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Higher serum levels of fibroblast growth factor 21 in old patients with cachexia

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ABSTRACT

Objective: Fibroblast growth factor (FGF)21 is promptly induced by short fasting in animal models to regulate glucose and fat metabolism. Data on FGF21 in humans are inconsistent and FGF21 has not yet been investigated in old patients with cachexia, a complex syndrome characterized by inflammation and weight loss. The aim of this study was to explore the association of FGF21 with cachexia in old patients compared with their healthy counterparts.

Methods: Serum FGF21 and its inactivating enzyme fibroblast activation protein (FAP)-α were measured with enzyme-linked immunoassays. Cachexia was defined as ≥5% weight loss in the previous 3 mo and concurrent anorexia (Council on Nutrition appetite questionnaire).

Results: We included 103 patients with and without cachexia (76.9 ± 5.2 y of age) and 56 healthy controls (72.9 ± 5.9 y of age). Cachexia was present in 16.5% of patients. These patients had significantly higher total FGF21 levels than controls (952.1 ± 821.3 versus 525.2 ± 560.3 pg/mL; $P = 0.012$) and the lowest FGF21 levels (293.3 ± 150.9 pg/mL) were found in the control group (global $P < 0.001$). Although FAP-α did not differ between the three groups (global $P = 0.082$), bioactive FGF21 was significantly higher in patients with cachexia (global $P = 0.002$). Risk factor-adjusted regression analyses revealed a significant association between cachexia and total (β = 649.745 pg/mL; $P < 0.001$) and bioactive FGF21 (β = 393.200 pg/mL; $P < 0.001$), independent of sex, age, and body mass index.

Conclusions: Patients with cachexia exhibited the highest FGF21 levels. Clarification is needed to determine whether this is an adaptive response to nutrient deprivation in disease-related cachexia or whether the increased FGF21 values contribute to the catabolic state.

Introduction

Endogenous fibroblast growth factor (FGF)21 is a pleiotropic mediator of metabolic homoeostasis and was first identified in mouse embryos as a novel member of the *FGF* gene family in 2000 [1]. FGF21 is primarily synthesized by hepatocytes after stimulation with free-fatty acids (FFAs) by peroxisome proliferator-activated receptor (PPAR)-α pathway [2], in addition to other tissues involved in glucose and lipid metabolism, such as the pancreas, testis and, to a lesser degree, in white and brown adipose tissue [3] and skeletal muscle [4]. Recent research has expanded the role of FGF21 to the metabolic

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regulation of glucose and fatty acid metabolism in mice adipocytes and in vitro in primary human adipocytes [5]. Circulating, bioactive FGF21 is inactivated through cleavage at the C-terminal site by the proteolytic enzyme fibroblast activation protein (FAP)-α [6].

Although the autocrine and paracrine function of FGF21 was described in adipose tissue during the fed state of mice to regulate adipocyte function [7], endocrine activity of FGF21 has been identified several times in rodents during a ketogenic diet [2,7] or during starvation or fasting [2,8,9]. The hepatic FGF21 levels and FGF21 mRNA expression increased in mice after a 12-h [8] and a 24-h fast [2,8,9] and later decreased with refeeding of the fasted mice [2]. These data indicate an involvement of FGF21 in the adaptive response to starvation or fasting. Other animal data demonstrated that low-protein intake but not energy restriction led to the induction of hepatic FGF21 [10].

To our knowledge, little and contradictory clinical data are available on the regulation of FGF21 in humans as compared to rodents. In the first small study on humans from 2008, serum FGF21 levels increased up to 74% after a 7-d fast, whereas overnight and 48-h fasting were not followed by an increase in serum FGF21 [11]. Interestingly, one study showed that plasma FGF21 levels in healthy women over a 24-h period increased at 02:30 h and decreased rapidly by 08:30 h the following morning, indicating a circadian rhythm [12]. A more recent study analyzed a medically monitored 10-d fast in healthy individuals, which also resulted in increased FGF21 levels [13], whereas other studies saw no influence in response to short fasting situations up to 48 [14] or 72 h [12,15]. Knowledge about FGF21 levels in patients with anorexia nervosa (AN), a state of chronic starvation, is inconsistent; FGF21 levels were either reduced or similar in AN group compared with a healthy control group with normal weight [16,17]. Cachexia is a complex catabolic syndrome characterized by anorexia, reduced food intake, and loss of muscle mass with or without loss of fat mass. It is triggered largely by multifactorial changes in various metabolic pathways as well as systemic inflammation [18]. Cachexia is common in advanced age (geriatric cachexia) [19,20] and disease (disease-related cachexia) [21–26], leading to increased morbidity and mortality [18].

