

Growth factors in relation to obesity, food habits, and microbiota among healthy Saudis: preliminary results

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Abstract. – OBJECTIVE: Obesity is a serious problem among Saudis because of the country's affluent lifestyle. Obesity is associated with various metabolic disorders and characterized by low-grade inflammation that leads to the release of pro-inflammatory cytokines, human growth factors (GFs), lipids, aberrant adipokines, and other chemokines from adipose tissue. The objective of this study is to delineate the effects of GFs on microbiota and their relationship to body mass index (BMI) and food habits.

SUBJECTS AND METHODS: In a cross-sectional study, 32 randomly selected participants (16 males and 16 females) were enrolled in a survey covering their sociodemographic information, medical history, lifestyle, and dietary practices. The information on diet, health condition, food and drink intake habits were examined under four distinct BMI categories: normal, underweight, overweight, and obese. The participants' serum samples were analyzed for the various GFs using a human magnetic 30-plex panel multiplex assay. Bioinformatics analysis was performed to investigate which bacterial taxa are enriched and to predict the functional profiles of the samples.

RESULTS: Correlational studies revealed sex-based differences between GFs and microbiota. Females exhibited a significant correlation between epidermal GF (EGF) and *Proteobacteria*, whereas males showed a significant correlation between fibroblast GF-basic and *Actinobacteria*. Interestingly, a combined analysis of both sexes showed a significant correlation between EGF and vascular endothelial GF with *Firmicutes*. The data in the underweight group revealed a correlation between granulocyte colony-stimulating factor (G-CSF) and hepatocyte GF with *Firmicutes*. In the obese group, a correlation was found between G-CSF and *Actinobacteria*.

CONCLUSIONS: Our results identified links between GFs, microbiota, and BMI in a Saudi cohort. The insights from this preliminary study will contribute to the predictive diagnosis of obesity. However, further research involving a larger cohort will be necessary to understand the mechanistic aspects of these GFs to provide biomarkers of potential obesity.

Key Words:

Growth factors, Microbiota, Obesity, Body mass index, Food.

Introduction

Most of the world's adult population is expected to be either overweight or obese by 2030¹. The latest data² (2016) suggest that 39% of adults aged 18 years or over (38% of males and 40% of females) are overweight. In Saudi Arabia, the prevalence of obesity among adults increased progressively from 22% in 1990-1993³ to 36% in 2005⁴. More recently, interest has emerged regarding the gut microbiota's role in determining the effects of diverse genetic combinations and environmental factors on the immune system and, thus, disease susceptibility⁵⁻⁷.

The first evidence of the role of microbiota in controlling adiposity and energy homeostasis came from animal studies⁸. The quality and composition of the diet have also been reported⁹ to affect the microbiota. In fact, consuming a high-fat diet results in a major shift in metagenomic characteristics as compared to a normal or balanced diet. This is best seen in high-fat Western diets, in which a reduction in *Bacteroidetes* and an increase in *Firmicutes*, especially Mollicutes, is associated with an increase in the genes responsible for handling sugars¹⁰.

A wide range of pro-inflammatory mediators, including growth factors (GFs), such as the nuclear protein high mobility group box-1 (HMGB1), are implicated in several pathogenic processes related to obesity¹¹. HMGB1 is an important mediator known to be involved in signaling by way of advanced glycation end products, particularly the receptor for advanced glycation products (RAGEs), and through the toll-like receptors (TLRs)^{12,13}. In spite of these differences in species composition, many functions of the gut microbiota are apparently shared among individuals. Such functions include the conversion of nondigestible carbohydrates (dietary fiber) to short-chain fatty acids, the provision of a barrier against pathogenic bacteria, and the modulation of the innate and adaptive immune systems¹⁴⁻¹⁶. Indeed, recent metagenomic and metabolomic studies^{10,17-20} demonstrate that gut microbiota may modulate the harvesting of energy from the diet, the storage of energy as triglyceride, and energy expenditure through fatty acid oxidation, in turn mediating diet-induced obesity.

The recent spate of knowledge suggests that studying gut microbiota could open new doors in the field of obesity management. Due to the effect of diet and host genes, gut microbiota composition will vary in different populations, but, to

date, no data are available on the largely affluent Saudi population. Thus, this project aimed to delineate the effects of GFs on microbiota and their relationship to body mass index (BMI) as well as the effects of food habits on GFs among the Saudi population.

Subjects and Methods

Study Sample

Two hundred healthy Saudi adults were surveyed using a preset questionnaire. Of the 117 who satisfied both the inclusion and exclusion criteria (56 females and 49 males), 32 participants (16 females and 16 males) were enrolled and stratified under four BMI categories (with four subjects each) following World Health Organization (WHO) criteria as follows: obese ≥ 30 kg/m²; overweight 25.0-29.9 kg/m²; normal 18.5-24.9 kg/m²; underweight < 18.5 kg/m²²¹. Fecal and blood samples of 2 mL were collected from all the participants. Participants were eligible for the study if they were ≥ 18 years of age, apparently healthy, and not on any medication affecting weight or sleeping habits. Among those recruited those with history of colon cancer, inflammatory bowel disease, or acute or chronic diarrhea in the previous eight weeks, those treated with antibiotics in the two months prior to fecal sampling, and those taking medication or supplements were excluded from the study. Informed consent was obtained from all the subjects prior to participation after they were informed of the study's objectives, their right to know, and their right to withdraw from the study. The investigation was ethically approved by the institutional review board of King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia (No. 361-14).

Study Design

This cross-sectional study surveyed healthy adult individuals accompanying relatives in primary health centers and hospitals in the cities of Jeddah and Makkah, Saudi Arabia. All the participants completed a predesigned questionnaire on their sociodemographic information, medical history, lifestyle, and dietary practices. The recruits were interviewed by trained medical students using a structured questionnaire that included demographic information and specific questions on dietary habits, patterns, and intake as well as specific questions on sleep patterns.

General Health Indicators and Anthropometric Measurements

The height of each participant was measured to the closest 0.5 cm using a stadiometer (Deteco, Webb City, MO, USA), and the lightly clothed participants were weighed to the nearest 0.5 kg using a portable calibrated scale (Omron BF511, Beringe, The Netherlands). Blood pressure was measured using a mercury blood pressure monitor (Kawamoto Corporation, Osaka, Japan), and heart rate was recorded. Anthropometric measurements, including waist, hip, and neck circumference, were taken using standard methods²². BMI – an individual's weight in kg divided by their height squared ($\text{BMI} = \text{kg}/\text{m}^2$) – was calculated for each participant.

