Increased serum IGF-1 levels protect the musculoskeletal system but are associated with elevated oxidative stress markers and increased mortality independent of tissue igf1 gene expression

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SHORT TAKE

Increased serum IGF-1 levels protect the musculoskeletal system but are associated with elevated oxidative stress markers and increased mortality independent of tissue *igf1* gene expression

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Summary

Although the literature suggests a protective (anabolic) effect of insulin-like growth factor-1 (IGF-1) on the musculoskeletal system during growth and aging, there is evidence that reductions in IGF-1 signaling are advantageous for promoting an increase in life span through reduction in oxidative stress-induced tissue damage. To better understand this paradox, we utilized the hepatocyte-specific IGF-1 transgenic (HIT) mice, which exhibit 3-fold increases in serum IGF-1, with normal IGF-1 expression in other tissues, and mice with an IGF-1 null background that exclusively express IGF-1 in the liver, which thereby deliver IGF-1 by the endocrine route only (KO-HIT mice). We found that in the total absence of tissue *igf1* gene expression (KO-HIT), increases in serum IGF-1 levels were associated with increased levels of lipid peroxidation products in serum and increased mortality rate at 18 months of age in both genders. Surprisingly, however, we found that in female mice, tissue IGF-1 plays an important role in preserving trabecular bone architecture as KO-HIT mice show bone loss in the femoral distal metaphysis. Additionally, in male KO-HIT mice, increases in serum IGF-1 levels were insufficient to protect against age-related muscle loss.

Key words: IGF-1; bone; aging; oxidative stress.

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Results and discussion

In mice, mutations that decrease secretion of growth hormone (GH) (*Prop1*, *Pit1*) lead to significant extensions in life span. The long-lived GH-deficient Ames dwarf mice have very low serum IGF-1 levels and increased activity of catalase and Cu/ZnSOD (Hauck & Bartke, 2000). These mice show reduced levels of DNA and protein oxidation in liver (Yamamoto *et al.*, 2005) and reduced levels of serum and liver F2-isoprostanes, which are stable lipid peroxidation products (Choksi *et al.*, 2007). Similar resistance to oxidative stress is observed in GH-resistant mice and in GH-deficient Snell and *lit/lit* dwarfs (Bartke & Brown-Borg, 2004; Salmon *et al.*, 2005). Likewise, female mice heterozygous for the *Igf1r* gene had extended life span and are resistant to the reactive oxygen species (ROS) generator paraquat (Holzenberger *et al.*, 2003). Furthermore, mice heterozygous for the brain *Igf1r* gene (b*Igf1r*+/−) had extended median life span (Kappeler *et al.*, 2008) accompanied by severely reduced serum IGF-1 as a result of reduced GH production.

IGF-1 is one of the most powerful regulators of muscle homeostasis and a significant regulator of bone mineral density. IGF-1R and IGF-1 null mice show severe retardation in skeletal muscle development and bone accrual (Liu *et al.*, 1993). On the other hand, IGF-1 transgenic mice exhibit a clear increase in body and organ weights and a 30% increase in muscle and bone mass (Mathews *et al.*, 1988). To begin to understand how autocrine/paracrine or endocrine IGF-1 actions affect the different organ/systems during aging, we utilized the hepatocyte-specific IGF-1 transgenic (HIT) mice, which exhibit 3-fold increases in serum IGF-1, with normal IGF-1 expression in other tissues and mice with IGF-1 null background that exclusively express IGF-1 in the liver and thereby deliver IGF-1 by the endocrine route only (KO-HIT mice) (Fig. 1A) (Elis *et al.*, 2010a,b; Wu *et al.*, 2009). We found that increases in serum IGF-1 levels in male and female HIT mice led to increased body weight throughout 18 months, while body weights of KO-HIT mice were indistinguishable from controls (Fig. 1B,C). Whole body assessment of lean and fat mass (by MRI) at 18 months of age did not reveal any differences between the groups (data not shown). However, although not significant, we found that gonadal fat pads of HIT and KO-HIT mice were reduced in females (−43%) and in males (−23%) when compared to controls. Similarly, in KO-HIT male mice, we found a 20% decrease