To our knowledge, there are no existing data on FGF21 levels in old patients with ongoing weight loss and anorexia. Thus, the main objective of this cross-sectional study was to investigate serum levels of total and bioactive FGF21 and FAP-α in old patients and in a healthy older control group, with a focus on FGF21 in patients with cachexia. We hypothesized that cachexia would be associated with increased serum levels of total and bioactive FGF21 and reduced FAP-α values.

Material and methods

Participants and study design

This was a prospective cross-sectional pilot study from the Department of Geriatrics at the Charité - University of Medicine Berlin (November 2016 to July 2017) conducted in two groups of participants 60 to 85 y of age. They were hospital patients who were consecutively recruited during a hospital stay and a healthy control group recruited with a flyer. In patients, exclusion criteria included impaired cognitive status according to the Mini-Mental State Examination (score <24) and life expectancy <3 mo. Healthy old participants were free of any acute diseases that could potentially affect their ability to complete the study. Healthy volunteers who reported weight loss and loss of appetite in the previous year were not included in the study. Demographic and clinical data (type of principal diagnosis, type and number of comorbidities, number of drugs per day) were recorded. Activities of daily living (ADLs) were evaluated using the Barthel Index [27]. A higher score indicates independence in ADL. Fatigue was identified using the validated questionnaire Brief Fatigue Inventory (BFI). A higher BFI score indicates fatigue symptoms [28]. Blood collection and all measurements were conducted within 48 h. The study was approved by the Ethics Committee of the Charité - University Medicine Berlin. All participants signed a written informed consent before inclusion in the study.

Anthropometry

Current edema-free body weight was measured with participants wearing lightweight clothing and using a portable electronic scale to the nearest 0.1 kg (Seca 910; Seca, Hamburg, Germany) and height was measured with a portable stadiometer (Seca 220 telescopic rod, Seca). Weight and height were used to calculate body mass index (BMI; kg/m^2).

Classification of cachexia

Patients were classified cachectic when they exhibited involuntary weight loss ≥5% within the previous 3 mo [20] and had concurrent anorexia determined by the validated eight-item Council on Nutrition appetite questionnaire (score ≤28) [29].

FGF21 and FAP-α quantification

Fasting blood samples were collected in the morning at the same time for all study participants. The blood was then centrifuged at room temperature at 1000*g* for 15 min and the serum was transferred into microtubes and frozen at −80°C until analysis. Serum concentrations of total and bioactive FGF21 and FAP-α were analyzed by enzyme-linked immunosorbent assays (ELISAs) as per the manufacturer's instructions. All parameters were determined in single measurement. The total FGF21 ELISA kit (R & D system, Minneapolis, MN, USA) quantifies both the intact, bioactive FGF21 and the cleaved, inactive FGF21 isoforms by using a monoclonal antibody targeting the core of the FGF21 protein structure [30]. The total FGF21 ELISA detection limit was 4.67 pg/mL. Intra-assay coefficient of variance (CV, %) was between 2.9% and 3.9%, and interassay CV was between 5.2% and 10.9%. The bioactive FGF21 ELISA kit (Eagle Biosciences, Amherst, NH, USA) uses two antibodies that selectively bind to different epitopes of active FGF21. One antibody binds to the N-terminal seven amino acid residues of FGF21 and the other to the six C-terminal amino acid residues, thus only identifying intact, bioactive FGF21 [31]. The sensitivity of the bioactive FGF21 ELISA was 1.7 pg/mL with an intra-assay CV of between 4.2% and 5.7% and interassay CV of between 1.9% and 6.9%. The sensitivity of the FAP-α ELISA (Abcam,Cambridge, United Kingdom) was 12 pg/mL with an intra-assay CV of <10% and an interassay CV of <12%.