Blood Sample Collection

Overnight fasting blood samples of 2 mL were drawn from the subjects and placed in sterile tubes. The tubes were centrifuged, and the serum was transferred to labeled Eppendorf tubes and stored at $-80\text{ }^{\circ}\text{C}$ until analysis.

Extraction of DNA from Stool Samples and 16S Ribosomal RNA Sequencing Using MiSeq Technology

A NucleoSpin[®] Tissue Mini Kit (Macherey Nagel, Hoerd, France) was used to extract DNA samples for sequencing of 16S ribosomal RNA using MiSeq technology as previously reported²³⁻²⁵.

Multiplex Growth Factors Assay

Multiplex GF analysis was performed on the participants' serum for hepatocyte growth factor (HGF), epidermal growth factor (EGF), fibroblast growth factor-basic (FGF-B), granulocyte-macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), and granulocyte colony-stimulating factor (G-CSF) using a multiplex immune-bead assay kit for human cytokines 30-plex panel (LHC6003M, Thermo Fisher Scientific, Waltham, MA, USA) end according to the manufacturer's instructions. Briefly, antibody-coated polystyrene magnetic beads (25 μL) with various spectral intensities were sonicated in solution, added to each well of a 96-well plate, and washed twice with $1\times$ wash buffer; this and subsequent washing steps were done using a handheld magnetic plate placed at the bottom of the 96-well plate to retain the magnetic beads and prevent their loss. Standards (1:3 serial dilution) and samples (undiluted serum) were prepared and added to the beads (100 μL), the mix-

tures were incubated at room temperature on an orbital shaker at 500 rpm for two hours to capture analytes, and then the plates were washed to remove all unbound analytes. The plates were then incubated with biotinylated detection antibodies (100 μL) for one hour and then washed twice with wash buffer (200 μL). Streptavidin-RPE antibodies (100 μL) were then added to the beads, which were incubated for 30 minutes and then washed three times with wash buffer (200 μL). The magnetic beads were then resuspended in 100 μL of wash buffer, and the data were acquired using a MAGPIX instrument (Luminex, Waltham, MA, USA). Finally, the GF expression data were analyzed using a Luminex xPONENT multiplex assay.

Bioinformatics

The raw NGS data produced by paired-end sequencing using the MiSeq technology were demultiplexed and analyzed using the Quantitative Insights into Microbial Ecology 2 (QIIME 2) pipeline (version 2021.2)²⁶. Quality control and denoising were performed using the DADA2 package (Bioconductor, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA), with forward and reverse truncation set to 210 bp and 185 bp, respectively, to remove low-quality and chimeric sequences. The taxonomic classification analysis was performed by assigning the denoised sequences to the representative reference sequences of bacterial genomes using an in-house trained classifier. The training was performed using the latest version of Greengenes (13_8) 99% Operational Taxonomic Units (OTUs) full-length sequences. The database contains reference sequences that are clustered using a 99% similarity threshold, offering the most taxonomic information and the highest accuracy of classification, especially when using clinical data and not simulated data²⁷. Decreasing the percentage of similarity cut-off results in a rapid loss of information; the number of unique taxonomic labels (genus and species level) declines as sequences are collapsed into larger OTUs²⁷. Each denoised sequence was assigned to a single OTU with a specific ID number and was subsequently considered to be representative of the specific taxonomic unit. To identify the varying abundance of taxa (OTUs) between groups with different EGF and FGF levels, thus explaining the differences between groups, we performed an analysis using the Linear Discriminant Analysis Effect Size (LEfSe tool 1.0.8, The Huttenhower Lab, Boston,

Table I. Demographics of the study population.

Total		Count 32	Percentage 100.0
BMI	Underweight	8	25.0
	Healthy	8	25.0
	Overweight	8	25.0
	Obese	8	25.0
Sex	Male	16	50.0
	Female	16	50.0

MA, USA)²⁸, setting the *p*-value at 0.05 and the logarithmic linear discriminant analysis (LDA) score cutoff at 2.0. LEfSe uses the nonparametric factorial Kruskal-Wallis' (KW) sum-rank test to detect features with significantly different abundance with respect to the class of interest. The association between the taxa reported as differently abundant and the specific metabolic functions in which microbiota take part were investigated using the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUST2) pipeline²⁹. PICRUST analysis was conducted to predict the total number of molecular pathways (and their relative abundances) to which the microbial community of each sample contributed. To extract pathway abundances as high-level prediction output, we used the MetaCyc database (an open-source alternative to KEGG) as suggested by the PICRUST2 manual. A subsequent LEfSe analysis was performed to identify the most differently abundant pathways among various groups²⁹. The threshold of the logarithmic LDA score for discriminative features was set to 2.0; as a result, only pathways meeting

an LDA significance threshold > 2 and with a statistically significant change (*p* < .05) in relative abundance are shown.

Statistical Analysis

Prism GraphPad version 6.0 (La Jolla, CA, USA) was used to evaluate the results. Pearson's moment correlation was employed to measure the strength of the relationship between microbiota and GFs with the effect of sex and BMI. The Jonckheere-Terpstra test for ordered variables was used to evaluate the effect of food intake on GFs. *p*-value considered to be significant when lower than 0.05.

Results

Microbiota Composition According to BMI Stratification

Table I shows the demographic characteristics for participants, while Table II shows the mean, median, Standard Deviation (SD), and ranges of microbiota counts and the investigated GFs in all participants.

Investigation of discriminant bacterial taxa and predicted metabolic potentials of the microbiota in individuals presenting different levels of growth factors

The samples were categorized in three groups (low, normal, and high) based on the level of the GF of interest identified in the patients' serum; they were subjected to LDA using the LEfSe tool. The analysis revealed which taxa were differently

Table II. Descriptive statistics.