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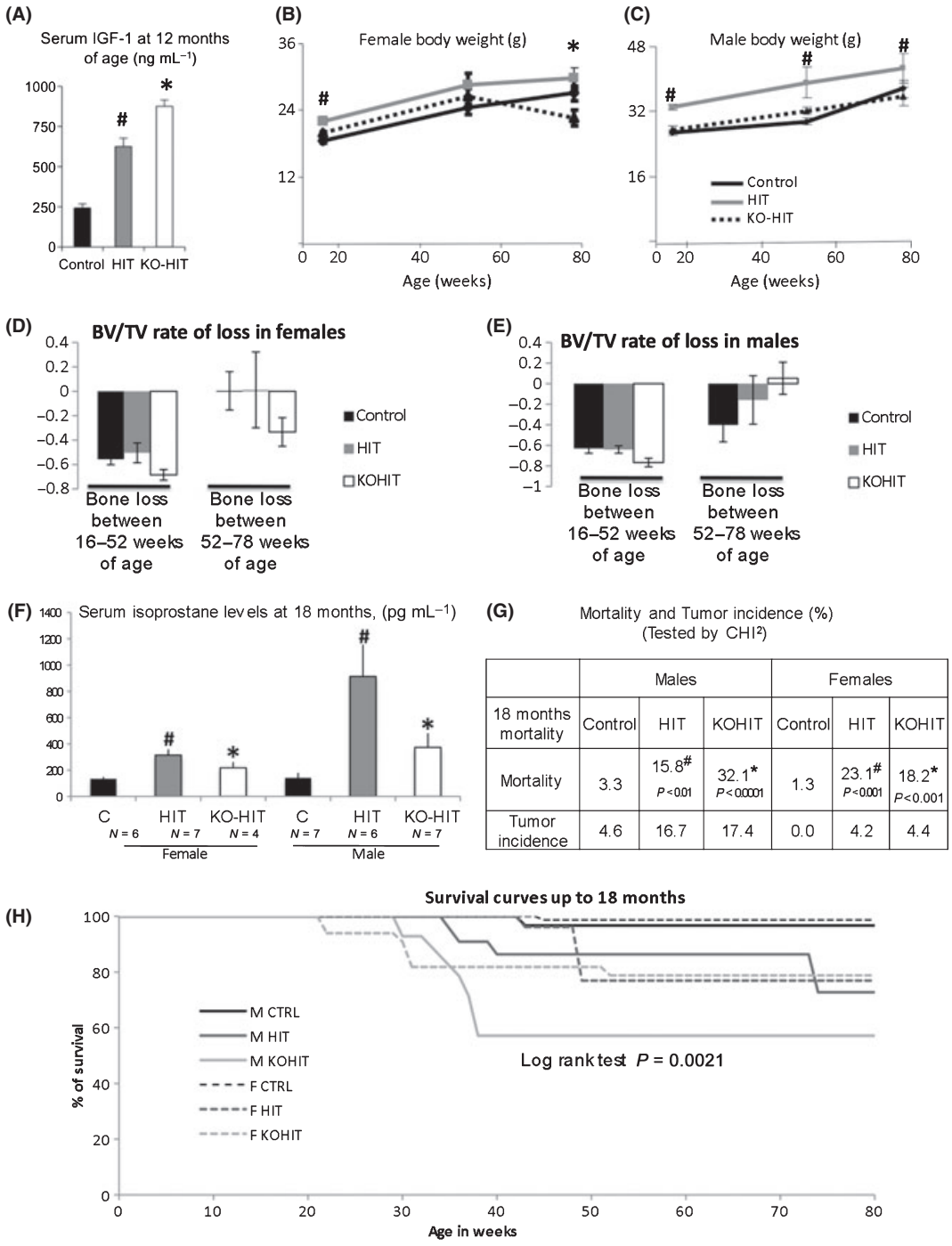