Statistical analysis

Statistical analysis was performed using the statistical software SPSS version 25 (IBM, Chicago, IL, USA). Descriptive analyses were carried out using mean and standard deviation (SD) or total number and percentage. Clinical data, FGF21, and FAP-α levels were analyzed to compare between patients with and without cachexia using the Student's *t* test for metric parameters or χ^2 test for nominal parameters and to compare these two groups with the healthy control group using the analysis of variance with the Welch test for unequal variances and sample sizes as well as Bonferroni post hoc test. Pearson's correlation coefficients were calculated to identify the relationship between FGF21 and age or BMI. General linear regression model (GLM) analysis was used to evaluate the association between FGF21 levels as a dependent variable and cachexia as an independent variable adjusted for sex, age, and BMI. Statistical significance was set a priori at $P < 0.05$. Boxplots represent median and 25th and 75th percentiles with whiskers representing 1.5 × interquartile range (IQR) and were created with GraphPad Prism 7.00 (GraphPad Software, La Jolla, CA, USA). This was a pilot study, therefore, the sample size was exploratory.

Results

Participant characteristics

We consecutively recruited 103 hospital patients from the Department of Geriatrics and 56 healthy controls from 60 to 85 y of age for the study. Of the patients, 51 (49.5%) had orthopedic disorders, 17 (16.5%) had cardiac issues, 13 (12.6%) had oncologic or hematologic disorders, 8 (7.8%) were diagnosed with neurologic diseases, 6 (5.8%) with pulmonary issues, and 8 (7.8%) with other diseases.

Prevalence of cachexia in old patients

Overall, cachexia was prevalent in 17 (16.5%) of the patients and none of the healthy controls. The characteristics of the study population according to group are shown in Table 1. The mean age was lower in the healthy control group than in the groups of patients with cachexia ($P = 0.043$) and without ($P < 0.001$). However, there was no difference in age between the two patient groups. The type

Table 1
Characteristics of the healthy participants from the control group and patients with and without cachexia

Parameter	Healthy control group (n = 56)	Patients		*P-value	†Global P-value
		Without cachexia (n = 86)	With cachexia (n = 17)		
Age (y)	72.9 ± 5.9[a]	76.9 ± 5.4[b]	76.7 ± 3.6[c]	1.000	<0.001
Height (m)	1.65 ± 0.09	1.66 ± 0.10	1.64 ± 0.09	1.000	0.590
Weight (kg)	73.0 ± 13.1	70.5 ± 12.2	64 ± 18.5	0.212	0.052
BMI (kg/m^2)	26.6 ± 4.1	25.5 ± 3.9	23.9 ± 7.2	0.520	0.061
Number of comorbidities	—	7.2 ± 3.2	9.5 ± 3.9	0.012	—
Number of medications (drugs/d)	—	9.9 ± 3.8	9.5 ± 3.7	0.681	—
Type of principal diagnosis (n, %)					
Orthopedic (n = 51)	—	46 (53.5)	5 (29.4)	0.035	
Cardiac (n = 17)	—	14 (16.3)	3 (17.6)		
Oncologic/hematologic (n = 13)	—	7 (8.1)	6 (35.3)		
Neurological (n = 8)	—	8 (9.3)	0 (0)		—
Pulmonary (n = 6)	—	5 (5.8)	1 (5.9)		
Other (n = 8)	—	6 (7.0)	2 (11.8)		
Serum bioactive FGF21 (pg/mL)	142 ± 72 [a]	284.7 ± 339.8 [a]	540.9 ± 523.2 [b]	0.021	0.002
Serum total FGF21 (pg/mL)	293.3 ± 150.9[a]	525.2 ± 560.3[b]	952.1 ± 821.3[c]	0.012	<0.001
Serum FAP-α (ng/ml)	90.2 ± 35.5	73.2 ± 46.4	77.9 ± 59.9	1.000	0.082
ADL (score)	98.6 ± 3[a]	85 ± 12.8[b]	81.5 ± 15.2[c]	0.317	<0.001
Fatigue (score)	1.5 ± 1.3 [a]	3.5 ± 1.7[b]	4.8 ± 2.2[c]	0.005	<0.001

ADL, activities of daily living; BMI, body mass index; FAP, fibroblast activation protein; FGF, fibroblast growth factor
Values are presented in mean ± standard deviation or categories in absolute number (percentage)
*P-value calculated between patients without and with cachexia.
†Global P-values calculated over the three groups. Differing superscripts (a, b, c) indicate significant differences among the three groups ($P < 0.05$).