Variables	N	Mean	Median	SD	Range
Microbiota					
Bacteroidetes	32	25,237.16	23,902.00	12,446.48	61,722.0
Actinobacteria	32	811.19	320.50	1,222.52	5,939.0
Verrucomicrobia	32	979.25	11.50	1,971.62	7,989.0
Proteobacteria	32	2,186.69	1,200.00	2,500.10	10,080.0
Firmicutes	32	22,581.59	17,808.00	12,364.87	58,753.0
Growth Factor					
G-CSF	32	67.03	73.49	17.37	67.9
GM-CSF	32	0.85	0.63	0.62	2.5
EGF	32	58.95	25.00	64.22	248.2
HGF	32	201.80	166.16	114.44	584.7
FGF-B	32	20.71	13.02	33.13	175.8
VEGF	32	2.59	0.00	7.65	35.31

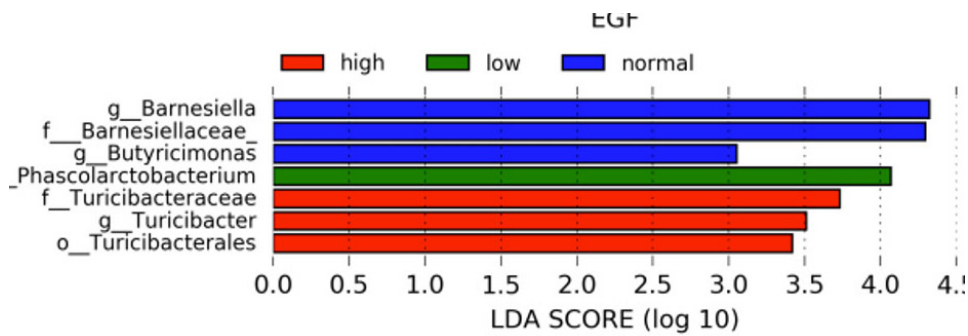


Figure 1. The bacterial taxa enriched in samples with high, low, and normal EGF levels are depicted with red, green, and blue bars, respectively.

abundant between samples with different levels of GFs, thus explaining the differences between these groups. It should be noted that the analysis was successfully completed for the GFs EGF, FGF and HGF; however, differently enriched taxa were identified only in samples with different EGF and FGF levels (Figure 1). No specific taxonomic rank was found to be discriminative between samples with normal and low HGF levels. In the case of the GFs G-CSF and GMCSF, all 32 samples were categorized in the same group (high and normal, respectively); thus, the LEfSe analysis to find taxa discriminative for the different groups could not be performed.

A significant enrichment in the genera *Butyr- icimonas*, *Phascolarctobacterium*, and *Turici- bacter* was observed in samples with normal, low, and high EGF levels, respectively (Figure 1). Specifically, the *Phascolarctobacterium* genus (*Firmicutes phylum*) presents the highest relative abundance in high-EGF samples, with the highest LDA score being slightly above 4.0. The scores for *Turicibacter* (*Firmicutes phylum*) and *Butyr- icimonas* (*Bacteroidetes phylum*) are 3.5

and > 3.0, respectively. The biological pathways analysis using PICRUSt found that the glycolytic pathway in *Pyrococcus* is significantly enriched in samples with normal EGF concentration, presenting an LDA score above 2.0 (Figure 2). In low-EGF samples, the processes of homolactic fermentation and anhydromuropeptides recycling are significantly enriched (LDA > 3.0) (Figure 2).

LEfSe analysis revealed that, at the genus level, members of *Lactobacillus* (*Lactobacillaceae* family) and *Weissella* (*Leuconostocaceae* family) were more relatively abundant in individuals with high levels of FGF, yielding LDA scores slightly above 4.0 and less than 3.5, respectively (Figure 3). According to the prediction of the functional profiles of the samples' microbiota, the biosynthesis of the amino acids L-glutamate and L-glutamine is the most differently enriched (with an LDA approximately equal to 3.0) in samples characterized by low levels of FGF. In high-FGF samples, the most differently enriched pathway is that of inosine 5'-phosphate biosynthesis III, followed by the degradation of glycerol to butanol (Figure 4).

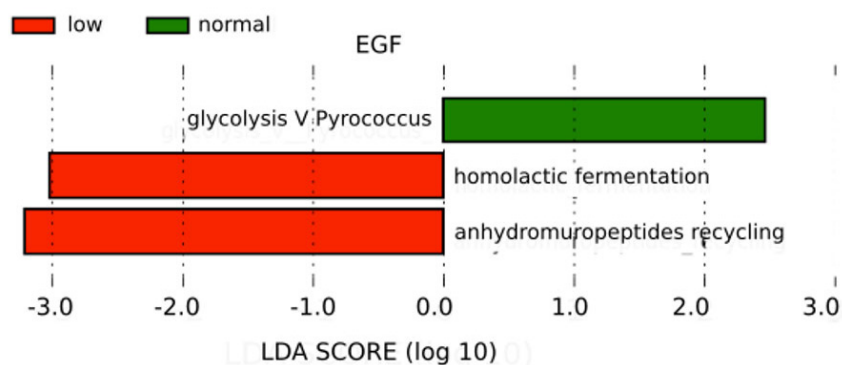


Figure 2. Prediction of the molecular pathways to which the microbial community of low- and normal-EGF samples contributed.

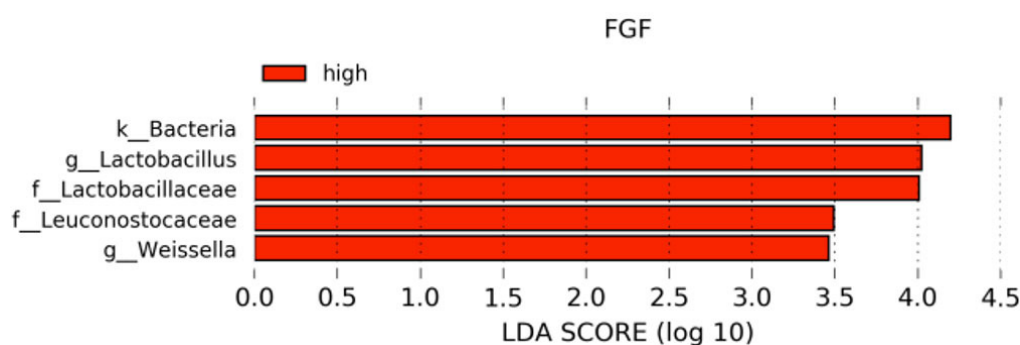


Figure 3. Histogram depicting the results of the LefSe analysis. The LDA scores of bacterial families and genera found to be enriched in samples with high FGF levels are compared to those with normal and low levels (see Figure 4).

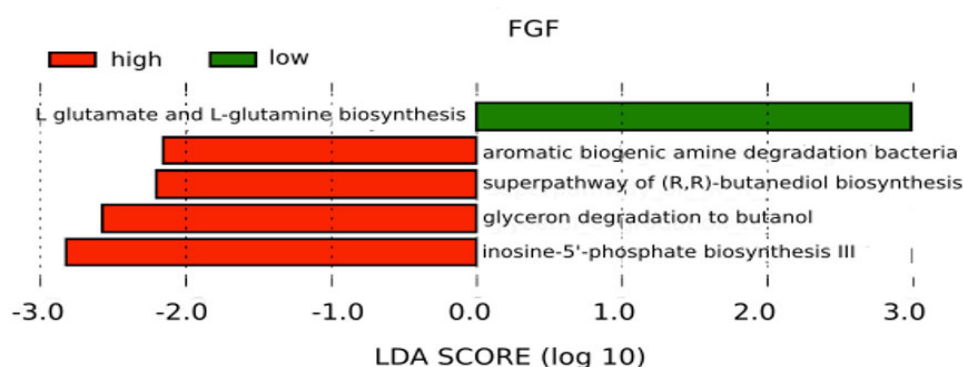


Figure 4. Prediction of the molecular pathways to which the microbial community of high- and low-FGF samples contributed.

