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GABRIELLA MELO FONTES SILVA

OSTEOARTRITE, GENO VALGO E DENSIDADE MINERAL ÓSSEA NA DEFICIÊNCIA ISOLADA GENÉTICA DO HORMÔNIO DO CRESCIMENTO

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Monografia apresentada à Universidade Federal de Sergipe como um dos pré-requisitos para a obtenção do título de Graduado no curso de Medicina.

Orientador: Prof. Dr. Manuel Hermínio de Aguiar Oliveira

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Monografia apresentada ao colegiado do curso de Medicina da Universidade Federal de Sergipe, como requisito parcial para obtenção do grau de bacharel em Medicina.

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Dedico este trabalho aos meus queridos pais, Givando e Giselda, por ter acreditado e investido nos meus estudos. Sem o amor, incentivo, a dedicação e presença de vocês, hoje eu não chegaria onde estou. Muito Obrigada!

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“A percepção do desconhecido é a mais fascinante das experiências. O homem que não tem os olhos abertos para o misterioso passará pela vida sem ver nada.”

Albert Einstein

RESUMO

O eixo GH/IGF-I é importante para o crescimento e desenvolvimento ósseo, mas seus efeitos na densidade mineral óssea (DMO) e na função articular não são completamente conhecidos. Indivíduos com deficiência de GH (DGH) no início da vida adulta têm frequentemente redução da DMO. Entretanto, existem dados limitados em indivíduos com deficiência isolada e congênita do hormônio do crescimento (DIGH). Mostramos que indivíduos adultos com DIGH, decorrente de uma mutação no *GHRHR* tipo c.57+1G>A, provenientes da coorte de Itabaianinha, não tratados, apresentam uma redução da rigidez óssea, mas a DMO e a função articular dessa coorte são desconhecidas. O objetivo desse trabalho foi estudar a DMO, a severidade da osteoartrite, a função e a anatomia articular em uma população de indivíduos com DIGH de ambos os sexos, portando a mutação c.57+1G > A no *GHRHR*, provenientes de Itabaianinha. Foi realizado um estudo transversal, através da realização da densitometria óssea com cálculo da DMO areal e volumétrica (DMOv) em coluna lombar, quadril total e corpo inteiro, em 25 indivíduos com DIGH e 23 controles. A função articular foi avaliada pela goniometria dos cotovelos, quadris e joelhos. Radiografias foram feitas para mensurar o eixo anatômico do joelho e a severidade da osteoartrite, baseada numa adaptação da classificação da Sociedade Internacional de pesquisa da osteoartrite a partir dos osteófitos (OF) e estreitamento do espaço articular (EEA). Os resultados mostraram que DMO areal foi menor que nos controles, mas a DMOv foi similar em ambos os grupos. A amplitude de movimentos dos cotovelos, quadris e joelhos foram semelhantes em ambos os grupos. Geno valgo foi mais prevalente nos indivíduos com DIGH que nos controles. No joelho, o escore de osteoartrite para OF foi similar em ambos os grupos e o escore para EEA mostrou uma tendência a ser mais elevado na DIGH. No quadril, os escores de OF e do EEA foram maiores no DIGH. Em conclusão, DIGH congênita não tratada causa osteoartrite no quadril e geno valgo, sem aparente importância clínica, reduz o tamanho do osso, mas não reduz a DMOv da coluna lombar e quadril.

Palavras Chave: deficiência de GH, densidade mineral óssea, osteoartrite, geno valgo.