of principal disease did not differ in patients with cachexia (Table 1). The patients with cachexia had more comorbidities than patients without cachexia but did not differ in the number of drugs taken per day. BMI did not differ between the control group and the groups of patients with cachexia ($P = 0.074$) and without ($P = 0.374$) or between patients with and without cachexia. As expected, the control participants were significantly more independent in their ADLs than the patients (both groups: $P < 0.001$), although there was no significant difference in ADL score between patients with and without cachexia. The fatigue score was significantly lower in the control group than in the two groups of patients (both groups: $P < 0.001$) and was the highest in the patients with cachexia.

The highest serum FGF21 levels in cachexia

Of the 159 participants, we were able to analyze 139 blood samples (n = 1 without sufficient blood volume, n = 16 hemolytic serum, and n = 3 non-detectable FGF21 values). Although total and bioactive FGF21 levels were significantly higher in patients with cachexia than in patients without cachexia and the healthy control group (Table 1 and Fig. 1), the proteolytic enzyme FAP-α did not differ significantly among the three study groups (Table 1). Overall, although involuntary weight loss ($r = 0.442$, $P = 0.001$) and loss of appetite ($r = -0.277$, $P = 0.001$) correlated with total FGF21 levels, there was no correlation between BMI and total FGF21 ($r = -0.148$, $P = 0.081$). In addition, there were no significant differences in FGF21 levels (total and bioactive) between disease categories (data not shown), and FGF21 levels did not correlate with the number of comorbidities in patients ($r = 0.152$, $P = 0.165$).

Regression model analyses

Tables 2 and 3 show the general linear regression model analyses of total and bioactive FGF21 levels (dependent variables) and cachexia (independent variable). Models 1 and 3 were adjusted for sex, age, and BMI in the overall study population. Patients with cachexia had higher total (β = 649.745 pg/mL, $P < 0.001$) and bioactive FGF21 levels (β = 393.200 pg/mL, $P < 0.001$) than patients without cachexia and the healthy control group. These associations remained statistically significant after additionally adjusting for the number of comorbidities in the group of hospital patients (models 2 and 4; Tables 2 and 3).

Discussion

In this cross-sectional study of patients and healthy participants ≥60 y of age, serum levels of FGF21 were higher in the patient group overall than in the healthy control group and highest in those patients with cachexia. Cachexia was associated with increased serum levels of FGF21, independent of sex, age, and BMI. In patients only, elevated total and bioactive FGF21 levels were associated with cachexia, independent of the number of comorbidities. Furthermore, we demonstrated a significant correlation between the degree of weight loss, anorexia, and FGF21 levels. Serum levels of the protease FAP-α did not significantly differ among the three groups, which may suggest an increased secretion of FGF21 as an age-related hepatokine within the patient cohort rather than reduced cleavage or prolonged half-life in the circulation.

As most studies in humans have measured total FGF21 or not specified, we measured both total (consisting all circulatory FGF21 isoforms [bioactive, inactive]) for comparative reasons and metabolically bioactive FGF21 as has been done previously [32]. Bioactive FGF21 has intact N– and C–termini essential for interactions with the cofactor β-Klotho and FGF receptors [33].

Although there are some preclinical and clinical data on fasting-induced FGF21, to our knowledge, this is the first study to investigate FGF21 in the cachexia-anorexia syndrome in humans. During nutritional deprivation, major physiologic changes are triggered as the body adapts to the fasting state or starvation. One of these changes is the upregulation of hepatic FGF21. We know from animal studies that the hepatic FGF21 pathway regulates substrate utilization and energy balance by inducing ketogenesis [8], gluconeogenesis, and oxidation of FFA in lean mice [34]. It is well known that the regulation of FGF21 is complex and the human amino acid sequence of FGF21 is highly similar to that of the mouse with 75% agreement [1]. However, the weight-specific metabolic rate is ~10-fold higher in mice than in humans, which explains the

