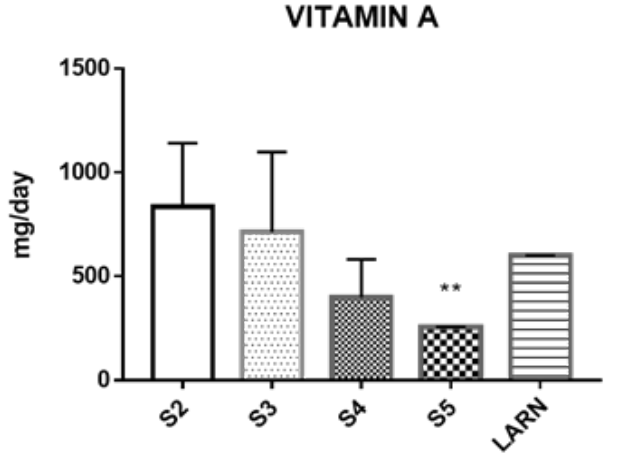

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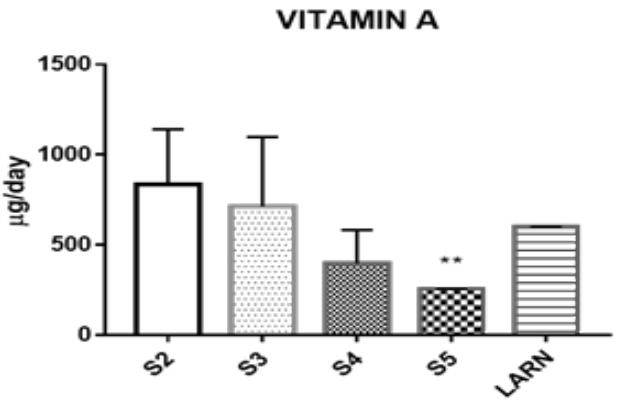

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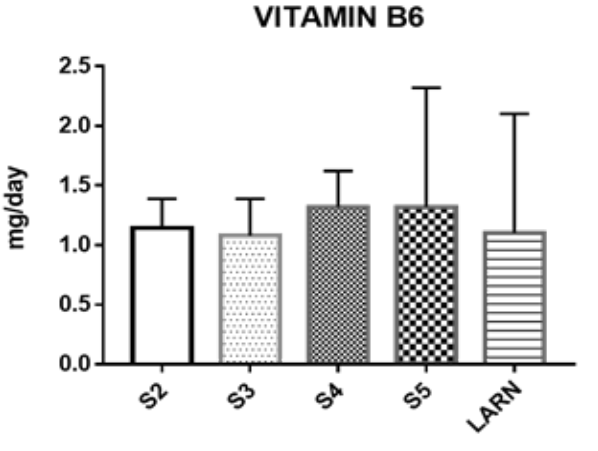

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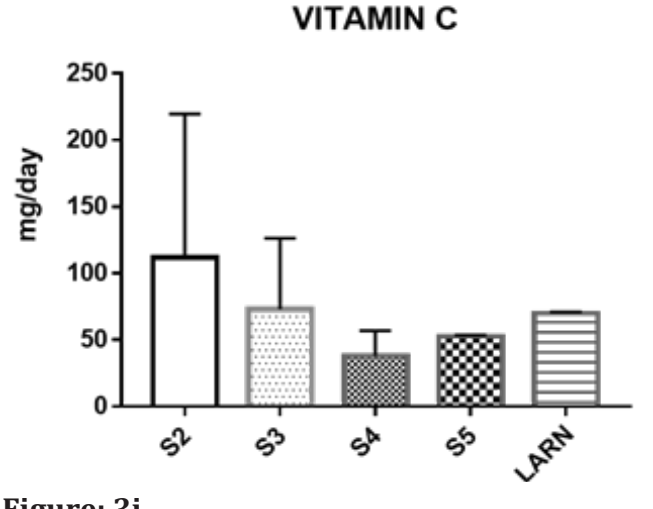