ABSTRACT

The GH/IGF-I axis is important for bone growth, but its effects on joint function are not completely understood. Adult onset GH deficient (GHD) individuals have often reduced bone mineral density (BMD). However, there are limited data on BMD in adult patients with untreated congenital isolated GHD (IGHD). We have shown that adult IGHD individuals from the Itabaianinha, homozygous for the c.57+1G>A *GHRHR* mutation, have reduced bone stiffness, but BMD and joint status in this cohort are unknown. The objective was to study BMD, joint function, and osteoarthritis score in IGHD adults harboring c.57+1G > A *GHRHR* mutation, previously untreated. It was performed a cross-sectional study. Areal BMD by dual-energy X-ray absorptiometry was measured in 25 IGHD and 23 controls. Volumetric BMD (vBMD) was calculated at the lumbar spine and total hip. Joint function was assessed by goniometry of elbow, hips and knees. X rays were used to measure the anatomic axis of knee and the severity of osteoarthritis, using a classification for osteophytes (OP) and joint space narrowing (JSN). Genu valgum was more prevalent in IGHD than controls. The osteoarthritis knees OP score was similar in both groups, and knees JSN score showed a trend to be higher in IGHD. The hips OP score, and JSN score were higher in IGHD. Areal BMD was lower in IGHD than controls, but vBMD was similar in the two groups. Range of motion was similar in elbow, knee and hip in IGHD and controls. Untreated congenital IGHD due inactivating GHRHR mutation causes hip joint osteoarthritis problems and genu valgum, without apparent clinical significance, reduces bone size but does not reduce vBMD of the lumbar spine and hip.

Key Words: GH deficiency, BMD, Osteoarhrites, genu valgum

LISTA DE ABREVIATURAS

ALS – Subunidade proteica ácido lábil

AOGHD - Adult-onset GH deficient

AP - anterior-posterior

BMD- Bone mineral density

BMDTB: Bone mineral density total body

BMDTH: Bone mineral density total hip

BMI- Body mass index

CO- Controls

DGH – Deficiência do Hormônio do crescimento

DIGH – Deficiência Isolada do Hormônio do crescimento

DMO – Densidade mineral óssea

DMOv – Densidade mineral óssea volumétrica

DMOvCI - Densidade mineral óssea volumétrica do corpo inteiro

DMOvL1-L4 - Densidade mineral óssea volumétrica de L1-L4

DMOvQT - Densidade mineral óssea volumétrica do quadril total

EEA – Estreitamento do espaço articular

GH - Hormônio do crescimento

GHD- GH deficient

***GH-R* –** Gene do receptor do Hormônio do crescimento

GH-R – Receptor do hormônio do crescimento

GHRH - Hormônio liberador do GH

***GHRHR* –** Gene do receptor do GHRH

GHRH-R – Receptor do hormônio liberador do GH

IGFBP-3 – Proteína carreadora de IGF, tipo 3

IGF-I – Fator de crescimento semelhante à insulina tipo 1

IGF-II - Fator de crescimento semelhante à insulina tipo 2

IGHD- Untreated congenital isolated GHD

IL-1 – Interleucina tipo 1

ISCD- International Society of Clinical Densitometry

JSN - Joint space narrowing

NO- Óxido Nítrico

OARSI - Sociedade Internacional de Pesquisa da Osteoartrite

OF – Osteófitos

OP - Osteophytes

ORSI - Osteoarthritis Research Society International

PTH - Paratormônio

SDS - Standard deviation scores

TGF - β - Fator transformador de crescimento tipo β

TNF - α – Fator de necrose tumoral tipo α

vBMD- Volumetric BMD

vBMDL14- Volumetric density of the lumbar spine

vBMDTB- Volumetric density of total body

vBMDTH- Volumetric density of total hip

LISTA DE SÍMBOLOS

g – gramas

cm^2 – centímetros quadrados

cm^3 – centímetros cúbicos

g/ cm^2 – grama por centímetros quadrados

g/ cm^3 - grama por centímetros cúbicos

+ - Mais ou menos

% - Porcentagem

√ **-** Raiz quadrada

< - Menor

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1. Hormônio do Crescimento (GH) e o Eixo GH- IGF-I

O Hormônio do Crescimento (GH) ou somatotropina é um hormônio polipeptídico secretado pela hipófise anterior, através das células somatotróficas, cuja principal função é a promoção do crescimento através do estímulo das cartilagens epifisárias de ossos longos e da massa muscular. Ele desempenha também funções metabólicas específicas: aumento da velocidade da síntese protéica (balanço nitrogenado positivo), maior metabolização e utilização de ácidos graxos do tecido adiposo e aumento da utilização dos carboidratos, possuindo assim efeito anabólico, lipolítico e antagonista da insulina. (CUNEO *et al.,* 1992; UNDERWOOD; VAN, 1992).

