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Reduced Insulin-Like Growth Factor-I Serum Levels in Formerly Obese Women Subjected to Laparoscopic-Adjustable Gastric Banding or Diet-Induced Long-term Caloric Restriction

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Life-span extension in laboratory rodents induced by long-term caloric restriction correlates with decreased serum insulin-like growth factor-I (IGF-I) levels. Reduced activity of the growth hormone/IGF-I signaling system slows aging and increases longevity in mutant mouse models. In the present study, we show that long-term caloric restriction achieved by two different interventions for 4 years, either laparoscopic-adjustable gastric banding or reducing diet, leads to reduced IGF-I serum levels in formerly obese women relative to normal-weight women eating ad libitum. Moreover, we present evidence that the long-term caloric restriction interventions reduce fasting growth hormone serum levels. The present study indicates that the activity of the growth hormone/IGF-I axis is reduced in long-term calorically restricted formerly obese humans. Furthermore, our findings suggest that the duration and severity of the caloric restriction intervention are important for the outcome on the growth hormone/IGF-I axis in humans.

Key Words: Aging—Caloric restriction humans—Gastric banding—IGF-I—IGFBP-3.

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LONG-TERM caloric restriction (CR) without malnutrition and/or dietary restriction induces healthy and maximum life-span extension by retardation of the aging process in a wide range of animal species [reviewed in refs. (1–3)]. Evidence for slower aging and delayed development of age-related diseases in response to long-term CR was found in rhesus monkeys (4,5), and the epidemiological evidence for beneficial effects of CR in humans is promising (6). This led to increasing interest in the development of CR mimetics, compounds that mimic the beneficial effects of CR without restricting the caloric intake (7). In rodents, long-term CR results in decreased serum concentrations of insulin (8) as well as insulin-like growth factor-I (IGF-I) (9). These alterations most likely mediate some of the beneficial aspects of CR, such as slowing biological aging and protecting against cancer (10) [reviewed in ref. (11)]. It was proposed that reduced circulating levels of insulin and IGF-I in calorically restricted rodents may lead to increased life span through diminished insulin/IGF-I signaling at the cellular level as it has been observed in lower organisms (12,13). These hypotheses, however, need to be further confirmed, particularly in humans.

The importance of IGF-I for longevity is demonstrated by studies showing that the reduction of IGF-I signaling caused by spontaneous or experimentally induced mutations is a robust intervention to increase healthy and maximum life span in rodents (14). In growth hormone (GH)–deficient and GH-resistant mice, which have low circulating IGF-I levels, the maximum life span is increased (15–18). In addition, decreased IGF-I signaling plays a role in the delayed aging phenotype of IGF-I receptor (IGF-IR)–deficient mice (19). Mainly stimulated by GH, secreted by somatotropic cells of the anterior pituitary gland, IGF-I is produced primarily by the liver as an endocrine hormone but also in peripheral tissues in a paracrine/autocrine fashion (20,21). Approximately 99% of IGF-I is bound to secreted IGF-binding proteins (IGFBP-1 to 6), which serve as transport vehicles for IGFs in the circulation and control their biological availability (22,23). IGFBP-3 is the most abundant circulating IGFBP, carrying over 80% of serum IGFs in heterotrimeric complexes that also contain the glycoprotein ALS (24). IGF-I binds to IGFBP-3 in a 1:1 molar ratio amplifying the half-life of IGF-I (25). The activity of IGFs in the target cells is mediated by the IGF-IR, a membrane receptor tyrosine

kinase activating growth, proliferation, and survival signals (20,26). IGFBP-3 can inhibit IGF-stimulated events in the local cellular environment by sequestering IGFs from the IGF-IR. Alternatively, IGFBP-3 interaction with cell or matrix components may concentrate IGFs near their receptor, enhancing IGF activity (22,27). The GH/IGF system determines normal somatic growth in mammals by regulating cell proliferation, survival, and differentiation in most tissues of the body (20,21,28). Moreover, IGFs play an important part in tumorigenesis acting as mitogens to promote cancer cell proliferation and survival (26,29–31). In fact, there is strong evidence that reduction in serum levels of GH and IGF-I mediate antiproliferative, proapoptotic, and anticancer effects of CR in animal models [reviewed in ref. (11)].

In humans, nutrition is a main regulator of IGF-I serum levels (32). Energy intake was shown to be positively correlated with circulating IGF-I (33), and short-term CR induces immediate and strong reduction in circulating IGF-I (32,34,35). Serum IGF-I levels were, however, found to be decreased neither in overweight people by medium-term CR regimes (6 or 12 months) resulting in moderate weight reduction (36,37) nor in lean weight-stable members of the Calorie Restriction Society after long-term CR diets for 6 years (36). Obesity was shown to result in a reduction in GH and IGF-I secretion. However, the impact of long-term CR, over several years, on the GH/IGF-I axis in formerly obese people is not precisely understood. Nowadays, bariatric surgery is frequently used to induce long-term CR in obese people either by modification of the anatomy of the gastrointestinal tract using bypass procedures (38) or by exclusively restrictive procedures (39). Its beneficial impact on health is demonstrated by the dramatic effects on the reduction of total morbidity and mortality rate in formerly obese humans, including deaths from cancer (40,41). Consistent with well-established endocrine features of CR animals (4,42), we (43) and others [reviewed in ref. (38)] showed that the insulin serum levels are decreased, and insulin sensitivity is improved in formerly obese women subjected either to bariatric surgery or to diet-induced long-term CR. The major aim of the present investigation was to evaluate IGF-I and IGFBP-3 serum levels in long-term calorically restricted formerly obese women subjected either to reducing diet or to laparoscopic-adjustable gastric banding (LAGB), a purely restrictive bariatric operation (39), and to compare them with age-matched normal-weight and obese women eating ad libitum. This should contribute to a better understanding of the impact of severe long-term CR on the IGF-I/IGFBP-3 axis in humans.