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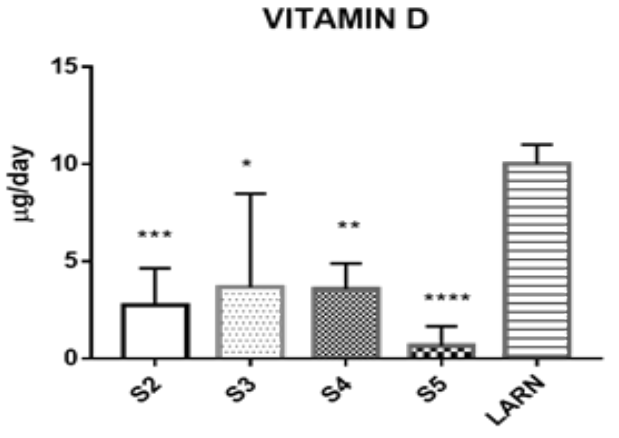

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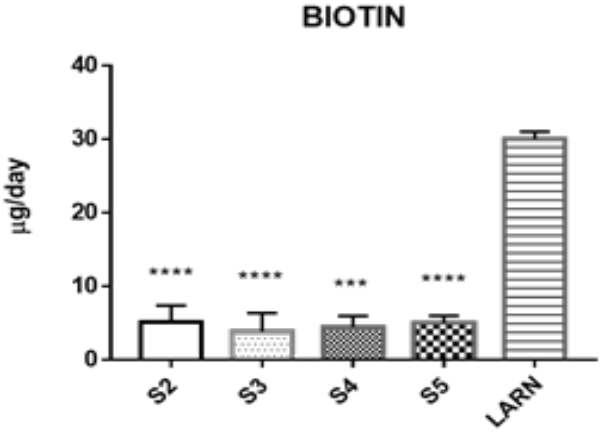

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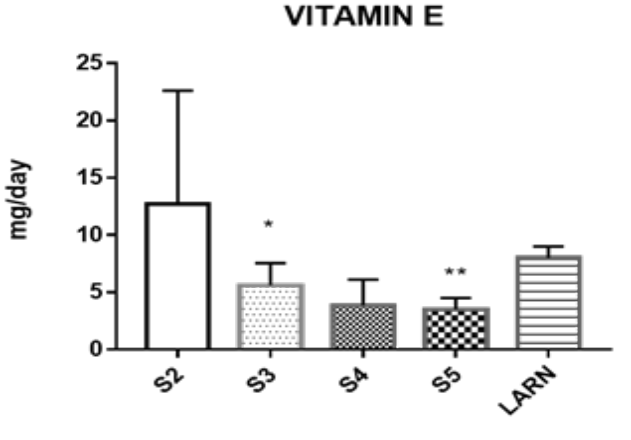

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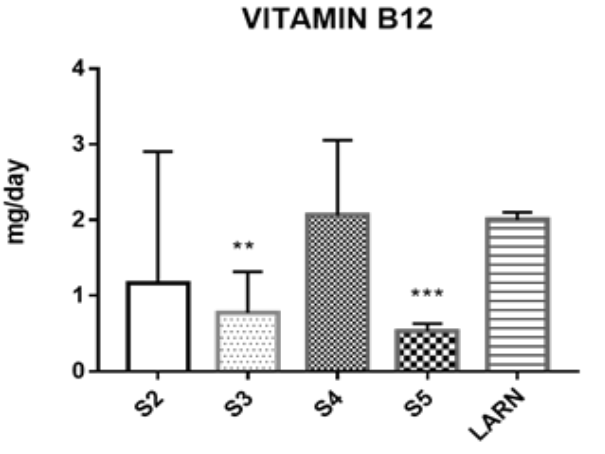


Figure: 3n

Figure 3. Daily reported micronutrients intake in subjects affected by alopecia areata (AA) (N=15), assessed by a 7-day weighed food record. LARN: Nutrition and Energy Reference Assuming Levels.

(a) Calcium; (b) Iron; (c) Zinc; (d) Folic acid; (e) Niacin; (f) Riboflavin; (g) Thiamine; (h) Vitamin A; (i) Vitamin B6; (j) Vitamin C (k) Vitamin D; (l) Biotin; (m) Vitamin E; (n) Vitamin B12. S2: 25%-49% hair loss; S3: 50%-74% hair loss; S4: 75%-99% hair loss; S5: 100% hair loss. * $p<0.05$; ** $p<0.01$.