A secreção hipofisária de GH tem controle hipotalâmico, exercido fundamentalmente pelo hormônio liberador de GH (GHRH), em menor intensidade pela grelina e de forma inibitória pela somatostatina. Outros fatores como a tiroxina, o glucagon, os esteróides sexuais, a dopamina, a hipoglicemia e alguns hexapeptídeos sintéticos (*GH releasing peptides*) também estimulam a secreção de GH, através de atuação no hipotálamo e/ou na hipófise.

Uma vez secretado, o GH age através do seu receptor (GH-R), estimulando a produção hepática e tecidual do fator de crescimento semelhante à insulina tipo I (IGF-I), um polipeptídio de 79 aminoácidos, semelhante à insulina e o maior responsável pelos seus efeitos biológicos (LARON, 1982; UNDERWOOD; VAN, 1992; SOUZA *et al*., 2004; CORREA-SILVA; CUNHA DE SÁ; LENGYEL, 2008) - Figura 1.

A secreção de GH ocorre em pulsos com um ritmo cicardiano, principalmente no início das fases III e IV do sono, com meia-vida de aproximadamente 20 minutos, sendo a amplitude e frequência dos pulsos maior em crianças e adultos jovens, uma hora após o início do sono profundo (SALVATORI *et al*., 1999; PEREIRA *et al*., 2007). O padrão de secreção do GH é modificado durante a idade: eleva-se na infância até a puberdade, depois permanece estável e após a meia-idade sua secreção cai progressivamente, em torno de 10 a 15% por década, sendo que na velhice os níveis de GH são semelhantes aos de um adulto jovem com deficiência de GH.

Outros fatores estão intimamente relacionados com a secreção de GH. Os fatores nutricionais é um exemplo, de modo que na desnutrição crônica ou no jejum prolongado ocorre a maior frequência dos pulsos de GH e as amplitudes máximas (KRONENBERG *et al*, 2010).

O IGF-I é encontrado na circulação como integrante de um complexo ternário de 150 KDa, formado pela proteína transportadora de IGF, tipo 3 (IGFBP-3) e pela subunidade

protéica ácido-lábil (ALS). Por causa do seu peso molecular, este complexo não transpõe a barreira endotelial e funciona como reservatório circulante, aumentando a vida média dos IGFs de 10 minutos, em sua forma livre, para 15 horas (JONES; CLEMMONS, 1995). Embora o fígado seja o principal produtor de IGF-I circulante, é possível que a contribuição óssea para o IGF-I circulante seja maior que acreditamos, e que, na osteoporose idiopática, a menor massa óssea pode causar os baixos níveis de IGF-I (MCQUILLAN *et al*., 1986).

A secreção do IGF-I é estimulada principalmente pelo GH na vida pós-natal, mas fatores como o aporte protéico-calórico e estado nutricional são importantes, sobretudo nos primeiros anos de vida. Durante o crescimento intrauterino, há uma menor dependência do IGF-I pelo GH, mas com o nascimento, o GH assume gradualmente posição de principal regulador do crescimento (MARTINELLI JR, C.E.; AGUIAR-OLIVEIRA, M.H., 2005; MARTINELLI JR, C.E; CUSTÓDIO, R.J.; AGUIAR-OLIVEIRA, M.H., 2008).