Methods

Study Groups

Obesity and normal weight were defined according to the World Health Organization criteria on the basis of the body mass index (BMI = weight [kg]/height [m^2]). Of 48 Caucasian women, we compared groups of healthy normal-weight (NW, BMI 19–25 kg/m^2), obese (OW, BMI > 30 kg/m^2), and long-term calorically restricted initially obese participants (current BMI ≤ 25) in a cross-sectional study. The groups were age matched with normal distribution of the variable "age" in all groups. None of the women had diabetes, liver, renal, or other severe metabolic diseases. The CR group consisted of 16 women, 8 reduced weight by LAGB and 8 by a reducing diet. The reducing diet was a combination of a formula diet with regular meals supervised by a nutritionist (43). The ratio of the major nutrients was about 50% carbohydrate, 30% fat, and 20% protein. The composition of the food and the physical activity was according to the improved American Food Guide Pyramid released by the U.S. Department of Agriculture 1992 and 2005. The caloric intake in the first 6–9 months was reduced by approximately 40% and then by 15% relative to the calorie intake at baseline. In the bariatric surgery group, weight loss was achieved by LAGB, a purely restrictive microinvasive operation that induces CR by a small gastric pouch (30 mL) and does not induce malabsorption (38,39). LAGB limits the capacity of the stomach to accommodate food and slows the flow of ingested nutrients, causing gastric and/or esophageal distension, eliciting early satiety, nausea and discomfort, or even vomiting if pouch capacity is exceeded (39). The composition of the food and the physical activity in the LAGB group was recommended by a supervising nutritionist according to the improved American Food Guide Pyramid released by the U.S. Department of Agriculture 1992 and 2005. The reduction in calorie intake resulted in a significant decrease in BMI in all participants over a time period of about 4 years. The body weight was constant for at least 1 year before sampling. Anthropometric measurements and blood samples were obtained ante meridiem following an overnight fast (43). The Medical University of Innsbruck Ethics Commission approved all studies, and written informed consent was obtained from each participant.

Anthropometric Measurements

Body weight was measured with an electronic balance and height using a calibrated height rod.

Measurements of Serum Parameters

The fasting serum concentrations of GH, IGF-I, and IGFBP3 were measured by enzyme-linked immunosorbent assays (ELISA), all samples were run three times. Enzyme immunoassay for quantitative determination of total human IGF-I (IGFBP-blocked), Mediagnost, Reutlingen, Germany; Active Free IGF-I ELISA DSL-10-9400; Diagnostic System Laboratories, Sinsheim, Germany; Enzyme immunoassay for quantitative determination of human IGFBP–3; Mediagnost, Reutlingen, Germany; IGFBP-3 ELISA, DSL-10-6600; Diagnostic System Laboratories; Quantikine Human Growth Hormone Immunoassay, DGH00, R&D Systems, Minneapolis, MN.

Table 1. Characteristics of the NW, DCR, LAGB, and OW Study Groups

	Normal (NW)	Diet-Induced CR (DCR)	Gastric Banding CR (LAGB)	Obese (OW)	*p* value, NW vs DCR	*p* value, NW vs LAGB	*p* value, NW vs OW	*p* value, DCR vs LAGB	*p* value, DCR vs OW	*p* value, LAGB vs OW
N	17	8	8	15						
Sex	F	F	F	F						
Age (y)	40 ± 12	38 ± 11	41 ± 7	45 ± 13	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Height (m)	1.66 ± 0.05	1.67 ± 0.05	1.68 ± 0.06	1.66 ± 0.05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Weight (kg)	62 ± 6	66 ± 9	72 ± 7	97 ± 17	n.s.	≤.001	≤.001	n.s.	≤.001	≤.001
Former BMI (kg/m^2)	—	33.5 ± 6.2	44.9 ± 8.4	—	—	—	—	≤.01	—	—
ΔBody weight (% body weight)	—	27 ± 8	42 ± 10	—	—	—	—	≤.01	—	—
CR period (y)	—	3.8 ± 1.5	3.9 ± 1.5	—	—	—	—	n.s.	—	—
Current BMI (kg/m^2)	22.5 ± 1.9	24 ± 3.7	25.5 ± 1.8	35.6 ± 5.7	n.s.	≤.001	≤.001	n.s.	≤.001	≤.001
Insulin (mU/L)	2.7 ± 2.3	1.4 ± 2	2.7 ± 0.9	9.4 ± 6.7	n.s.	n.s.	≤.01	n.s.	≤.01	≤.05
IGF-I (ng/mL)	243.7 ± 67.7	145.5 ± 42	127.8 ± 46.4	171.9 ± 74.4	≤.001	≤.001	≤.01	n.s.	n.s.	n.s.
Free IGF-I (ng/mL)	0.9 ± 0.4	0.5 ± 0.2	0.6 ± 0.3	0.6 ± 0.3	≤.05	n.s.	n.s.	n.s.	n.s.	n.s.
IGFBP3 (μg/mL)	5.3 ± 1.7	4.1 ± 0.6	3.3 ± 1.4	5 ± 1.3	≤.05	≤.01	n.s.	n.s.	n.s.	≤.05
Ratio (IGF-I/IGFBP3)	0.26 ± 0.08	0.25 ± 0.15	0.23 ± 0.06	0.17 ± 0.07	n.s.	n.s.	≤.01	n.s.	n.s.	n.s.
GH (ng/mL)	2.1 ± 0.7	0.9 ± 0.9	0.2 ± 0.3	0.9 ± 0.8	≤.01	≤.001	≤.001	≤.05	n.s.	≤.01

Notes: BMI = body mass index (weight [kilograms]/height [square meters]); GH = growth hormone; IGF-I = insulin-like growth factor-I; IGFBP-3 = IGF-binding protein 3; n.s. = not significant. Clinical, anthropometric, and endocrine parameters are indicated. Data presented as mean ± *SD*.

*$p < .05$, **$p < .01$, ***$p < .001$.

Statistics

The data were analyzed by SPSS 17.0, 2008 (SPSS, Chicago, IL). Data are presented as the means ± standard deviation of absolute values. Statistical significance was considered at $p < .05$ used for a significant two-tailed p value. To evaluate the mean differences of each factor between the groups, the Mann–Whitney U test was used. The Kolmogorov–Smirnov one-sample test was used to confirm a Gaussian distribution of the variable "age" among all participants. Associations between given variables were investigated using the Spearman's rank correlation coefficient. R = correlation coefficient.