The daily amount of proteins was significantly lower in S3, S4 ($p< 0.05$) and, especially, S5 group ($p< 0,01$) (Fig. 2a). The analysis of the food survey also highlighted a lower intake of lipids for S3 ($p<0,005$) and S5 group ($p<0,001$) (Fig. 2b). S2, S3, and S4 also showed lower and comparable intake of carbohydrates compared to LARN values ($p< 0,005$) (Fig. 2c). All analyzed groups also reported a very small intake of amide (S2: $p<0,001$; S3: $p<0,005$; S4: $p<0,01$; S5: $p<0,001$) (Fig. 2d) and fiber (S2: $p<0,01$; S3: $p<0,05$; S4: $p<0,01$; S5: $p<0,001$) (Fig. 2e). No differences were found as regard cholesterol (Fig. 2f) and saturated fatty acids (Fig. 2g) intake compared to LARN values. Most interesting, a significant lower intake of polyunsaturated fatty acids (S2: $p<0,05$; S3: $p<0,001$; S4: $p<0,01$; S5: $p<0,001$) (Fig. 2h) was reported in all groups. On the other side, all groups showed a higher intake of monounsaturated fatty acids (S2: $p<0,01$; S3: $p<0,005$; S4: $p<0,01$; S5: $p<0,001$) (Fig. 2i).