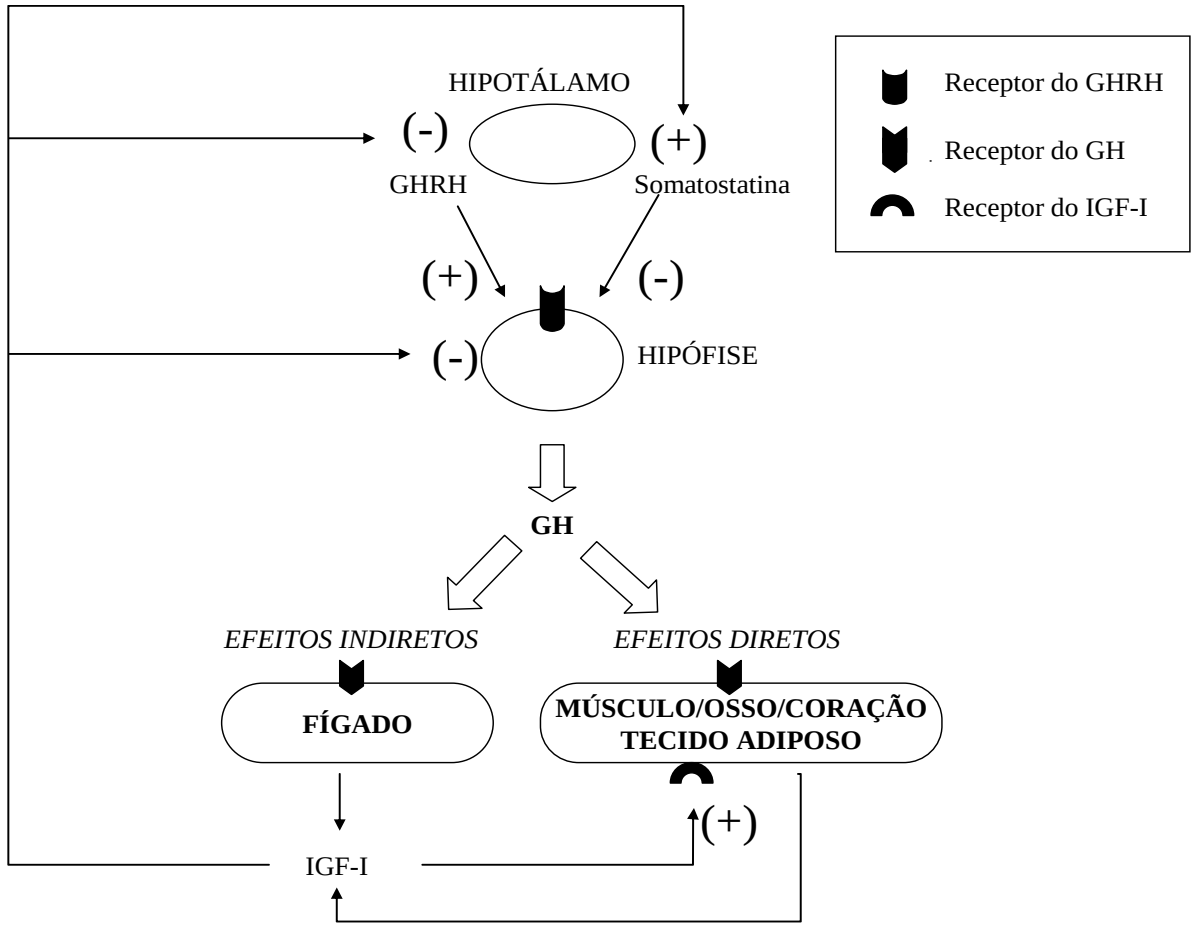

Figura 1. Esquema da regulação intrínseca do eixo GH-IGF-I. **Fonte:** AGUIAR-OLIVEIRA *et al.*, 2010.

2. Deficiência do GH

A deficiência do GH (DGH) pode ser classificada nos seguintes tipos: esporádica ou familiar. No primeiro tipo, encontramos as causas congênitas (deficiência ou ausência do gene para o GH, defeitos da linha média, síndromes de Prader Willi e Laurence-Moon Biedl, síndrome da sela vazia, agenesia, hipoplasia ou ectopia da hipófise), adquiridas (tumores intracranianos, doenças hematológicas, auto-imunes e granulomatosas, infecções pré-natais e do sistema nervoso central, radioterapia craniana), variantes (síndrome de Laron, GH biologicamente inativo) e idiopáticas (associação com trauma intra-uterino ou perinatal) (SALVATORI *et al.*, 1999, SALVATORI *et al.*, 2001).

A DGH pode ocorrer de forma isolada (DIGH) ou associada a déficits de múltiplos hormônios hipofisários. A incidência de DIGH é estimada em 1:3.480 a 1:10.000 nascidos vivos. A DIGH genética compreende 4 formas de acordo com o grau de deficiência de GH e modo de herança (PHILLIPS *et al.*, 1981, PHILLIPS; COGAN, 1994, SOUZA, 1997, BOGUSZEWSKI, 2001):

• Tipo IA: herança autossômica recessiva, com níveis séricos ausentes de GH. Ela é a forma mais grave de DIGH, onde os afetados apresentam uma baixa estatura ao nascer, hipoglicemia na infância, mas uniformemente desenvolve severo nanismo até os seis meses de idade. Nesse tipo, os pacientes desenvolvem anticorpos anti-GH com o uso da terapia com GH (PHILLIPS, 1981, WAJNRAJCH, 1996, COGAN, J.D.; PHILLIPS III, J.A., 1998, CARAKUSHANSKI, 2003).