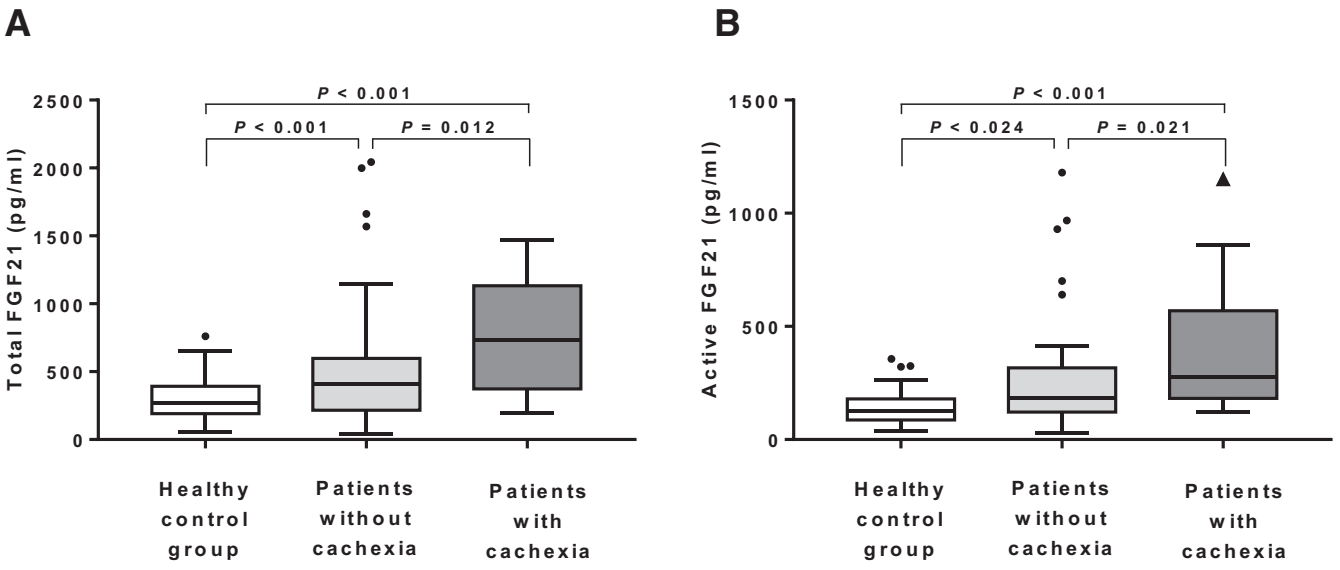

Fig. 1. Quantification of serum total (**A**) and bioactive (**B**) FGF21 levels in the healthy control group and patients without and with cachexia. Boxplots represent median and 25th and 75th percentiles and whiskers represent 1.5 × interquartile range. One extreme value within each hospital group is not shown in the figure but included in the statistical analysis. FGF, fibroblast growth factor.

Table 2
Association between cachexia and total FGF21 in a general linear regression model

Parameter	β coefficient	95% CI	*P*-value
Model 1			
Age (y)	−2.224	−17.264 to 12.816	0.770
Male sex*	29.677	−135.828 to 195.181	0.723
BMI (kg/m^2)	−5.314	−27.208 to 16.580	0.632
Cachexia†	649.745	328.833 to 970.657	<0.001
Non-cachexia†	232.543	43.022 to 422.064	0.017
Model 2			
Comorbidities (n)	17.842	−21.064 to 56.747	0.364
Cachexia‡	387.338	14.140 to 760.537	0.042

BMI, body mass index; CI, confidence interval; FGF, fibroblast growth factor
Model 1 in patients and control group adjusted for age, sex, and BMI
Model 2 in patients adjusted for number of comorbidities
*Compared with female sex.
†Compared with healthy control group as reference.
‡Compared with patients without cachexia as reference.