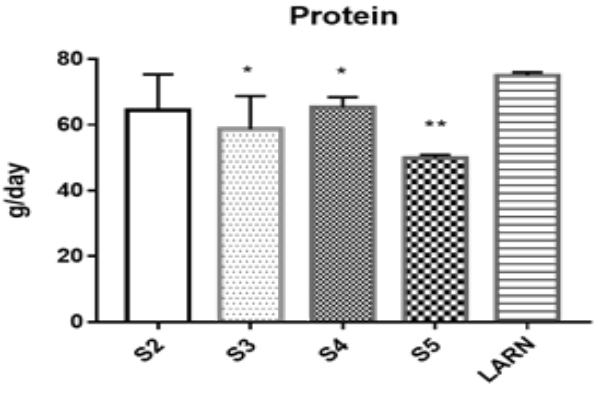


Figure: 2a

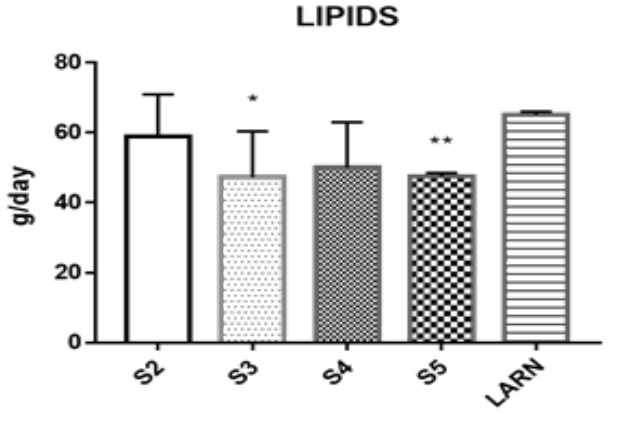


Figure: 2b

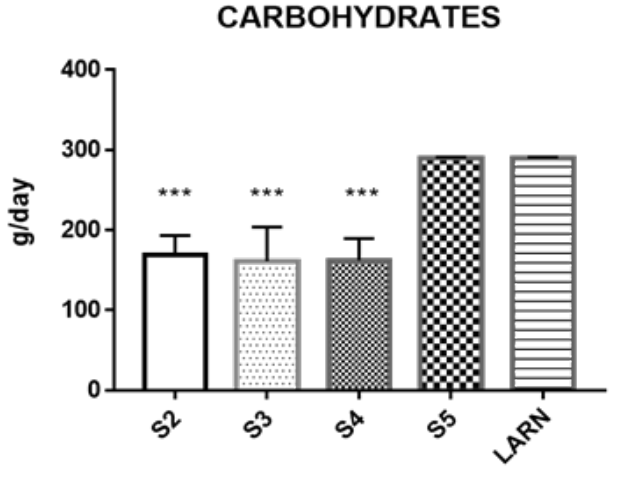
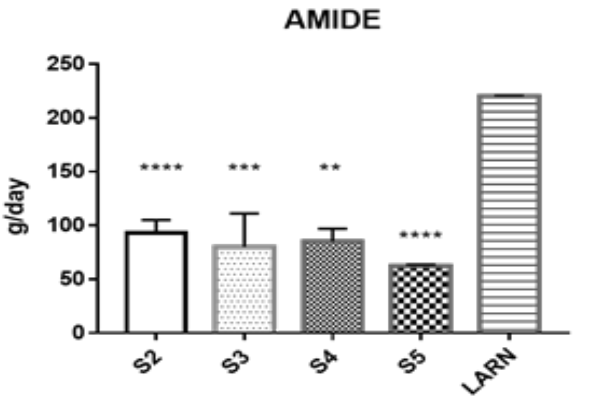


Figure: 2c



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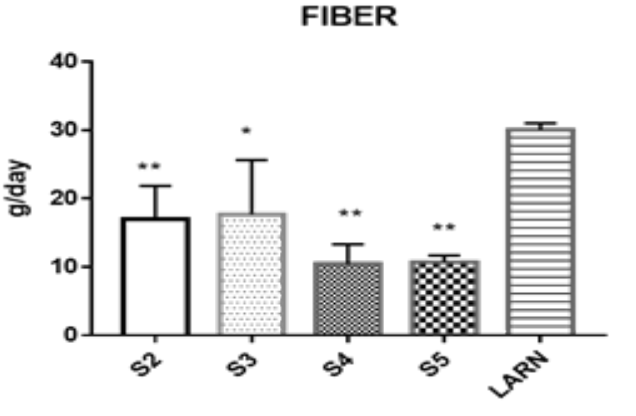

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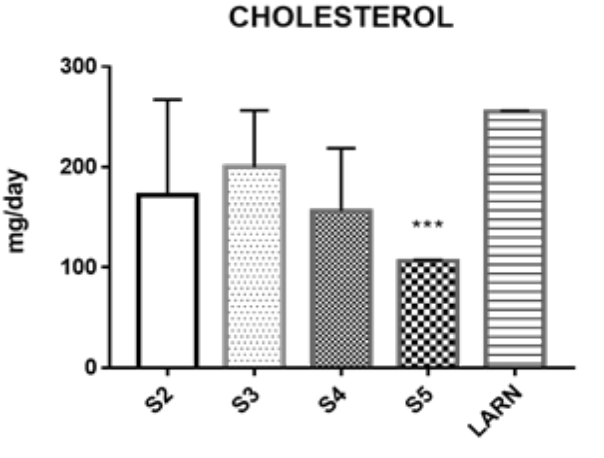

Figure: 2f

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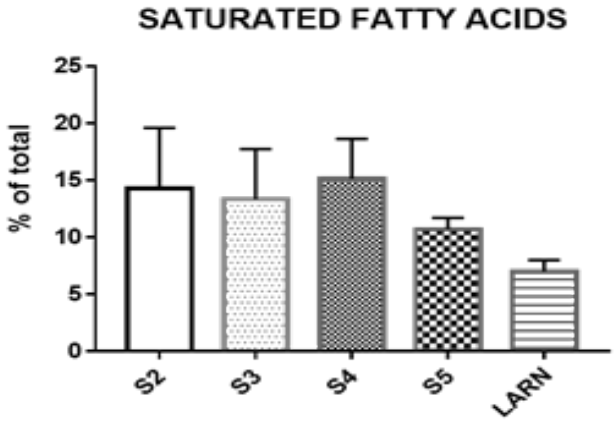