• Tipo IB: herança autossômica recessiva com níveis diminuídos de GH. Essa é a forma mais frequente da DIGH, com apresentação de baixa estatura menor de 2 desvios padrões para o sexo e idade e uma boa resposta ao tratamento com GH exógeno, sem desenvolver anticorpos anti-GH.

• Tipo II: herança autossômica dominante, com níveis séricos de GH severamente diminuídos. Há uma variada severidade clínica entre as famílias afetadas, geralmente com boa resposta ao tratamento com GH exógeno. Não desenvolvem anticorpos anti-GH e respondem bem à terapia com GH. Os indivíduos afetados são heterozigotos e as mutações são dominantes negativas, isto é, o alelo mutante prevalece sobre o alelo não afetado e consegue interferir na síntese e/ou liberação da molécula normal de GH, apresentando níveis séricos de GH reduzidos, porém detectáveis.

• Tipo III: herança ligada ao X podendo ser associada com hipogamaglobulinemia, com clínica variada, sendo a forma mais rara. Não há alteração do gene GH-1.

A primeira mutação no gene do receptor do hormônio liberador do GH (GHRH-R) ocasionando a DIGH tipo IB foi descrita sub-continente indiano, em três famílias, com uma popularmente conhecida como os "anões de Sindh". Na população de Sindh encontrou-se a mutação GAG → TAG no códon 72, correspondente ao aminoácido residual 50 na proteína madura do GHRH-R. O "nanismo de Sindh" representa o modelo humano homólogo ao "*little mouse*" descrito em 1976, em que a mutação no GHRH-R extingue o sítio de ligação para o GHRH, causando resistência a esse hormônio, hipoplasia pituitária e DIGH (BAUMANN, G; MAHESHWARI, H., 1997; MAHESHWARI, 1998).

Nosso grupo descreveu a DIGH tipo IB em uma extensa família com 100 indivíduos em Carretéis, povoado de Itabaianinha, município de Sergipe. Essa foi a segunda mutação descrita para o DIGH e nela ocorre uma mutação homozigótica tipo splicing no início do íntron 1 no sítio doador de emenda onde a guanina foi substituída pela adenina (c.57+1g>A) no receptor GHRH. Essa mutação impede a formação do RNA mensageiro do GHRH-R, abolindo completamente sua expressão e consequentemente impedindo a ligação do GHRH na superfície da célula somatotrófica, a ativação da adenilciclase, a proliferação celular e a secreção do GH. Como o GHRH é importante para o desenvolvimento dos somatotrofos hipofisários, os indivíduos homozigóticos para essa mutação apresentam uma hipoplasia pituitária anterior pela acentuada redução dos mesmos. Os indivíduos afetados são mut/mut, os indivíduos homozigotos normais são wt/wt, e os heterozigotos são wt/mut (SALVATORI *et al.*, 1999, HAYASHIDA et al., 2000, SALVATORI *et al.*, 2001, SALVATORI *et al.*, 2002, SOUZA *et al.*, 2004, AGUIAR-OLIVEIRA *et al*., 2004).

Esses indivíduos apresentam baixa estatura acentuada, aumento da percentagem de massa gorda, redução da massa magra (MENEZES OLIVEIRA *et al*., 2006) e diminuição da rigidez óssea (DE PAULA *et al*., 2009), medida através da ultrassonometria quantitativa de calcâneo. Entretanto, a influência do tamanho dos ossos na avaliação ultrassonométrica não pode ser excluída.