Table 3
Association between cachexia and bioactive FGF21 in a general linear regression model

Parameter	β coefficient	95 % CI	*P* -value
Model 3			
Age (y)	−0.800	−9.871 to 8.271	0.862
Male sex*	41.069	−60.522 to 142.660	0.425
BMI (kg/m^2)	−3.490	−17.253 to 10.273	0.617
Cachexia†	393.200	191.413 to 594.988	<0.001
Non-cachexia†	138.716	25.013 to 252.420	0.017
Model 4			
Comorbidities (n)	11.912	−2.894 to 26.718	0.114
Cachexia‡	323.222	113.178 to 533.266	0.003

BMI, body mass index; CI, confidence interval FGF, fibroblast growth factor
Model 3 in patients and control group adjusted for age, sex, and BMI
Model 4 in patients adjusted for number of comorbidities
*Compared with female sex.
†Compared with healthy control group as reference.
‡Compared with patients without cachexia as reference.

difference in the dynamics of circulating FGF21 levels between the species and the fact that stored energy is exhausted faster in mice than in humans during fasting [11]. Although FGF21 is rapidly induced by overnight or short fasting in rodents [2,8,9], FGF21 levels do not change during such a short fast in humans [11,12,14,15] but in response to prolonged fasting (>7–10 d) [11,13]. On the other hand, in AN, a state of chronic starvation, data on FGF21 values are contradictory. FGF21 levels have been shown to be either markedly decreased or comparable to normal weight control groups [16,17]. A recent study reported decreased FGF21 levels in girls with AN, whereas obese female adolescents exhibited higher FGF21 levels than the normal weight control participants [35]. Although the elevated levels in obesity are thought to be an adaptive mechanism to insulin resistance, the decreased FGF21 levels in AN might serve to maintain glucose levels [35]. Furthermore, the same study demonstrated a positive relationship between elevated FGF21 and parameters of obesity, such as BMI, in the overall study sample [35]. This association has been reported elsewhere [16,36]. The duration of AN or the phase of the illness also may influence the results. The adaptive mechanism to nutritional deprivation in patients with cachexia and anorexia differs from that of simple but persistent starvation typical of AN. In the old loss of appetite and subsequent weight loss is a frequent complication in disease, triggered in part by inflammatory processes, although the underlying mechanisms are complex [37]. Cachexia, however, is reflected by ongoing weight loss, especially loss of muscle mass owing to increased protein breakdown, in addition to anorexia and is accompanied by inflammation [20,38]. In AN, energy is mainly derived from ketone body production rather than from amino acids, thus preserving muscle mass [39].

Several studies have shown that FGF21 levels increase with age [40–42]. It has been suggested that elevated FGF21 levels contribute to a systemic, pro-aging metabolic shift, which was reverted by deletion of FGF21 in animal models. Tezze et al. also noted that FGF21 does not appear to play a defining role in protein breakdown and muscle loss but rather whole body metabolic changes [42]. However, the reason for the increased levels in higher age remains unclear and needs further investigation in the light of potential side effects of increased FGF21. FGF21 gain of function or high plasma FGF21 levels are, for example, associated with a marked loss of bone mass [43,44]. In addition, clinical studies have reported

increased FGF21 values in various diseases such as coronary heart disease, carcinoid atherosclerosis, type 2 diabetes, and mitochondrial disease [45] so that a paradigm has been suggested with lower concentrations being beneficial and highly elevated, chronic levels harmful [40,46]. We found FGF21 to be highly expressed in the overall patient sample but found no difference between patients with or without cachexia regarding the disease category. In light of these findings, the effects of the higher FGF21 values in the old participants, especially in those with nutritional deprivation, needs to be further investigated.

The results of the present study are subject to limitations. First was the relatively small sample size, especially regarding the number of patients with cachexia, and further the unequally sized groups, representing a heterogeneous old patient population. The small number of patients with cachexia can be attributed to the rather restrictive definition of cachexia that we used with clinically relevant weight loss of >5% in 3 mo and concurrent anorexia. Second, we did not measure further biochemical parameters that could be associated with cachexia and FGF21. Third, evaluable blood samples were only available in 139 individuals owing to common problems in the geriatric setting. Finally, because of the cross-sectional design, we could not explore the cause–effect association between FGF21 and cachexia-related parameters.

Conclusions

We demonstrated that both FGF21 isoforms are increased in old patients compared with a healthy same-aged control group and further increased in old patients with cachexia, independent of sex, age, BMI, and number of comorbidities. In contrast, we did not find any significant differences regarding FAP-α between patients and the healthy control group. Whether FGF21 acts as a rescue hormone to regulate glucose and fat metabolism as a result of nutritional deprivation or whether it contributes to the catabolic state in cachexia remains to be elucidated. Furthermore, the effect of chronically increased FGF21 levels in the old needs to be assessed.

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