The Effect of Growth Factors on Microbiota in Relation to Sex

Table III shows the significant correlations between microbiota and GFs, namely EGF, VEGF, and FGF-B, with and without the effect of sex. Sex has a significant effect on the correlation between

GFs and microbiota. In females, there is a moderate significantly positive correlation between EGF and *Proteobacteria*. In males, a moderate significantly positive correlation is evident between FGF-B and *Actinobacteria*. Combining both sexes revealed that EGF had a weak significantly positive correla-

Table III. Relationship between GFs and microbiota in relation to sex.

Spearman Correlation	rho	Bacteroidetes	Actino-bacteria	Verruco-microbia	Proteo-bacteria	Firmicutes
<i>All</i>						
EGF	r	-0.267	-0.008	-0.084	0.382	0.233
	p	.140	.966	.648	.031*	.200
VEGF	r	-0.167	-0.042	0.180	-0.040	0.364
	p	.362	.818	.324	.827	.041*
<i>M</i>						
FGF-B	r	-0.089	0.576	-0.230	0.375	0.245
	p	.744	.019*	.391	.152	.361
<i>F</i>						
EGF	r	-0.318	-0.282	-0.046	0.571	-0.376
	p	.230	.290	.866	.021*	.151

F: female; M: male; * Statistically significant results ($p < 0.05$).

tion with *Proteobacteria*, while VEGF had a weak significantly positive correlation with *Firmicutes*. Correlations with the remaining GFs were not significant and are not listed.

The LEfSe analysis of the taxa differently enriched between male and female individuals revealed in females a significant presence (LDA score above 3.6) of two genera, *Porphyromonas* and *Paraprevotella*, belonging to the *Bacteroidales* order, *Bacteroidia* class, and *Bacteroidetes*

phylum (Figure 5a). The genera found enriched in male individuals were *Prevotella* (*Bacteroidia* class), *Lachnospira*, and *Dorea* (both *Clostridia* class). The cladogram produced by the LEfSe analysis (Figure 5b) identifies the most differently abundant taxa in the two groups. The green-colored branches depict the taxa enriched in the group of samples derived from male individuals, while those in red represent the taxa enriched in females.

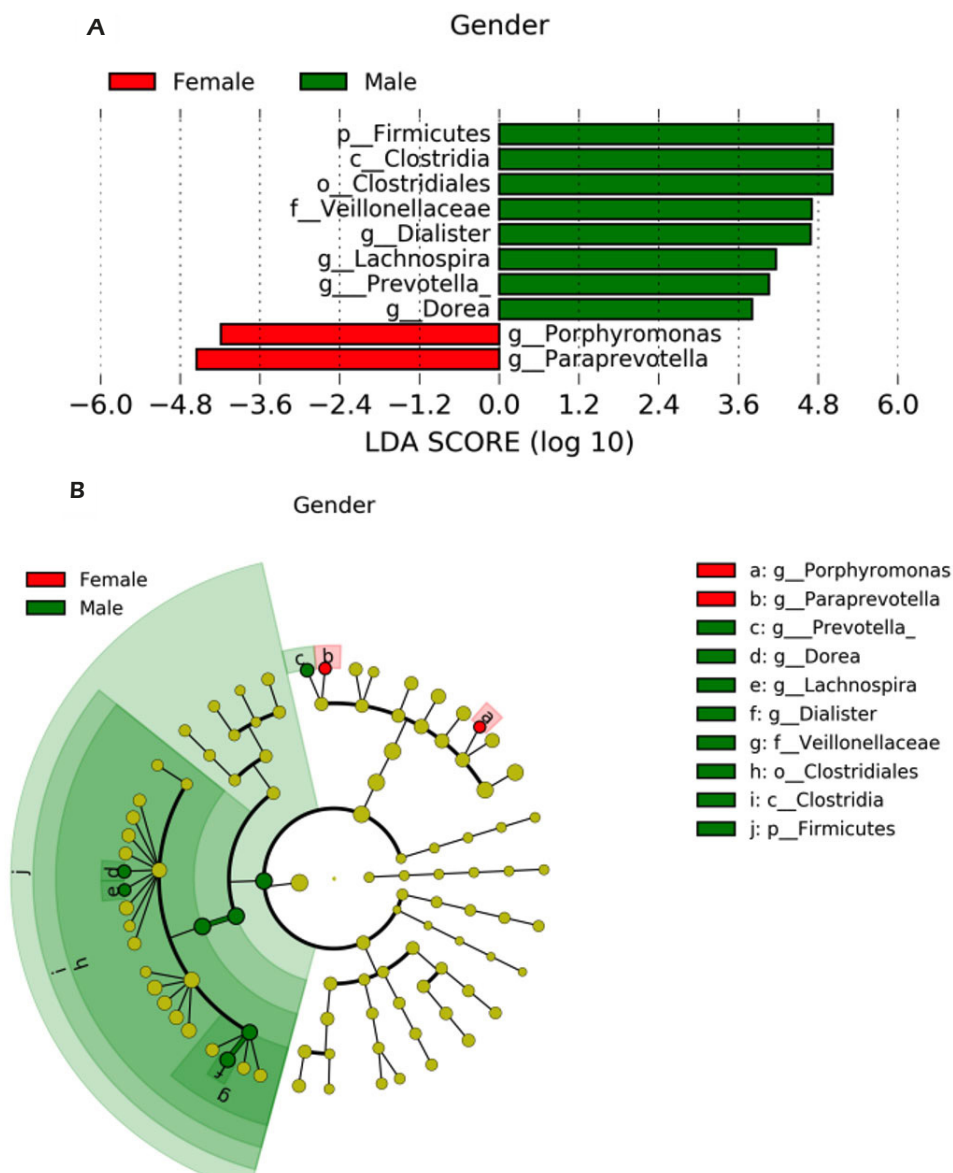


Figure 5. A, Histogram of the LDA scores for bacterial taxa differently abundant between male and female individuals. B, Taxonomic cladogram presenting taxa found significantly differently represented in samples from male and female individuals by LDA effect size. Significantly discriminant taxon nodes are colored, and the branch areas are shaded according to the highest-ranked group for that taxon. Highly abundant and selected taxa are indicated with the letters a-j.

Table IV. Relationship between GFs and microbiota in relation to BMI.