3. Densidade Mineral Óssea e Deficiência do GH

O GH e o IGF-I são substâncias essenciais para o desenvolvimento ósseo. Este crescimento decorre da proliferação e diferenciação dos condrócitos na placa epifisária, formando uma cartilagem nova que é invadida por vasos sanguíneos, levando a formação de osso trabecular, caracterizando a formação óssea do tipo endocondral. Fatores genéticos,

hormonais e nutricionais estão envolvidos nesse processo (NILSON *et al*., 2005). A deficiência do GH e IGF-I na infância provoca uma diminuição da proliferação de condrócitos na placa epifisária, resultando numa falha do crescimento longitudinal do osso causando uma diminuição do tamanho dos ossos longos. Entretanto a contribuição desses hormônios para o aumento da massa óssea e de sua densidade mineral é pouco conhecida (HIRALAL *et al.*, 2003). Na idade adulta, GH e IGF-I são importantes na manutenção da massa óssea (MONSON *et al*., 2002; BARONCELLI *et al*., 2003).

Tanto o GH quanto o IGF-I são hormônios anabólicos que, além de promover o crescimento longitudinal ósseo, eles regulam a modelação e remodelação óssea estimulando a proliferação, diferenciação e função dos osteoblastos (BACHRACH *et al.*, 1998).

Durante o crescimento embrionário, o IGF- I e o IGF- II determinam o crescimento independentemente do GH. No período pós-natal até a puberdade, o GH e o IGF-I possuem uma função primordial no crescimento longitudinal do osso (WOODS *et al.*, 1996). A disfunção osteoblástica foi relacionada às baixas concentrações de IGF-I no sangue ou na matriz óssea. Existe uma relação entre o IGF-I sérico e o esquelético, de maneira que, a quantidade do IGF-I no osso reflete a produção local pelos osteoblastos corticais e trabeculares e também o IGF-I circulante. Embora a maior produção de IGF-I no organismo seja pelo fígado, o osso representa o maior reservatório de IGF total no organismo. O IGF-I e o IGF-II são os mais abundantes fatores de crescimento ósseo, sua síntese e atividade é regulada por hormônios sistêmicos, como o GH e o Paratormônio (PTH) (CANALIS *et al*., 1988; MOHAN *et al*., 1988). Na osteoporose idiopática, a contribuição esquelética pode estar diminuída, contribuindo para os baixos níveis circulantes de IGF-I (LJUNGHAL *et al*., 1992). De fato, há uma correlação positiva entre densidade mineral óssea (DMO) e níveis séricos de IGF-I na osteoporose idiopática (PATEL *et al*., 2005).

Crianças e adultos com deficiência de GH apresentam uma redução da massa óssea que pode ser aumentada com a reposição do GH. Além disso, uma menor DMO areal foi observada em quatro indivíduos com deficiência grave de IGF-I secundária a deficiência isolada do GH causada por deficiência no GHRH-R (MAHESHWARI *et al.*, 2003) e também em adultos com deficiência do GH-R (BACHRACH *et al.*, 1998).

4. Osteoartrite e Deficiência de GH

A osteoartrite é uma doença caracterizada pela degradação de cartilagem articular,

esclerose óssea e formação de osteófitos (MEULENBELT *et al*.,1998). É a causa articular que mais comumente provoca incapacidade física. Aproximadamente, uma em três pessoas com idade superior a 25 anos apresenta evidência radiológica de osteoartrite em, pelo menos, uma articulação. Sua etiologia é desconhecida e os gastos com seu tratamento refletem importante problema socioeconômico nos países ocidentais (PETER; WIM, 2000).

A cartilagem articular consiste em condrócitos embebidos em extensa matriz extracelular. Essas células são responsáveis pela síntese, organização e regulação da deposição e degradação da matriz (JAMES *et al*., 1996).

Aparentemente, todos os estágios da osteoartrite, independente de sua causa inicial, são causados por mediadores anabólicos e catabólicos que desempenham um papel fundamental nos processos de reparação e destruição da articulação com osteoartrite. Os efeitos desses mediadores dependem não somente de sua quantidade absoluta, mas também da presença de seus inibidores e do equilíbrio entre os vários mediadores, determinando, como resultado final se o processo será de reparação ou destruição articular (PETER; WIM, 2000). Na osteoartrite, o estado de equilíbrio entre a síntese e a degradação dos componentes da cartilagem é comprometido e a taxa de perda de proteoglicanos e outros componentes da matriz eventualmente excede a taxa de formação (CLEASSEN *et al*., 2012).