Results

Long-term CR Interventions and Body Weight Changes

In a cross-sectional study, the IGF-I, IGFBP-3, and GH concentrations were compared in fasting serum samples from age-matched groups of normal-weight (NW), obese (OW), and long-term calorically restricted women (Table 1). Long-term CR was achieved either by LAGB or by long-term reducing diet (DCR). The LAGB group ($n = 8$) had a significantly higher BMI (44.9 ± 8.4 kg/m^2) than the DCR group ($n = 8$, BMI = 33.5 ± 6.2 kg/m^2) at the beginning of the interventions ($p \leq .01$). The weight loss in the LAGB group was higher (54.4 ± 22 kg; 42% ± 10%) than in the DCR group (25.5 ± 10.5 kg; 27% ± 8%). The LAGB group reached a new significantly decreased steady-state BMI (25.5 ± 1.8 kg/m^2), 3.9 ± 1.5 years, after baseline. The DCR subgroup reached a new significantly decreased steady-state BMI (24.0 ± 3.7 kg/m^2), 3.8 ± 1.5 years after baseline. The average BMI in the two CR intervention groups was in the upper normal weight range according to the World Health Organization criteria on the basis of the BMI, whereby the stable current BMI in LAGB was significantly higher relative to the normal weight control group BMI (22.5 ± 1.9 kg/m^2) in the middle normal range (Table 1).

IGF-I and IGFBP-3 Secretion

The total fasting IGF-I levels were the highest in NW (Figure 1A). In keeping with previous work showing that the total IGF-I levels are lower and inversely correlated with BMI in obesity (44), the total IGF-I levels were significantly lower in OW than in NW (Figure 1A). In both long-term CR intervention groups, the total serum IGF-I level were also significantly lower relative to NW. The IGF-I levels were the lowest in the LAGB group and in both long-term CR groups by trend lower than in OW. This suggests that severe long-term CR over several years in humans, induced either by dietary restriction or by LAGB, leads to a reduction in the total IGF-I serum levels. The serum IGF-I levels were negatively correlated with donor age ($R = -0.33$; $p \leq .05$; Table 2), reflecting the well-known age-dependent decline in the IGF-I serum levels (45).

The serum IGFBP-3 levels (Figure 1B) were lowest in LAGB, followed by DCR. Both CR intervention groups had significantly lower IGFBP-3 levels than the normal-weight group. The IGFBP-3 serum levels in LAGB were also significantly lower than in OW. No difference was found between the IGFBP-3 serum concentration in obese and normal weight women. IGFBP-3 levels were strongly correlated with IGF-I ($R = 0.66$; $p \leq .001$; Table 2), underlining that the control of the serum level of both proteins is strongly coregulated, as shown before (46) [reviewed in ref. (27)]. Because IGFBP-3 is the main IGF-I-binding protein in the serum, constituting both a reservoir and a carrier system for

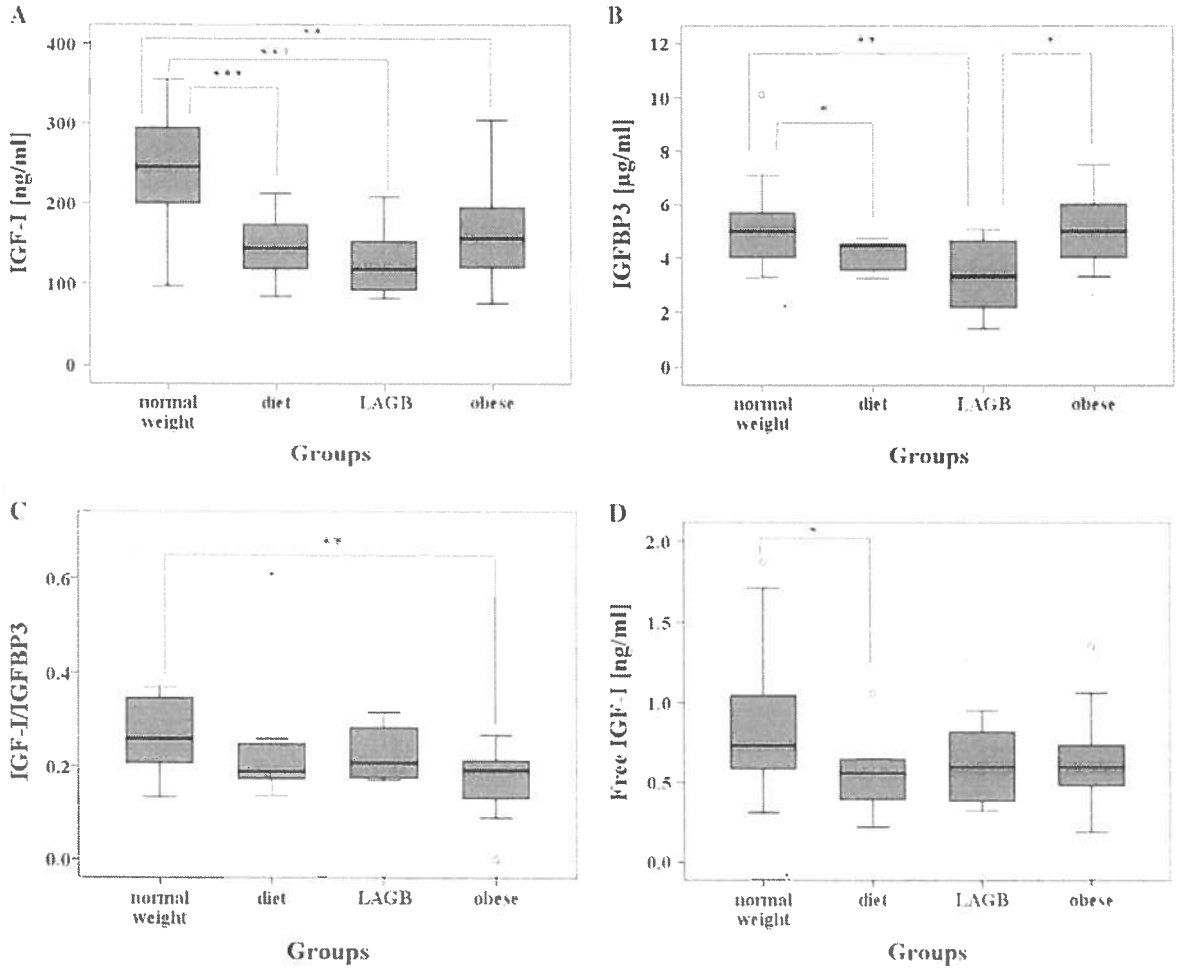

Figure 1. Insulin-like growth factor (IGF)/IGFBP3 axis in the study groups. The serum concentrations of total IGF-I, IGFBP3, and free IGF-I were measured by enzyme-linked immunosorbent assays ante meridiem following overnight fasting. Median values are indicated ± standard deviation. (**A**) Total IGF-I. (**B**) IGFBP-3. (**C**) IGF-I/IGFBP-3. (**D**) Free IGF-I.