Spearman Correlation	rho	Bacteroidetes	Actino-bacteria	Verruco-microbia	Proteo-bacteria	Firmicutes
Underweight						
G-CSF	r	0.169	-0.116	0.040	0.167	-0.898
	p	.688	.784	.925	.693	.002**
HGF	r	0.277	0.084	0.049	0.210	-0.764
	p	.506	.843	.909	.618	.027*
No						
FGF-B	r	-0.475	-0.020	0.770	-0.194	0.601
	p	.235	.963	.025*	.645	.115
Ov						
EGF	r	-0.110	-0.417	-0.183	0.835	0.037
	p	.795	.304	.665	.010**	.932
Ob						
EGF	r	0.322	0.755	-0.250	-0.114	0.073
	p	.437	.030*	.550	.787	.864

No: normal BMI; Ov: overweight; Ob: obese; *Statistically significant results. ($p < 0.05$); **Statistically high significant results p -value $< .01$.

The Effect of Growth Factors on Microbiota in Relation to BMI Category

Table IV shows the significant effect of BMI on the correlations between microbiota and GFs, namely G-CSF, HGF, and VEGF. The data show strong significantly negative correlations of G-CSF and HGF with *Firmicutes* in subjects categorized as underweight, a strong significantly positive correlation between VEGF and *Verrucomicrobia* in subjects with a normal BMI, a strong significantly positive correlation between VEGF and *Proteobacteria* in overweight subjects, and a strong significantly positive correlation between G-CSF and *Actinobacteria* in obese subjects. Correlations with the remaining GFs were not significant and are not listed.

The LDA size effect analysis did not reveal any representative genus or species for the four BMI groups; however, PICRUST2 analysis provided insight into the functions of the microbiota. The pyrimidine deoxyribonucleosides salvage pathway is the top differently abundant process in underweight as compared to normal, overweight, and obese individuals, with an LDA score close to 3.0 (Figure 6 A and B). The relative abundance of the super-pathway of UDP N acetylglucosamine-derived O-antigen building blocks biosynthesis is high in obese individuals, with an LDA score close to 2.5 (Figure 6 A and C).

Effects of the Consumption of Various Foods on Growth Factors

We studied the effect of various food habits on the tested parameters. Dietary habits were defined as poor when individuals consumed high levels of junk food and highly caffeinated drinks (e.g., French fries, canned juice, black tea, Arabic coffee, Turkish coffee, cappuccino, and energy drinks) and low levels of healthy foods and drinks (e.g., fruits, vegetables, dairy, green tea, fresh juice, roselle, and cinnamon). Only bread exhibited a significant correlation between the amount consumed and levels of the GF VEGF; no other food, healthy or unhealthy, exhibited any significant correlation with other GFs (Table V).

Relationship Between BMI and Tested Growth Factors

The Luminex xMAP analyses of GFs revealed no significant differences between any of the subjects as stratified by BMI category (Figure 7).

However, when subjects were segregated according to sex and stratified under BMI categories, the Luminex xMAP analyses for GFs indicated that HGF levels in the serum of underweight males differed significantly at the 95% confidence level from those of overweight males ($p = .0517$). A nonsignificant decrease in the levels of EGF was observed in the underweight males compared to other groups. However, as for the rest of the factors, the levels did not differ significantly between groups (Figure 8).

Figure 6. A, The most differently abundant predicted function of the bacterial communities in obese and underweight individuals. B-C, Histogram presenting the relative abundance of the two predicted biological functions found enriched in obese and underweight individuals.

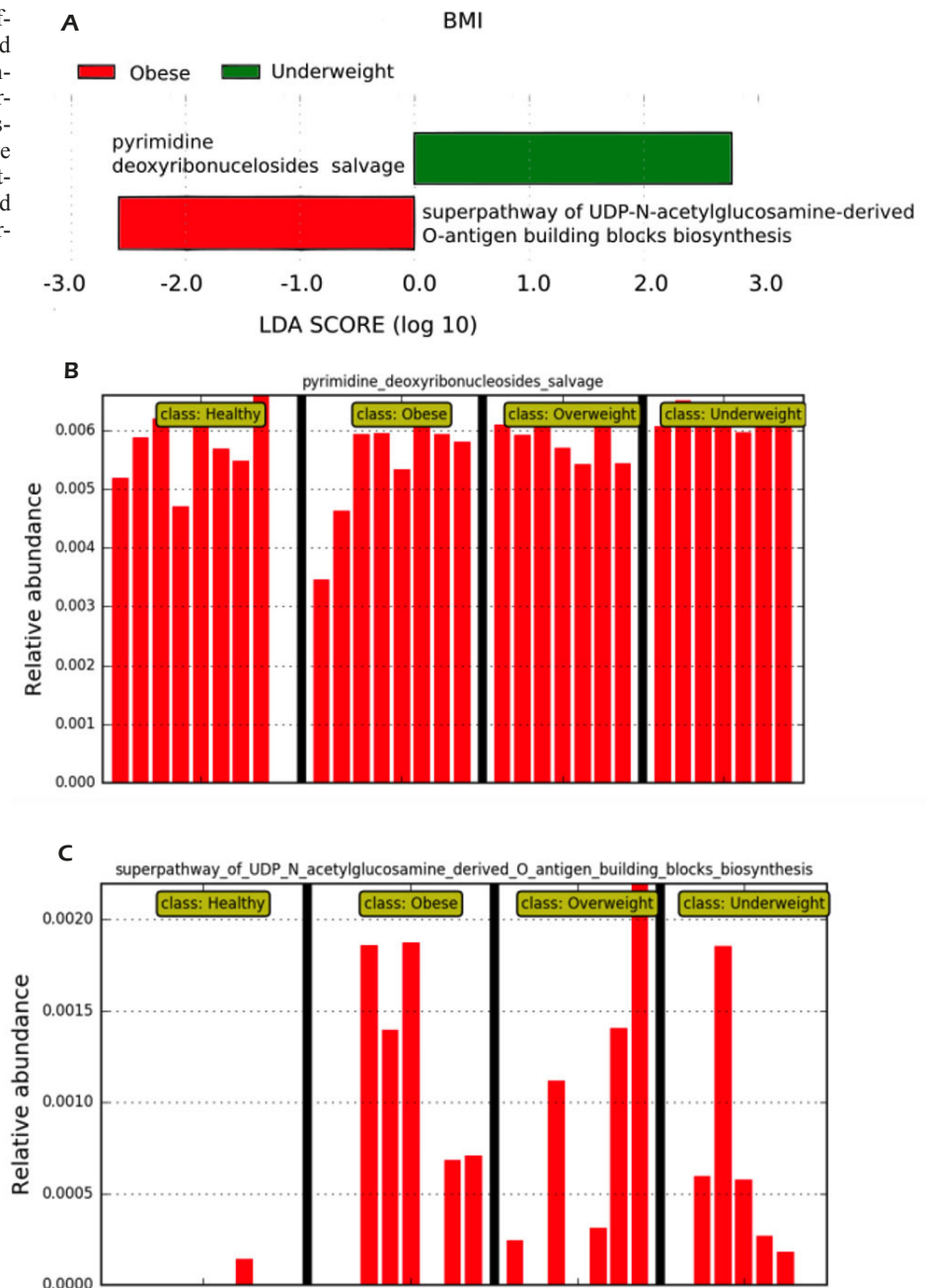


Table V. Effect of bread consumption on VEGF level.