	Males			Females		
18 months mortality	Control	HIT	KOHIT	Control	HIT	KOHIT
Mortality	3.3	15.8# $P<0.01$	32.1* $P<0.0001$	1.3	23.1# $P<0.001$	18.2* $P<0.001$
Tumor incidence	4.6	16.7	17.4	0.0	4.2	4.4

Fig. 1 Physiological traits, oxidation, and mortality in hepatocyte-specific IGF-1 transgenic (HIT) and KO-HIT mice. Generation and detailed characterization of the HIT and KO-HIT mice were previously described (Elis *et al.*, 2010a,b). (A) Serum levels of IGF-1 (n = 10 mice per group) were determined by radioimmunoassay as described earlier (Elis *et al.*, 2010a,b). Body weight of female (B) and males (C) were followed from 16 to 78 weeks of age (n = 8–10 mice per group). Tabecular bone loss in female (D) and males (E) calculated between 16–52 and 52–78 weeks of age. 8-iso PGF2a levels in serum were measured by GC/MS and were normalized to sample volume (F) (n = 5 mice per group) (Roberts & Morrow, 2000). Data presented in panels A–F were tested by one-way analysis of variance (ANOVA) followed by Fisher's test, considering $P < 0.05$ as significant. (*) Denotes significant difference between control and KO-HIT mice, and (#) denotes significant difference between control and HIT mice. (G) Compiled mortality and tumor incidence up to 18 months of age (n = 22 to 78 mice per group per gender) analyzed by chi-square test. (H) Survival curves of the different groups up to 18 months of age of both genders. Log rank test revealed significant difference between all the curves (P = 0.0021).

Table 1 Tissue weights of control, HIT, and KO-HIT mice at time of sacrifice (18 months of age)

Gender	Genotype (*N*)	Body weight, grams	Gonadal fat, % of body weight (weight in grams)	Quadriceps % of body weight (weight in grams)	Gastrocnemius % of body weight (weight in grams)
Female	Control (10)	27.08 ± 1.27	4.2 ± 0.9 (1.23 ± 0.31)	0.85 ± 0.04 (0.22 ± 0.009)	0.76 ± 0.02 (0.20 ± 0.006)
Female	HIT (8)	28.63 ± 2.12	2.6 ± 1.1 (0.83 ± 0.39)	0.86 ± 0.03 (0.24 ± 0.012)	0.72 ± 0.10 (0.20 ± 0.016)
Female	KO-HIT (10)	20.10 ± 0.95	2.4 ± 0.7 (0.55 ± 0.13)	0.85 ± 0.04 (0.17 ± 0.012)	0.79 ± 0.04 (0.16 ± 0.008)
Male	Control (10)	37.70 ± 2.18	3.1 ± 0.4 (1.21 ± 0.17)	0.77 ± 0.04 (0.28 ± 0.003)	0.73 ± 0.04 (0.27 ± 0.008)
Male	HIT (8)	42.70 ± 3.56	2.6 ± 0.6 (1.23 ± 0.40)	0.68 ± 0.04 (0.28 ± 0.009)	0.68 ± 0.04 (0.28 ± 0.011)
Male	KO-HIT (8)	35.70 ± 2.40	2.4 ± 0.5 (0.92 ± 0.22)	0.68 ± 0.04 (0.23 ± 0.005)	0.62 ± 0.04* (0.21 ± 0.014)

*Statistically ($P < 0.05$) different from control tested by ANOVA.
HIT, hepatocyte-specific IGF-1 transgenic.

in the relative weights of the gastrocnemius muscle, when compared to controls (Table 1). Together with previous observations, our study suggests that muscle IGF-1 along with other local factors regulates muscle mass and that increases in serum IGF-1 alone are insufficient for muscle maintenance during aging (at least in males). This was also evident in HIT mice, where despite significant elevations in serum IGF-1 levels, muscle mass was similar to controls (Table 1). Nonetheless, future studies will be necessary to determine how these changes affect muscle function.