IGF-I (22), the molar ratio of the two proteins could be of importance for the biological activity of IGF-I. A significant difference in the IGF-I/IGFBP-3 ratio was only found between NW and OW, with the ratio being lower in the obese women (Figure 1C; Table 1). In agreement with these data, the IGF-I/IGFBP-3 ratio was found to be negatively correlated with BMI ($R = -0.36$; $p \leq .05$; see also Figure 2). Our data suggest that the coregulation of the serum levels of IGF-I and IGFBP-3 is impaired in obesity but restored in severe long-term calorically restricted formerly obese women.

To obtain more information about the amount of IGF-I available to IGF-I receptor binding, the serum levels of free IGF-I were measured. The results demonstrated that the levels of free IGF-I were significantly lower in women subjected to long-term reducing diet than in NW (Figure 1D). In LAGB and OW, free circulating IGF-I was by trend lower than in NW (Figure 1D). These data suggest that the levels of free IGF-I are differentially regulated in the corresponding cohorts. With regard to the IGFBP-3 serum level, the results suggest that the level of IGF-I in DCR women is not only regulated by the IGFBP-3 serum levels, thus warranting further studies. In agreement with our findings, the serum levels of free IGF-I were found to be positively correlated with total IGF-I ($R = 0.46$; $p \leq .01$; Table 2). Together, these data

Table 2. Correlations Between Age, IGFBP-3, Free IGF-I, GH, and Total IGF-I

Correlations	Total IGF-I
Age (y)	−0.33*
IGFBP3 (µg/mL)	+ 0.66**
Free IGF-I (ng/mL)	+ 0.46***
GH (ng/mL)	+ 0.40**

Notes: GH = growth hormone; IGF-I = insulin-like growth factor-I; IGFBP-3 = IGF-binding protein 3. The dependence of one variable on the other is measured with the correlation coefficient (R). Data presented as mean ± *SD*. The asterisks indicate the p value: *$p < .05$, **$p < .01$, *** $p < .001$.

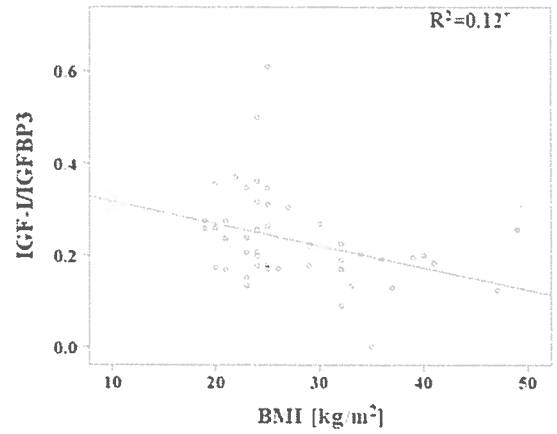

Figure 2. Linear regression between the Insulin-like growth factor (IGF)-I/IGFBP-3 ratio and the body mass index (BMI). The IGF-I/IGFBP-3 ratio was significantly negatively correlated with the BMI.

suggest that the levels of free IGF-I in the circulation do indeed correspond to the total IGF-I serum levels. However, depending upon GH-IGF-I signaling, additional parameters, such as the IGFBP-1 and -2 serum levels, may also contribute to free circulating IGF-I, as previously shown [reviewed in ref. (23)]. It should be noted that the free IGF-I levels in the circulation are not simply equivalent to the levels of bioavailable IGF-I because only very low amounts of IGF-I, that is, approximately 1%–2% of total IGF-I, are free, and binding to the IGFBPs increases the stability and mediates the transport of IGF-I in the circulation. Moreover, the positive and negative effects of IGFBP binding for the bioavailability of IGF-I to the IGF-IR in the given target tissues are not fully understood [reviewed in refs. (22,23)].

To further evaluate the effectiveness of the long-term CR interventions, we regarded the DCR and LAGB groups as one group and compared this group of all 16 long-term CR women (CRW) with NW and OW. We found that the total serum IGF-I levels in CRW were considerably lower in CRW than in NW and by trend lower than in OW (Table 3). Moreover, the levels of free IGF-I were also significantly lower in CRW than in NW (Table 3). This further supports the data that long-term CR in humans leads to a reduction in the IGF-I serum levels.

Growth Hormone Secretion

Because the principal stimulus for the synthesis of IGF-I in liver and in most other tissues is provided by GH (20,21), we analyzed the GH serum levels in the fasting blood samples from our cohorts. As expected, fasting GH showed a broad standard deviation (Figure 3), reflecting the fluctuating release of this hormone in women (47,48). In accordance with the current literature, GH levels in obese women were significantly decreased relative to normal weight women (Figure 3). This is in keeping with the finding that obesity leads to a cutoff in the normal amplitude of GH release (49,50). Long term CR induced by reducing diet promoted a significant decrease of GH serum levels compared with NW and with OW by trend. A strong reduction of the fasting GH levels compared with all other groups was found in LAGB-induced long-term CR (Figure 3). Moreover, the amplitude of GH was very low, suggesting that GH release is largely abrogated in LAGB. Furthermore, the fasting GH serum levels were significantly lower in the group of all CRW relative to NW as well as OW (Table 3). The positive correlation found between the fasting serum GH and IGF-I levels ($R = .40$; $p \leq .01$; Table 2) underscores the GH dependency of IGF-I secretion. In conclusion, these data suggest that long-term CR in formerly obese humans reduces the fasting GH levels.