Variable	Growth Factor	Jonkcheere-Terpstra Test Consumption Level			p-value
		Low	Moderate	High	
Bread	VEGF	21.43	13.00	13.84	.011*

*Statistically significant results ($p < 0.05$).

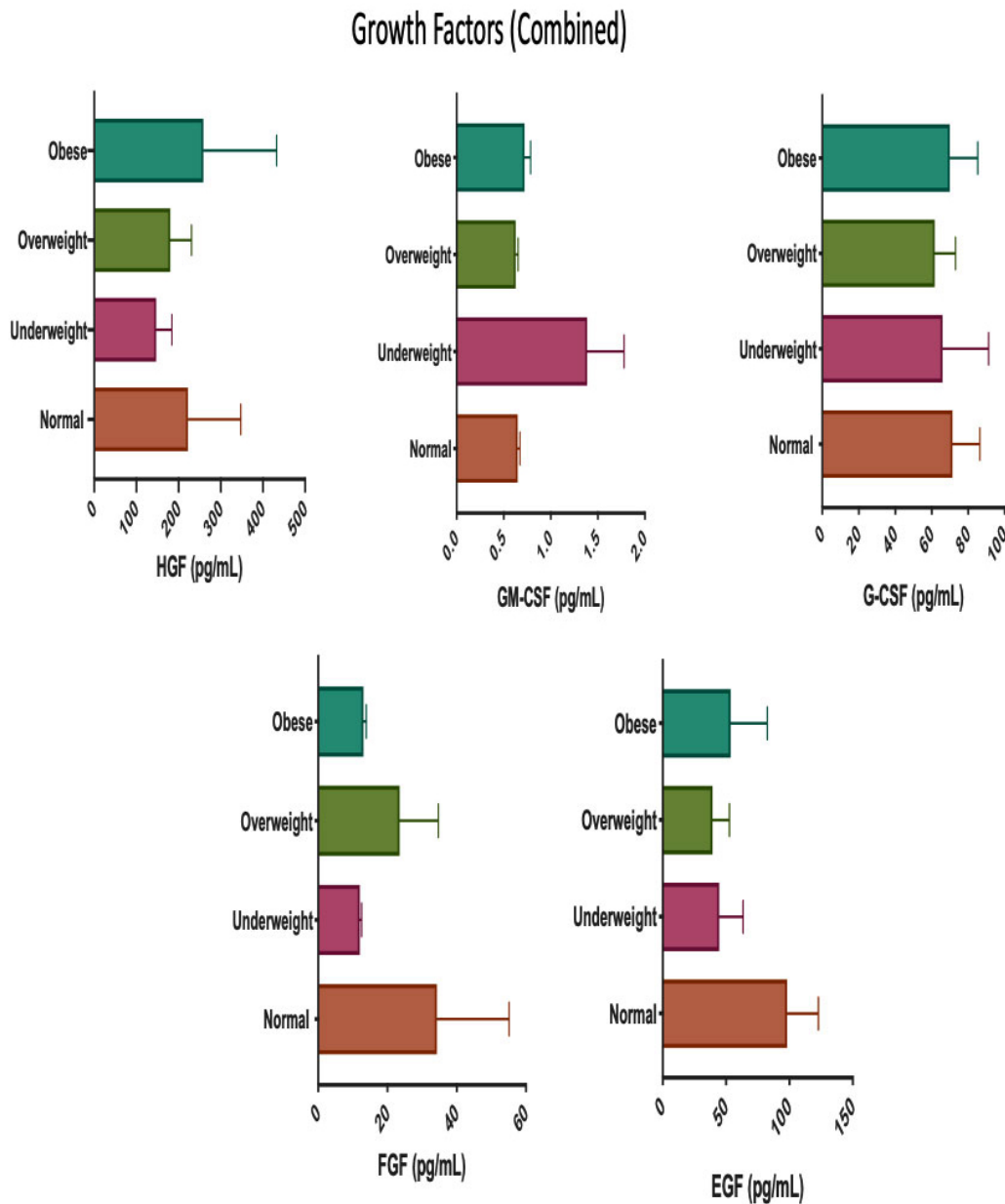


Figure 7. GF levels stratified by BMI category for all subjects.

Finally, the Luminex xMAP analyses for GFs indicated that HGF levels in the serum of underweight females were lower than those of the obese and were nearly significant ($p = .0969$). A near-significant decrease in the level of EGF was observed in underweight females ($p = .0796$) and overweight females as compared to the normal control ($p = .0185$). As for the remaining GFs, neither FGF-B, GM-CSF, nor G-CSF differed significantly between the BMI categories among women (Figure 9).

Discussion

Gut microbiota plays a critical role in human metabolism and health³⁰, and research³¹ has focused on identifying these microorganisms, their functions, and their role in metabolic pathways. Ample evidence shows that microbiota is likely to be involved in obesity and diabetes³²⁻³⁴, but no studies have yet clearly described the correlation between GFs and microbiota in relation to sex, BMI, and food habits.