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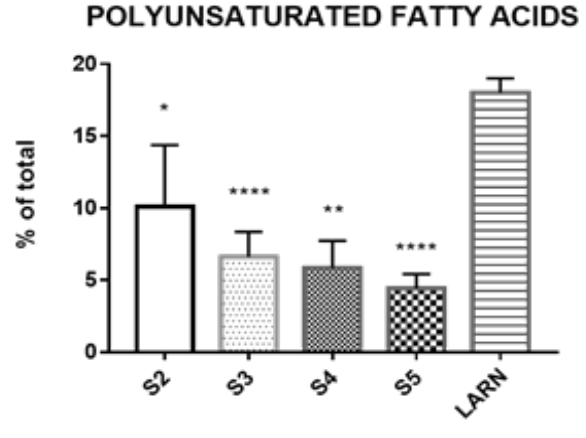

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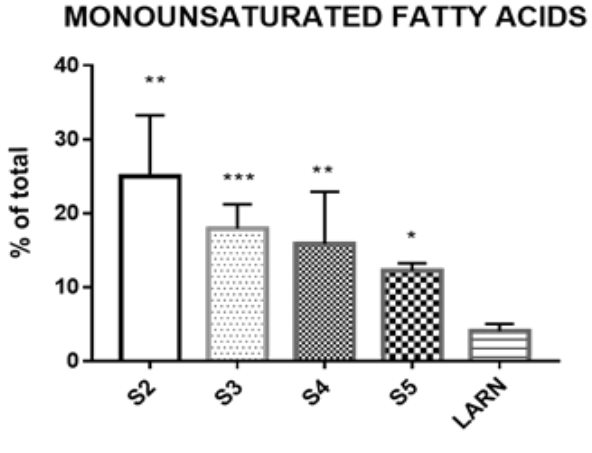

Figure: 2i

Figure 2: Daily reported macronutrients intake in subjects affected by alopecia areata (AA) (N=15), assessed by a 7-day weighed food record. LARN: Nutrition and Energy Reference Assuming Levels.
(a) Proteins; (b) Lipids; (c) Carbohydrates; (d) Amide; (e) Fiber; (f) Cholesterol; (g) Saturated fatty acids; (h) Polyunsaturated fatty acids; (i) Monounsaturated fatty acids. S2: 25%-49% hair loss; S3: 50%-74% hair loss; S4: 75%-99% hair loss; S5: 100% hair loss. * $p<0.05$; ** $p<0.01$.

With regards to the micronutrients intake (Fig. 3), we noticed a significantly lower intake of calcium (S2: $p<0,005$; S3: $p<0,001$; S4: $p<0,01$; S5: $p<0,001$) (Fig. 3a), and iron (S2: $p<0,001$; S3: $p<0,001$; S4: $p<0,05$; S5: $p<0,001$) (Fig. 3b) in all analyzed groups. No differences were reported with regards to the zinc intake between groups and compared to LARN values (Fig. 3c). A significantly lower intake of folic acid was reported only in S4 and S5 groups (S4: $p<0,05$; S5: $p<0,005$) (Fig. 3d). No significant differences were reported in vitamins intake (Fig. 3e-j) with the exception of Vitamin D (Fig. 3k) (S2: $p<0,005$; S3: $p<0,05$; S4: $p<0,01$; S5: $p<0,001$) and biotin (Fig. 3l) (S2: $p<0,001$; S3: $p<0,001$; S4: $p<0,005$; S5: $p<0,001$). A significantly lower intake of vitamin E and vitamin B12 was also reported in S3 and S5 group, respectively (S3: $p<0,05$; S5: $p<0,01$ and S3: $p<0,01$; S5: $p<0,001$, respectively) (Fig. 3m-n).

The findings of the present work are in line with our previous data. Here we added more knowledge as regards to bacterial dysbiosis and AA severity score. Our findings suggested the presence of a unique ecosystem in AA patch which leads to an unbalance in P. acnes species at the expense of S. epidermidis species. Even if an interindividual difference has to be considered, this unbalancing did not seem to be correlated with the severity of AA, with the exception of the more severe grade (S5, as SALT score level) in which there has also been a significant decrease of S. aureus species reported.

The aim of the present work was to investigate if different dietary habits can contribute to microbial dysbiosis of the scalp of AA subjects.