Table 3. Comparison of the CRW, NW, and OW study groups

	Normal (NW)	Caloric Restriction (CRW)	Obese (OW)	*p* value, NW vs CRW	*p* value, NW vs OW	*p* value, CRW vs OW
N	17	16	15			
Sex	F	F	F			
Age (y)	40 ± 12	40 ± 10	45 ± 13	n.s.	n.s.	n.s.
Height (m)	1.66 ± 0.05	1.67 ± 0.06	1.66 ± 0.05	n.s.	n.s.	n.s.
Weight (kg)	62 ± 6	68 ± 8	97 ± 17	≤.01	≤.001	≤.001
Former BMI (kg/m²)	—	38.6 ± 9	—	—	—	—
Δ Body Weight(% body weight)	—	27 ± 8	—	—	—	—
CR period (y)	—	3.8 ± 1.5	—	—	—	—
Current BMI (kg/m²)	22.5 ± 1.9	24.6 ± 3.1	35.6 ± 5.7	≤.01	≤.001	≤.001
Insulin (mU/L)	2.7 ± 2.3	1.9 ± 1.7	9.4 ± 6.7	n.s.	≤.01	≤.01
IGF-I (ng/mL)	243.7 ± 67.7	137.9 ± 43.1	171.9 ± 74.4	≤.001	≤.01	n.s.
Free IGF-I (ng/mL)	0.9 ± 0.4	0.6 ± 0.2	0.6 ± 0.3	≤.05	n.s.	n.s.
IGFBP3 (μg/mL)	5.3 ± 1.7	3.7 ± 1.1	5 ± 1.3	≤.01	n.s.	≤.01
Ratio (IGF-I/IGFBP3)	0.26 ± 0.08	0.24 ± 0.12	0.17 ± 0.07	n.s.	≤ .01	n.s.
GH (ng/mL)	2.1 ± 0.7	0.6 ± 0.8	0.9 ± 0.8	≤.001	≤.001	≤.05

Notes: BMI = body mass index (weight [kilograms]/height [square meters]); GH = growth hormone; IGF-I = insulin-like growth factor-I; IGFBP-3 = IGF-binding protein 3; n.s.= not significant. Clinical, anthropometric, and endocrine parameters are indicated. Data presented as mean ± *SD*.

* $p < .05$, ** $p < .01$, *** $p < .001$.

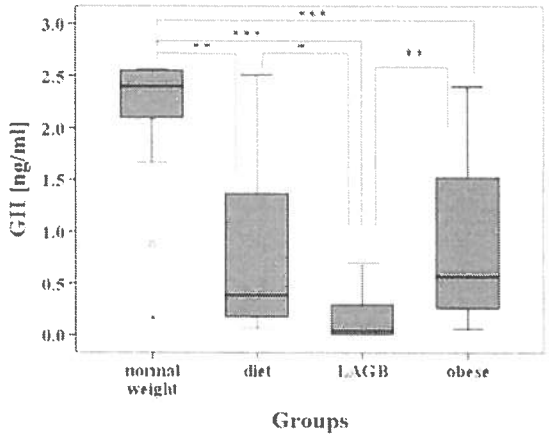

Figure 3. Fasting growth hormone (GH) serum levels in the study groups. The serum concentrations of GH were measured by enzyme-linked immunosorbent assays ante meridiem following overnight fasting. Median values are indicated ± standard deviation.

Discussion

The key finding of this investigation is that 4 years of severe CR induced by two different interventions, LAGB and diet, led to significantly lower total IGF-I serum levels in formerly obese women relative to normal weight women. Moreover, we found that the fasting GH serum levels were considerably lower in long-term calorically restricted formerly obese women compared with NW as well as with OW. In fact, severe long-term CR in formerly obese women over several years led rather to a reduction or a sustainment of the low IGF-I levels found in obese women, respectively, than to an increase as found in earlier studies (51). These studies showed that obesity results in a reduction in GH and IGF-I secretion, which is reversible on weight reduction by severe diet-induced CR. The length of the CR intervention was, however, not indicated (51). Well-controlled studies in CR rodents have shown that a long period of severe CR is important for CR outcomes, including effects on serum IGF-I levels (1,9,14). It is quite likely that the duration of the severe CR interventions in our investigation (4 years) was much longer than in the other studies. This is crucial because most of the beneficial effects in animal studies were obtained by long-term CR over a long time period relative to the life span of the organism (1). In two recent studies, circulating IGF-I was found to be not decreased relative to non-CR controls after a moderate CR (25%) for 6 and 12 months in overweight people (36,37). This could be due to the relatively short CR regimes employed in these studies, which may mainly reflect the initial period of weight loss but not the effects of sustained CR beyond the point of weight stability as discussed recently (52). In fact, in animal studies demonstrating the beneficial effects of CR, after an initial phase of weight loss, the body weight is relatively stable for a large part of the CR period (53). In our study, the strongly reduced calorie intake resulted in pronounced weight loss in all participants followed by a phase of at least 1-year weight stability before sampling. Thus, both the long duration and the severe caloric reduction in our intervention groups may explain at least in part why the total IGF-I levels in our CR study groups were below the IGF-I levels in normal-weight women eating ad libitum.

Large-scale cross-sectional studies indicate a nonlinear relationship between the total circulating IGF-I levels and BMI. IGF-I levels rise with increasing BMI up to 25–26 kg/m^2 but then progressively fall again with a further increase in BMI (44,54,55) [for reviews, see refs. (31,56)]. After 4 years of CR, our two long-term CR study groups showed a BMI of 24–25 kg/m^2 in contrast to the considerably lower BMI (22.5 kg/m^2) of NW. Thus, despite the fact that the BMI of the two long-term CR groups is more close to 25–26 kg/m^2 as the BMI of NW, the IGF-I levels are still lower in CRW than in NW. This further underscores our finding that the two long-term CR interventions lead to decreased circulating IGF-I levels. There is very little, if any, diurnal variation in circulating concentrations of IGF-I and IGFBP-3 in healthy participants (56).

What may contribute to the relatively low IGF-I serum level in the severe long-term calorically restricted formerly obese women? IGF-I synthesis can be modulated by several nutritional and endocrine factors (32,35). Thus, caloric intake was shown to be a major determinant of insulin serum level, and insulin can increase the serum levels and bioactivity of IGF-I in humans [reviewed in refs. (31,35)]. It was shown that the effects of nutritional energy balance on hepatic IGF-I synthesis and availability of circulating IGF-I are mediated, at least in part, by differences in pancreatic insulin secretion. Insulin sensitizes liver cells for the stimulatory effects of GH on IGF-I synthesis by increasing GH-receptor levels and by enhancing cellular protein synthesis in general. Moreover, insulin can augment IGF-I bioactivity by inhibiting the production of IGFBP-1 and IGFBP-2 [reviewed in ref. (35)]. We showed that the insulin serum levels are decreased in formerly obese women subjected to bariatric surgeries, such as LAGB, or diet-induced long-term CR (Table 1) (43). This suggests that lowered insulin levels contribute to the relatively low IGF-I levels found in long-term calorically restricted women.