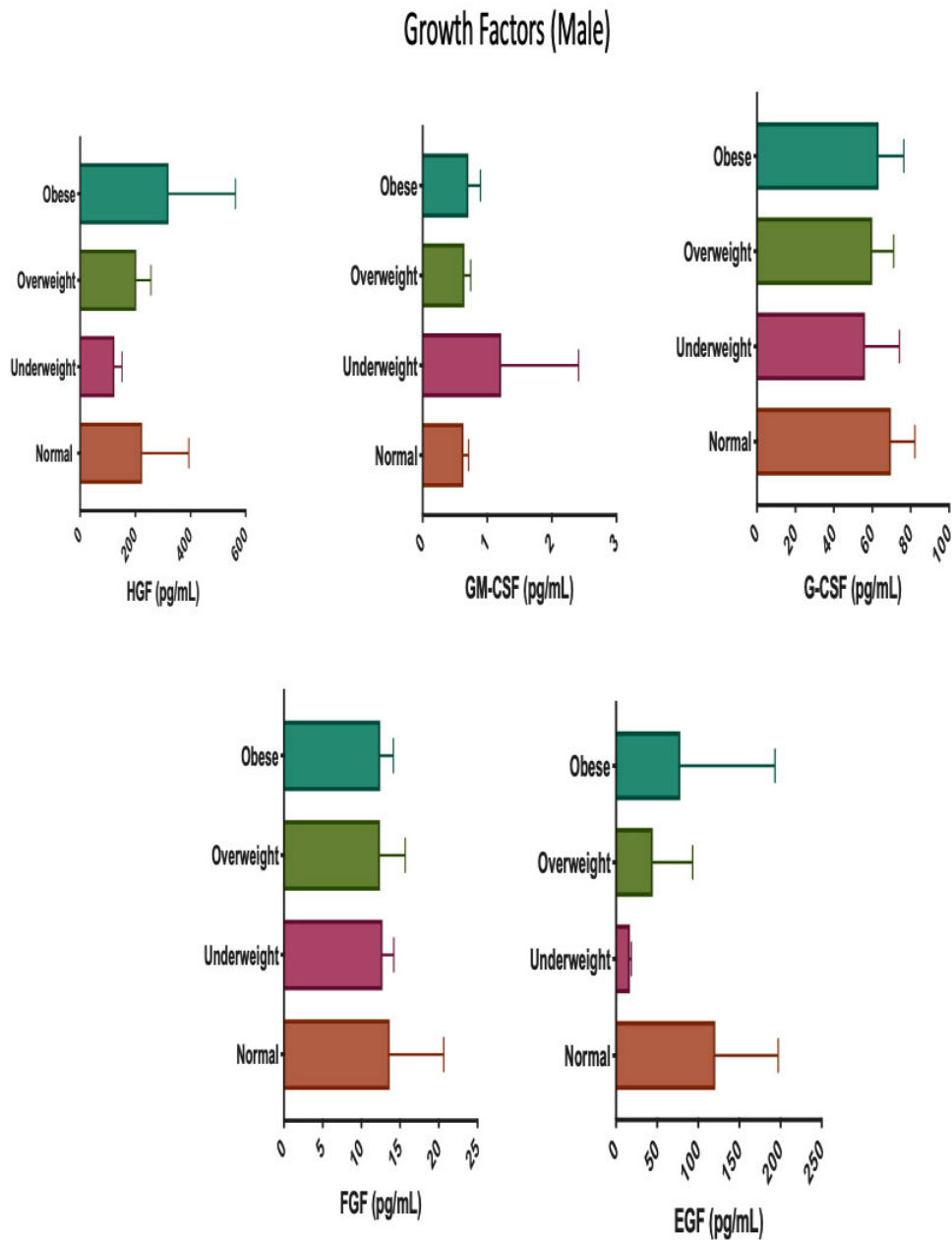


Figure 8. GF levels of male subjects stratified under BMI categories.

Studies³⁵ have shown that bacteria in the intestinal flora play a vital role in controlling postnatal growth in rats; they ensure optimal postnatal growth and play an essential role in determining the size of adult individuals, mainly in cases of undernutrition. One of the key factors in this relationship is insulin-like growth factor-1 (IGF-1), as its production and activity are controlled by microbiota^{36,37}. Less activity and lower levels of IGF-1 have been observed in germ-free mice than in normal mice. Interestingly, the injection of IGF-1

into germ-free mice and interfering with IGF1 activity in normal mice have revealed that the favorable growth of intestinal microbiota is associated with an enhanced production and activity of IGF-1, while the reduced growth of the same microbiota is associated with reduced activity of the GF³⁸.

Growth retardation may be caused by chronic undernutrition. Retardation is a consequence of resistance to the activity of the pituitary growth hormone, which usually stimulates the production of various GFs, including IGF-1³⁹⁻⁴¹. This resistance to