The bone phenotype of HIT and KO-HIT mice was maintained throughout 18 months of age, such that HIT bones were larger and mechanically stiffer and stronger in both genders, and KO-HIT bones did not differ significantly from controls (Table 2). Unexpectedly, we found that in female HIT mice, trabecular bone volume per total volume (%BV/TV) was higher than controls and trabecular thickness (Tb.Th) and tissue mineral density (TMD) increased significantly (Table 2). In contrast, in female KO-HIT mice, %BV/TV was lower than controls, and trabecular number (Tb.N) decreased significantly, suggesting that in females, trabecular bone IGF-1 is important for preserving normal %BV/TV, and this cannot be compensated by elevations in serum IGF-1 (as seen in KO-HIT mice). Trabecular bone loss between 52 and 78 weeks of age did not show significant differences between the groups in both genders (Fig. 1D,E). However, KO-HIT female mice tended to

Table 2 Skeletal assessment of 18-month-old mice with altered IGF-1 levels in tissues and serum. Femural bone morphology at the mid-diaphysis, and trabecular bone volume fraction and microarchitecture in the excised distal femoral metaphysis were assessed by micro-CT. Mechanical testing was performed on mouse femora from 18-month-old control, HIT, and KO-HIT mice. Bones were tested to failure by 4-point bending using a servohydraulic materials testing system (Instron Corp., Canton, MA, USA). (Elis *et al.*, 2010a,b)

	Males			Females		
Trait	Control ($n = 10$)	HIT ($n = 8$)	KO-HIT ($n = 8$)	Control ($n = 10$)	HIT ($n = 10$)	KO-HIT ($n = 10$)
Bone length	15.77 ± 0.30	15.9 ± 0.24	15.59 ± 0.88	15.14 ± 0.27	15.29 ± 0.36	14.24 ± 0.44*
Cortical bone (femural mid-diaphysis)						
Total cross-sectional area Tt.Ar. (mm^2)	1.89 ± 0.06	2.38 ± 0.16*	1.95 ± 0.13	1.59 ± 0.09	1.98 ± 0.10*	1.40 ± 0.11
Cortical bone Ct.Ar. (mm^2)	0.91 ± 0.04	1.23 ± 0.08*	0.95 ± 0.06	0.95 ± 0.06	1.16 ± 0.06*	0.83 ± 0.04
Relative cortical area (RCA; Ct.Ar/Tt.Ar)	0.60 ± 0.02	0.59 ± 0.01	0.61 ± 0.02	0.48 ± 0.01	0.52 ± 0.01 ($P = 0.06$)	0.49 ± 0.02
Cortical thickness Ct.Th. (mm)	0.20 ± 0.01	0.24 ± 0.01*	0.20 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.23 ± 0.00
Medullary area Ma.Ar (mm^2)	0.98 ± 0.03	1.15 ± 0.08	1.00 ± 0.10	0.64 ± 0.04	0.82 ± 0.06*	0.57 ± 0.07
Polar moment of inertia Jo (mm^4)	0.44 ± 0.03	0.72 ± 0.09*	0.47 ± 0.06	0.35 ± 0.04	0.53 ± 0.05*	0.27 ± 0.04
Tissue mineral density TMD (mg cm^{-3})	1388 ± 15	1423 ± 12	1406 ± 16	1449 ± 14	1439 ± 11	1443 ± 9
Trabecular bone (Femoral metaphysis)						
Bone volume fraction BV/TV (%)	0.030 ± 0.008	0.038 ± 0.011	0.028 ± 0.004	0.046 ± 0.007	0.055 ± 0.015	0.023 ± 0.004 ($P = 0.09$)
Trabecular thickness Tb.Th. (mm)	0.033 ± 0.001	0.034 ± 0.002	0.034 ± 0.003	0.034 ± 0.001	0.042 ± 0.003*	0.031 ± 0.002
Trabecular number Tb.N. (1 per mm)	0.91 ± 0.24	1.06 ± 0.26	0.86 ± 0.10	1.34 ± 0.20	1.20 ± 0.27	0.73 ± 0.11*
Trabecular spacing Tb.Sp. (mm)	1.68 ± 0.38	1.45 ± 0.36	1.26 ± 0.16	0.98 ± 0.22	1.20 ± 0.24	1.70 ± 0.32
Tissue mineral density TMD (mg cm^{-3})	747 ± 34	777 ± 29	757 ± 29	762 ± 17	914 ± 37*	831 ± 32
Mechanical properties (femora tested to failure by 4-point bending)						
Stiffness (*N* per mm)	163.5 ± 10.6	192.9 ± 19.0	180.1 ± 16.9	183.0 ± 14.9	246.8 ± 19.1*	141.6 ± 9.5
Max load (*N*)	28.6 ± 1.6	37.2 ± 3.3*	34.0 ± 3.8	34.2 ± 2.7	46.2 ± 3.7*	24.7 ± 1.7