We found in the present investigation that the fasting GH serum levels were very low in long-term calorically restricted women relative to normal-weight and obese groups. Taking into account the parameters, BMI, IGF-I serum levels, and glucose tolerance (43), these findings suggest that GH contributes, at least in part, to the lower circulating IGF-I in the long-term calorically restricted formerly obese women. Our data are further corroborated by the fact that, although abdominal obesity results in a secondary reduction in GH secretion (50), we found that very long (4 years) LAGB-induced long-term CR further reduced fasting GH to the very low levels in formerly obese women (Figure 3). Given that the GH levels were analyzed as fasting ambulatory levels, we cannot, however, exclude

different results in 24-hour spontaneous GH release, which might be due to the pulsatile nature of GH secretion (57,58). Approximately 80% of daily GH secretion proceeds by periodic bursts (with nocturnal augmentation of GH secretion), which are superimposed on basal secretion (58). It should further be noted that short-term fasting (calorie-free diet) leads to increased mean GH levels due to an increase in mean GH burst mass and amplitude (59).

Measuring the IGF-I, IGFBP-3, and GH levels before and after the weight loss in addition to compare with normal weight and obese control groups might have add additional aspects to the present study. This is a limitation due to our concept to employ only age-matched normal-weight women as control group to provide the opportunity to compare the corresponding hormone levels in previously obese humans after long-term CR with people with normal weight who were never obese.

Results similar to our data regarding the impact of severe CR on circulating IGF-I were obtained in previous investigations on obese people. There is evidence that massive weight loss after bariatric surgery does not always restore GH and IGF-I levels but can instead lead to persistent GH/IGF-I deficiency (60–62). Six months after surgery, about 20% of obese women undergoing LAGB were found to be GH and IGF-I deficient and another fifth had IGF-I levels still below the normal range (61,62). Similar results were observed after malabsorptive bariatric surgical procedures (60). On the other hand, recovery of the components of the GH/IGF axis has been found in other studies 6–12 months after bariatric surgery (63–65). It is, however, important to note that most of these studies were conducted on gastric bypass surgeries, suggesting that the mode of gastrointestinal surgery may contribute to the differences. Undernutrition such as anorexia nervosa was also shown to lead to reduced IGF-I serum concentrations in low weight people but does increase GH level (66–69). This might be due to severe undernutrition, however, the underlying mechanisms are not precisely understood.

Beginning in early adulthood, circulating GH and IGF-I levels decline strongly with age, and relatively low GH and IGF-I serum levels are characteristics of normal aging (70,71). Studies of the effects of GH treatment in healthy elderly participants showed improvements in body composition (72). However, undesirable side effects of treatment of endocrinologically normal healthy older people with GH, offsetting major benefits of GH therapy, were also found (73). Thus, the role of the GH/IGF-I axis in human aging is currently a topic of controversial discussion. Data from animal models and epidemiological studies suggest that reduced somatotropic signaling provides protection from cancer in humans (74–76) [reviewed in ref. (77)] and may promote old age survival [for reviews, see refs. (21,78)]. On the other hand, the natural decline of the GH/IGF-I axis during aging probably contributes to deteriorations in body composition and youthfulness. In accordance with these considerations, studies in several animal models suggest that a decrease in GH/IGF-I action prolongs longevity and increases health span [reviewed in refs. (14,21)]. This has been supported by human epidemiological studies (79). Other human studies suggest, however, that enhancing the activity of the GH/IGF-I axis in the elderly participant may extend the healthy life span (78).

In conclusion, the present study indicates that the activity of the GH/IGF-I axis is lower in formerly obese women subjected to severe long-term CR without malnutrition (induced either by LAGB or by diet) relative to normal-weight women. Our data also suggest that the duration and severity of the CR intervention might be important for the outcome on the GH/IGF-I axis in humans. Further studies are, however, necessary to more precisely understand the impact of long-term CR on the age-related decline of the GH/IGF-I axis in humans.

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REFERENCES

1. Weindruch R, Walford RL. The Retardation of Aging and Disease by Dietary Restriction. Springfield, IL: C.C. Thomas; 1988.
2. Helfand SI BJ, Wood JG. Calorie restriction in lower organisms. In: Guarente LPL, Wallace D, eds. *Molecular Biology of Aging*. New York: Cold Spring Harbor Laboratory Press; 2008:73–93.
3. Minor RK, Allard JS, Younts CM, et al. Dietary interventions to extend life span and health span based on caloric restriction. *J Gerontol A Biol Sci Med Sci*. 2010;65:695–703.
4. Mattison JA, Lane MA, Roth GS, et al. Calorie restriction in rhesus monkeys. *Exp Gerontol*. 2003;38:35–46.
5. Colman RJ, Anderson RM, Johnson SC, et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science*. 2009;325:201–204.
6. Willcox BJ, Yano K, Chen R, et al. How much should we eat? The association between energy intake and mortality in a 36-year follow-up study of Japanese-American men. *J Gerontol A Biol Sci Med Sci*. 2004;59:789–795.
7. Le Couteur DG, McLachlan AJ, Quinn RJ, et al. Aging biology and novel targets for drug discovery. *J Gerontol A Biol Sci Med Sci*. 2011;66:3–8.
8. Kalant N, Stewart J, Kaplan R. Effect of diet restriction on glucose metabolism and insulin responsiveness in aging rats. *Mech Ageing Dev*. 1988;46:89–104.
9. Breese CR, Ingram RL, Sonntag WE. Influence of age and long-term dietary restriction on plasma insulin-like growth factor-1 (IGF-1), IGF-1 gene expression, and IGF-1 binding proteins. *J Gerontol A Biol Sci*. 1991;46:B180–B187.
10. Sonntag WE, Lynch CD, Cefalu WT, et al. Pleiotropic effects of growth hormone and insulin-like growth factor (IGF)-1 on biological aging: inferences from moderate caloric-restricted animals. *J Gerontol A Biol Sci*. 1999;54:B521–B538.
11. Hursting SD, Smith SM, Lashinger LM, et al. Calories and carcinogenesis: lessons learned from 30 years of calorie restriction research. *Carcinogenesis*. 2010;31:83–89.
12. Gems D, Partridge L. Insulin/ IGF-1 signalling and ageing: seeing the bigger picture. *Curr Opin Genet Dev*. 2001;11:287–292.
13. Kenyon C. A conserved regulatory system for aging. *Cell*. 2001;105:165–168.
14. Bartke A. Growth hormone, insulin and aging: the benefits of endocrine defects. *Exp Gerontol*. 2011;46:108–111.