Hypocaloric regimen or scarcity of proteins, minerals, amino acids, vitamins, and essential fatty acids derived from an unbalanced diet can lead to structural changes in the hair follicle and, eventually, to hair loss (Rushton, 2002). Therefore, micronutrients have been implicating in affecting chronic telogen effluvium, androgenetic alopecia (AGA), female pattern hair loss (FPHL), and AA (Spivak & Jackson, 1977;

Goldberg & Lenzy, 2010; Mubki et al., 2014). Indeed, many of the above micronutrients are reported to affect the hair follicle regarding restoration of hair growth, cell division, cycling (Finner, 2013).

Even though recent evidence (Scott et al., 2013) strongly highlighted the ability of diet to impact on gut and oral (Kato et al., 2017) microbiome, poor knowledge is still available about the influence of diet on microbial dysbiosis in skin disorders (Bowe et al., 2010; Gallo et al., 2011). Evidence is mainly linked to acne vulgaris (Borde & Astrand, 2018; Zouboulis et al., 2014; Grossi et al., 2016), atopic dermatitis (Manam et al., 2014) and psoriasis (Zákostelská et al., 2016).

More and more evidence has been accumulated with regards to the link between gut and hair disorders (Rebello et al., 2017; Borde & Astrand, 2018). In autoimmune disease, among which AA, immune response leads to tissue damage and loss of function of the intestinal barrier (Mu et al., 2017). Therefore, the permeability of the epithelial lining may be compromised; antigens, toxins, and bacteria migrated from the lumen to the bloodstream leading to a syndrome known as "leaky gut" (Mu et al., 2017). Modulating the gut microbiome also by mean of diet represents a valid approach for regulating and restoring such damage, leading to an improvement of the autoimmune disease.

Combining data from microbial dysbiosis of the scalp and diet a clear link between dietary food intake and the severity grade of AA could not be hypothesized. On the other hand, the impact of lower intake of some macronutrient and micronutrients lead to hypothesize an impact on microbial dysbiosis on the scalp and suggests the possibility to modulate microbial dysbiosis by targeted dietary approaches.

For example, modulating the intake of monounsaturated fatty acids by lowering them in favor of some polyunsaturated fatty acids could help reduce general inflammatory status (Raphael et al., 2013).

Similarly, improving the intake of some micronutrients such as calcium, iron and folic acid could lead to an improvement of hair follicle healthiness. Most interestingly, all analyzed groups reported a lower intake of vitamin D and biotin, two micronutrients strongly involved in hair follicle development (Finner, 2013; Chiu et al., 2015; Patel et al., 2017). The benefits of dietary intervention would be not be limited to hair follicle development itself, but also extended to the modulation of the microbial ecosystem around the hair follicle. Thus, increasing dairy intake of biotin and in general, vitamins leads to an increase of nutrients for the hair follicle contributing to a healthier ecosystem for microbial communities of the scalp which can result in themselves be stimulated into micronutrients biosynthesis.

The data from the present study is just a limited representation of a larger set of data we have accumulated in our clinical practice. Indeed, we have noticed, for example, how a gluten-free diet could strongly affect AA evolution in patients affected by non-celiac gluten sensitivity (NCGS), in which AA manifestations systematically recurred following a non-gluten free diet. Therefore, even though not conclusive, our data also opens to a new diet and microbiome-based adjuvant approaches in the management of hair disorders such as AA. Larger studies are still needed to better investigate the role of the microbiome in scalp diseases and different drivers involved in this process.

AUTHOR STATEMENTS

Conceptualization, Methodology, and Investigations: DP and FR. Data curation and Formal Analysis: DP. Resources: ES and FR. Wrote the paper: DP, BM, and FR. Funding acquisition: GG and FR. Supervision: ES and FR.

CONFLICT OF INTEREST

FR and ES serve as a consultant for Giuliani S.p.A. DP are employed by Giuliani S.p.A.

FUNDING INFORMATION

This study was supported by the Giuliani SpA.

ETHICAL APPROVAL

The study was approved by the Ethical Independent Committee for Clinical, not a pharmacological investigation in Genoa (Italy) and in accordance with the ethical standards of the 1964 Declaration of Helsinki. Volunteers signed informed consent.

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