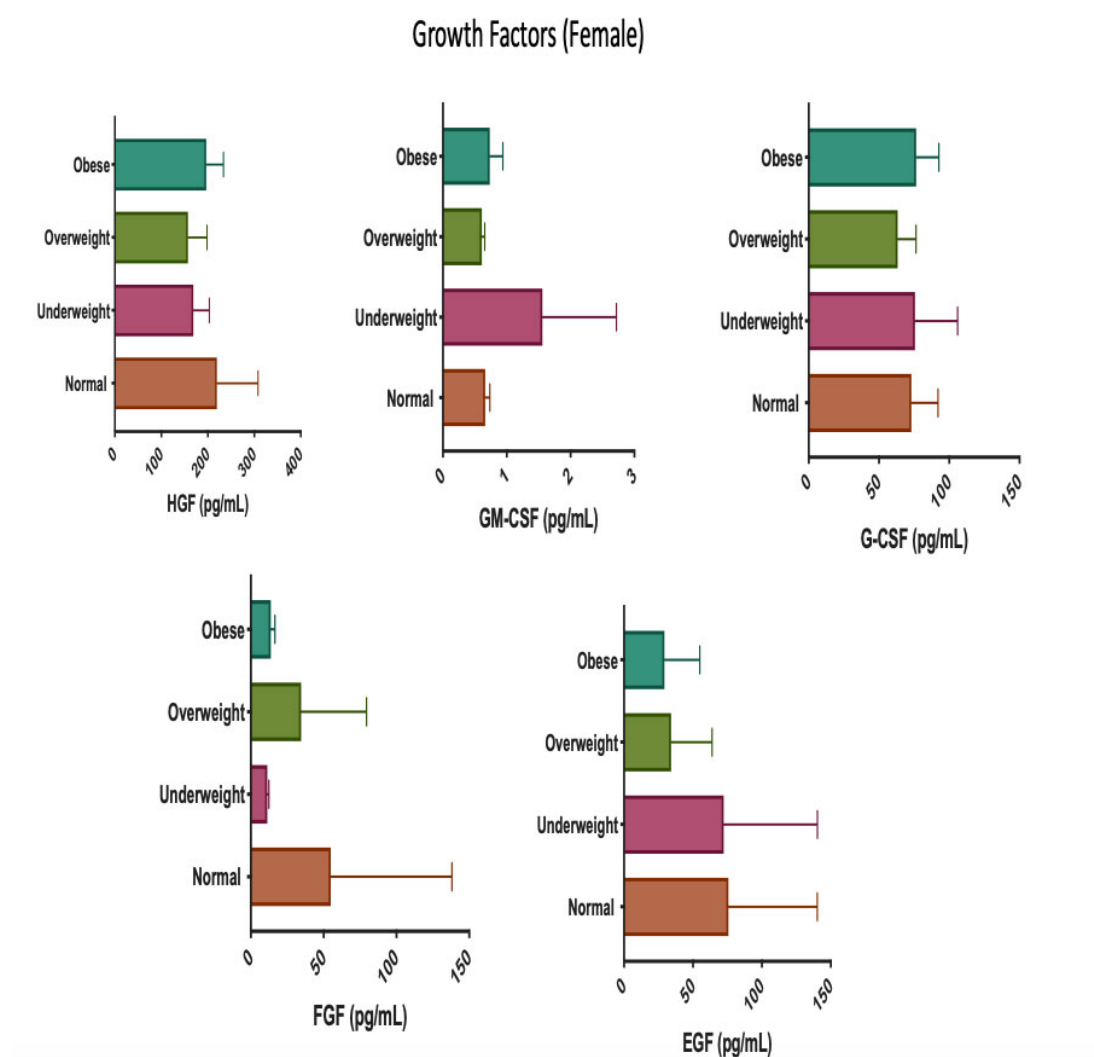


Figure 9. GF levels of female subjects stratified under BMI categories.

the pituitary growth hormone results in a decreased IGF-1 production, causing a retardation in normal development and a reduction in the size of the individual relevant to their age. The exact role of microbiota in such mechanisms remains to be elucidated³⁶.

Hypothalamic transforming growth factor- β (TGF- β) is reportedly excessively produced in the brain as a result of obesity³⁸. It is worth noting that TGF- β plays a vital role in cell growth, differentiation, and maturation, and its presence is vital for cell development. TGF- β is needed by the brain to regulate the neurological development of synapse functions, and its absence may be detrimental and unhealthy^{42,43}.

A study on breast cancer (BC) patients found that obese BC patients had systemically higher levels of interleukin-6 (IL-6) and/or fibroblast growth factor 2 (FGF-2) and were less sensitive

to anti-VEGF treatment⁴⁴. Researchers⁴⁴ using a mouse model have demonstrated that obesity increases with higher FGF-245. The use of metformin or a specific FGF receptor inhibited the normalization of FGF-2 expression, lowered the vessel density, and re-established tumor sensitivity to anti-VEGF therapy in obese mice. This indicates that obesity promotes resistance to anti-VEGF therapy in BC patients, mediated by the production of angiogenic and inflammatory related factors⁴⁴. Obesity impaired the effects of anti-VEGF therapy on angiogenesis, tumor growth, and metastasis. The data of a study⁴⁴ using a murine BC model indicate an association between obesity and higher IL-6 production by adipocytes and myeloid cells in tumors. Blocking the IL-6 reversed the obesity-induced resistance

to anti-VEGF therapy in primary and metastatic sites. A positive correlation was noted between the plasma concentrations of IL-6 and FGF-2 and the visceral adipose tissue (VAT). IL-6, in particular, was elevated during treatment in patients with elevated VAT and BMI. In addition, there was an abundance of IL-6 and FGF-2 expression in tumors, especially in adipocyte-rich regions. Ample evidence indicates a major role of microbiota in the metabolism of dietary components and on its impact on health^{31,34,45,46}. Inflammation was induced by microbiota in GF mice through the inhibition of FGF-23 to eventually reinstate homeostasis, and it was observed that FGF-23 regulates vitamin D metabolism⁴⁷. Studies^{48,49} have shown that coffee has antibiotic effects, and its consumption has resulted in a significant decrease in *E. coli* and *Clostridium* spp. counts and caused a higher *Bifidobacterium* spp. count.

According to our LDA, individuals with high levels of FGF are characterized by the presence of two representative genera, *Lactobacillus* and *Weissella*, both belonging to the *Lactobacillaceae* family. Two of the more enriched biological processes in these samples are the biosynthesis (III) of inosine-5'-phosphate and the degradation of glycerol to butanol. The species of the *Clostridia* genus as well as some *Lactobacillus brevis* strains naturally produce butanol, but efforts have been made to genetically manipulate other bacteria of the *Lactobacillaceae* family to produce butanol⁵⁰. Our prediction of the functional profiles in low-FGF samples revealed that only the L-glutamate and L-glutamine biosynthesis pathways were more abundant than in high- and normal-FGF samples. Regarding the relationship between GFs and microbiota according to sex, the Spearman's correlation revealed that EGF had a significant but weak positive correlation with *Proteobacteria*, but this association was not observed at the genus and species level through the LDA. Moreover, the weak positive correlation between VEGF and *Firmicutes* was observed only at the phylum level, although the genera *Dialister*, *Lachnospira*, and *Dorea* as well as bacteria of the *Clostridia* class belonging to *Firmicutes* were found to be enriched in males.

It has also been reported that the consumption of Arabic coffee as well as various kinds of honey can regulate gut microbiota, helping to maintain intestinal balance⁴⁸. Coffee consumption results in a reduction of *Bacteroides* spp. and *Clostridium* spp. counts in the proximal colon, but this was not the case with *Bifidobacterium* spp. Further-

more, *Lactobacillus* counts increased significantly because of coffee consumption⁴⁹. Coffee intake may also decrease the risk of nonalcoholic fatty liver disease (NAFLD), and researchers have discussed the potential biological mechanisms underlying the benefits of coffee consumption for NAFLD⁵⁰.

Various food components, especially proteins, may increase the inflammatory effects of human diets. In fact, the modern diet consumed daily by humans has many peptides and proteins that can activate TGF- β and TLRs and induce inflammation⁵¹. Interestingly, Khorasan wheat affected the reduction of pro-inflammatory VEGF regardless of the baseline level, which was higher in two chronic disease populations⁵². Strategies aimed at reducing VEGF levels are considered to have therapeutic significance, as increased (overexpressed) VEGF is a proven causative agent of the increased permeability of endothelial cells (leakage) and in regulating subsequent inflammatory responses, resulting in the progression of vascular complications⁵³. This agrees with our findings, in which the consumption of bread and a healthy diet resulted in the downregulation of VEGF levels.

Conclusions

The information presented in this study – that changes in levels of specific circulating GFs are highly associated with obesity – offers potential as a diagnostic tool to predict obesity among adult Saudis in clinical settings. It is recommended that future research decipher the role of GFs in obesity and further explore the involved mechanisms.

Informed Consent

Informed consent was obtained from all the subjects prior to participation after they were informed about the study's objectives, their right to know, and their right to withdraw from the study.

Ethics Approval

The investigation was ethically approved by the Institutional Review Board of King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia (Reference No. 150-14).

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Conflict of Interests

The authors declare that there is no conflict of interests.

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