15. Brown-Borg HM, Borg KE, Meliska CJ, et al. Dwarf mice and the ageing process. *Nature*. 1996;384:33.
16. Flurkey K, Papaconstantinou J, Miller RA, et al. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc Natl Acad Sci U S A*. 2001;98: 6736–6741.
17. Coschigano KT, Holland AN, Riders ME, et al. Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor i levels and increased life span. *Endocrinology*. 2003;144:3799–3810.
18. Bonkowski MS, Rocha JS, Masternak MM, et al. Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction. *Proc Natl Acad Sci U S A*. 2006;103:7901–7905.
19. Holzenberger M, Dupont J, Ducos B, et al. IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature*. 2003;421:182–187.
20. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. *Endocr Rev*. 1995;16:3–34.
21. Kopchick JJBA, Berryman DE. Extended life span in mice with reduction in the GH/ IGF-1 axis. In: Guarente LPL, Wallace D, eds. *Molecular Biology of Aging*. New York: Cold Spring Harbor Laboratory Press; 2008:347–369.
22. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev*. 2002;23:824–854.
23. Frystyk J. Free insulin-like growth factors—measurements and relationships to growth hormone secretion and glucose homeostasis. *Growth Horm IGF Res*. 2004;14:337–375.
24. Baxter RC, Martin JL, Beniac VA. High molecular weight insulin-like growth factor binding protein complex. Purification and properties of the acid-labile subunit from human serum. *J Biol Chem*. 1989;264: 11843–11848.
25. Guler HP, Zapf J, Schmid C, et al. Insulin-like growth factors I and II in healthy man. Estimations of half-lives and production rates. *Acta Endocrinol (Copenh)*. 1989;121:753–758.
26. Valentinis B, Baserga R. IGF-I receptor signalling in transformation and differentiation. *Mol Pathol*. 2001;54:133–137.
27. Yamada PM, Lee KW. Perspectives in mammalian IGFBP-3 biology: local vs. systemic action. *Am J Physiol Cell Physiol*. 2009;296: C954–C976.
28. Baker J, Liu JP, Robertson EJ, et al. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell*. 1993;75:73–82.
29. LeRoith D, Roberts CT Jr The insulin-like growth factor system and cancer. *Cancer Lett*. 2003;195:127–137.
30. Yakar S, Leroith D, Brodt P. The role of the growth hormone/insulin-like growth factor axis in tumor growth and progression: lessons from animal models. *Cytokine Growth Factor Rev*. 2005;16:407–420.
31. Dossus L, Kaaks R. Nutrition, metabolic factors and cancer risk. *Best Pract Res Clin Endocrinol Metab*. 2008;22:551–571.
32. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. *Endocr Rev*. 1994;15:80–101.
33. Holmes MD, Pollak MN, Willett WC, et al. Dietary correlates of plasma insulin-like growth factor i and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomarkers Prev*. 2002;11:852–861.
34. Clemmons DR, Underwood LE. Nutritional regulation of IGF-I and IGF binding proteins. *Annu Rev Nutr*. 1991;11:393–412.
35. Kaaks R, Lukanova A. Energy balance and cancer: the role of insulin and insulin-like growth factor-I. *Proc Nutr Soc*. 2001;60:91–106.
36. Fontana L, Weiss EP, Villareal DT, et al. Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans. *Aging Cell*. 2008;7:681–687.
37. Redman LM, Veldhuis JD, Rood J, et al. The effect of caloric restriction interventions on growth hormone secretion in nonobese men and women. *Aging Cell*. 2010;9:32–39.
38. Thaler JP, Cummings DE. Minireview: hormonal and metabolic mechanisms of diabetes remission after gastrointestinal surgery. *Endocrinology*. 2009;150:2518–2525.
39. O'Brien PE, Dixon JB, Laurie C, et al. Treatment of mild to moderate obesity with laparoscopic adjustable gastric banding or an intensive medical program: a randomized trial. *Ann Intern Med*. 2006;144:625–633.
40. Adams TD, Gress RE, Smith SC, et al. Long-term mortality after gastric bypass surgery. *N Engl J Med*. 2007;357:753–761.
41. Sjostrom L, Narbro K, Sjostrom CD, et al. Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med*. 2007;357:741–752.
42. Weindruch RCR, Perez V, Richardson AG. How does caloric restriction increase the longevity of mammals? Guarente LPL, Wallace D, eds. *Molecular Biology of Aging*. New York: Cold Spring Harbor Laboratory Press; 2008:409–425.
43. Mitterberger MC, Mattesich M, Klaver E, et al. Adipokine profile and insulin sensitivity in formerly obese women subjected to bariatric surgery or diet-induced long-term caloric restriction. *J Gerontol A Biol Sci Med Sci*. 2010;65:915–923.
44. Gomez JM, Maravall FJ, Gomez N, et al. The IGF-I system component concentrations that decrease with ageing are lower in obesity in relationship to body mass index and body fat. *Growth Horm IGF Res*. 2004;14:91–96.
45. Juul A, Bang P, Hertel NT, et al. Serum insulin-like growth factor-i in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J Clin Endocrinol Metab*. 1994;78:744–752.
46. Clemmons DR, Thissen JP, Maes M, et al. Insulin-like growth factor-1 (IGF-1) infusion into hypophysectomized or protein-deprived rats induces specific IGF-binding proteins in serum. *Endocrinology*. 1989; 125:2967–2972.
47. Veldhuis JD. Neuroendocrine control of pulsatile growth hormone release in the human: relationship with gender. *Growth Horm IGF Res*. 1998;8(suppl B):49–59.
48. Goldenberg N, Barkan A. Factors regulating growth hormone secretion in humans. *Endocrinol Metab Clin North Am*. 2007;36:37–55.
49. Makimura H, Stanley T, Mun D, et al. The effects of central adiposity on growth hormone (GH) response to GH-releasing hormone-arginine stimulation testing in men. *J Clin Endocrinol Metab*. 2008;93:4254–4260.
50. Rasmussen MH. Obesity, growth hormone and weight loss. *Mol Cell Endocrinol*. 2010;316:147–153.
51. Rasmussen MH, Hvidberg A, Juul A, et al. Massive weight loss restores 24-hour growth hormone release profiles and serum insulin-like growth factor-i levels in obese subjects. *J Clin Endocrinol Metab*. 1995;80:1407–1415.
52. Rochon J, Bales CW, Ravussin E, et al. Design and conduct of the calerie study: comprehensive assessment of the long-term effects of reducing intake of energy. *J Gerontol A Biol Sci Med Sci*. 2011; 66:97–108.
53. Masoro EJ, Yu BP, Bertrand HA. Action of food restriction in delaying the aging process. *Proc Natl Acad Sci U S A*. 1982;79:4239–4241.
54. Lukanova A, Lundin E, Zeleniuch-Jacquotte A, et al. Body mass index, circulating levels of sex-steroid hormones, IGF-I and IGF-binding protein-3: a cross-sectional study in healthy women. *Eur J Endocrinol*. 2004;150:161–171.
55. Gram IT, Norat T, Rinaldi S, et al. Body mass index, waist circumference and waist-hip ratio and serum levels of IGF-I and IGFBP-3 in European women. *Int J Obes (Lond)*. 2006;30:1623–1631.
56. Frystyk J, Freda P, Clemmons DR. The current status of IGF-I assays –a 2009 update. *Growth Horm IGF Res*. 2010;20:8–18.
57. Thorner MO, Chapman IM, Gaylinn BD, et al. Growth hormone-releasing hormone and growth hormone-releasing peptide as therapeutic agents to enhance growth hormone secretion in disease and aging. *Recent Prog Horm Res*. 1997;52:215–244.
58. Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. *Endocr Rev*. 1998;19:717–797.
59. Nass R, Farhy LS, Liu J, et al. Evidence for acyl-ghrelin modulation of growth hormone release in the fed state. *J Clin Endocrinol Metab*. 2008;93:1988–1994.