*Different from control ($P < 0.05$) by ANOVA. Statistics were run separately for males and females.
HIT, hepatocyte-specific IGF-1 transgenic.

loose more bone than control or HIT mice, while in males, increases in serum IGF-1 (in HIT and KO-HIT mice) seem to be protective.

In the past 10 years, it has become evident that insulin/IGF-1 receptor activation leads to generation of ROS (Mahadev *et al.*, 2001a,b, 2004); however, the exact link between insulin/IGF receptors and the anti-oxidant pathways has not been fully elucidated. We found that increased serum IGF-1 in HIT and KO-HIT mice was accompanied by increased isoprostane levels in serum of both genders, and this was independent of tissue igf-1 gene expression (KO-HIT mice) (Fig. 1F). It is believed that the duration and the magnitude of exposure to ROS play a critical role in the cellular defense response and ultimately in acceleration/delay of aging.

Cross-sectional pathology at 18 months of age revealed that 4–5% of female HIT and KO-HIT mice developed (hepatic or gastric tract) tumors, while control mice appeared healthy at that age (Fig. 1F, i.e., 0.0% tumor incidence). Additionally, we found that 5% of control male mice developed tumors at 18 months of age, while 17% of HIT mice showed tumors. Although tumor incidence data did not differ significantly between the groups of each gender, these data suggest that excess serum IGF-1 tends to increase tumor incidence, irrespective of tissue IGF-1 levels. We also found that mortality (analyzed by chi-square test) increased significantly in both male and female HIT and KO-HIT mice when compared to controls (Fig. 1G). Additionally, survival curves up to 78 weeks of age revealed significant decrease (tested by log rank) in life span of HIT and KO-HIT mice in both genders (Fig. 1H). Nonetheless, it is important to note that we did not follow the animals longer than 78 weeks, and further studies will be needed to determine the cause of death. Additionally, we found an increased death rate (up to a year) among the KO-HIT male and female mice (Fig. 1H) that stabilize thereafter. This may imply to hormetic effects of increases in serum IGF-1 levels or simply suggest a stochastic process. However, to provide a conclusive explanation to this phenomenon, further studies are needed.

In summary, we found that constant elevations in serum IGF-1 levels (from birth) are associated with increased oxidative stress markers in tissues and serum as well as increased mortality, independent of local igf-1 gene expression. Concurrently, we found that constant elevations in serum IGF-1 were insufficient to protect the musculoskeletal system in the absence of tissue *igf*-1 gene expression in males. Similarly, elevations in serum IGF-1 were insufficient to protect from trabecular bone loss in the absence of tissue *igf*-1 gene expression in females.

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