60. De Marinis L, Bianchi A, Mancini A, et al. Growth hormone secretion and leptin in morbid obesity before and after biliopancreatic diversion: relationships with insulin and body composition. *J Clin Endocrinol Metab*. 2004;89:174–180.
61. Di Somma C, Angrisani L, Rota F, et al. GH and IGF-I deficiency are associated with reduced loss of fat mass after laparoscopic-adjustable silicone gastric banding. *Clin Endocrinol (Oxf)*. 2008:69: 393–399.
62. Savastano S, Di Somma C, Angrisani L, et al. Growth hormone treatment prevents loss of lean mass after bariatric surgery in morbidly obese patients: results of a pilot, open, prospective, randomized, controlled study. *J Clin Endocrinol Metab*. 2009;94:817–826.
63. Holdstock C, Engstrom BE, Ohrvall M, et al. Ghrelin and adipose tissue regulatory peptides: effect of gastric bypass surgery in obese humans. *J Clin Endocrinol Metab*. 2003;88:3177–3183.
64. Mancini MC, Costa AP, de Melo ME, et al. Effect of gastric bypass on spontaneous growth hormone and ghrelin release profiles. *Obesity (Silver Spring)*. 2006;14:383–387.
65. Eden Engstrom B, Burman P, Holdstock C, et al. Effects of gastric bypass on the GH/ IGF-I axis in severe obesity—and a comparison with gh deficiency. *Eur J Endocrinol*. 2006;154:53–59.
66. Counts DR, Gwirtsman H, Carlsson LM, et al. The effect of anorexia nervosa and refeeding on growth hormone-binding protein, the insulin-like growth factors (IGFs), and the IGF-binding proteins. *J Clin Endocrinol Metab*. 1992;75:762–767.
67. Argente J, Caballo N, Barrios V, et al. Multiple endocrine abnormalities of the growth hormone and insulin-like growth factor axis in patients with anorexia nervosa: effect of short- and long-term weight recuperation. *J Clin Endocrinol Metab*. 1997;82:2084–2092.
68. Scacchi M, Pincelli AI, Caumo A, et al. Spontaneous nocturnal growth hormone secretion in anorexia nervosa. *J Clin Endocrinol Metab*. 1997:82:3225–3229.
69. Fazeli PK, Misra M, Goldstein M, et al. Fibroblast growth factor-21 may mediate growth hormone resistance in anorexia nervosa. *J Clin Endocrinol Metab*. 2009;95:369–374.
70. Finkelstein JW, Roffwarg HP, Boyar RM, et al. Age-related change in the twenty-four-hour spontaneous secretion of growth hormone. *J Clin Endocrinol Metab*. 1972;35:665–670.
71. Maggio M, Ble A, Ceda GP, et al. Decline in insulin-like growth factor-i levels across adult life span in two large population studies. *J Gerontol A Biol Sci Med Sci*. 2006:61:182–183.
72. Rudman D, Feller AG, Nagraj HS, et al. Effects of human growth hormone in men over 60 years old. *N Engl J Med*. 1990:323:1–6.
73. Liu H, Bravata DM, Olkin I, et al. Systematic review: the safety and efficacy of growth hormone in the healthy elderly. *Ann Intern Med*. 2007;146:104–115.
74. Yang XF, Beamer WG, Huynh H, et al. Reduced growth of human breast cancer xenografts in hosts homozygous for the lit mutation. *Cancer Res*. 1996;56:1509–1511.
75. Ng ST, Zhou J, Adesanya OO, et al. Growth hormone treatment induces mammary gland hyperplasia in aging primates. *Nat Med*. 1997; 3:1141–1144.
76. Le Marchand L, Donlon T, Seifried A, et al. Association of a common polymorphism in the human gh1 gene with colorectal neoplasia. *J Natl Cancer Inst*. 2002;94:454–460.
77. Perry JK, Mohankumar KM, Emerald BS, et al. The contribution of growth hormone to mammary neoplasia. *J Mammary Gland Biol Neoplasia*. 2008;13:131–145.
78. Thorner MO. Statement by the growth hormone research society on the GH/ IGF-I axis in extending health span. *J Gerontol A Biol Sci Med Sci*. 2009;64:1039–1044.
79. Suh Y, Atzmon G, Cho MO, et al. Functionally significant insulin-like growth factor i receptor mutations in centenarians. *Proc Natl Acad Sci U S A*. 2008:105:3438–3442.