Os principais fatores de crescimento anabólicos são o IGF-I, o fator transformador de crescimento tipo beta (TGF-β) e a proteína morfogenética óssea. O IGF-I, no soro humano e nos fluidos sinoviais, é o principal estimulador da síntese de proteoglicanos pelos condrócitos. Além disso, desempenham um papel central, na formação de osteófitos, com a continuidade do processo da osteoartrite. Muito embora o IGF-I possa estar envolvido na produção de moléculas da matriz extracelular, observadas nos estágios iniciais da osteoartrite, foi proposto que a deficiência desse fator está envolvida no processo de degradação da cartilagem articular.

Os mais importantes fatores catabólicos da osteoartrite são o óxido nítrico (NO), fator de necrose tumoral tipo alfa (TNF-α) e a interleucina-1 (IL-1) (PETER; WIM, 2000). Foi descrita relação entre os níveis séricos de alguns grupos desses marcadores e severidade da osteoartrite radiográfica (ANITUA *et al*., 2009).

Três linhas de pesquisa sugerem o envolvimento do IGF-I na patogênese da osteoartrite. A primeira avalia o estímulo à síntese de colágeno e proteoglicanos e à proliferação de condrócitos in vivo e in vitro na cartilagem normal, assim como durante a exposição às citocinas, levando a um maior predomínio do processo catabólico da cartilagem. Além disso, inibe o estímulo a degradação dos proteoglicanos da cartilagem normal in vitro

pelas citocinas. Uma segunda linha de pesquisa relaciona- se ao aumento dos níveis de IGF-I na acromegalia, doença caracterizada pela superprodução de GH pela hipófise, resultando no aumento dos níveis séricos de GH e IGF-I que foi associado ao aumento de osteoartrite secundária, apresentando uma prevalência e severidade da artropatia que se relaciona com a duração do descontrole da doença. Por fim, uma terceira linha de estudos mostrou que genes regulam a formação, degradação e reparação da cartilagem articular e do osso subcondral. Esse processo pode estar envolvido na patogênese da osteoartrite (CLAESSEN *et al*., 2012).

5. Teoria Mecanostato

De acordo com essa teoria, o músculo é conhecido por ser o principal orientador da adaptação óssea. Como o osso se adapta às forças a que ele é exposto, em pessoas com baixa estatura e déficit muscular, há uma menor força agindo sobre o seu esqueleto, o que pode afetar a geometria e o desenvolvimento ósseo. A DGH produz um déficit importante de massa muscular, em conseqüência da redução dos efeitos sinérgicos do GH e IGF-I, conforme mostrado na coorte de Itabaianinha (MENEZES OLIVEIRA *et al*., 2006). A teoria mecanostato propõe que o osso se adapta a força para manter a tensão das cargas fisiológicas exercidas pelo músculo próximo ao ponto de ação (SCHOENAU, 2007). Isso pode explicar porque os ossos desses indivíduos com DIGH, mesmo sendo menores, podem ter se adaptado ao tamanho corporal e sua reduzida massa muscular (KATZMAN *et al*., 1991; HOGLER; SHAW, 2010), dessa forma, mantendo uma resistência normal a fraturas.

6. GH, PTH e Vitamina D

O GH tem um efeito indireto na remodelação óssea através da regulação do PTH, cálcio e fosfato, sendo envolvido na 1α hidroxilação da 25 hidroxivitamina-D para a 1,25 diidroxivitamina-D (WHITE *et al*., 2007). Pacientes com DGH demonstram uma insensibilidade de suas células renais, ósseas e intestinais ao PTH, levando a uma resistência a esse hormônio e, portanto, aumentando seus níveis séricos. Essa diminuição de sensibilidade faz com que a resposta do cálcio ao PTH se torne prejudicada (GIUSTINA; MAZZIOTTI; CANALIS, 2008). A razão para o aumento dos níveis de PTH é comumente multifatorial e a deficiência de vitamina D está associada ao aumento dos níveis séricos de PTH. Sabe-se que o

aumento dos níveis de PTH se associa a uma maior reabsorção óssea que leva a um aumento do cálcio extracelular. Este aumento é compensado pelo aumento da excreção de cálcio renal, diminuição da reabsorção intestinal do mesmo e supressão da paratireóide à secreção do PTH, prevenindo o desenvolvimento da hipercalcemia (CLARKE; KHOSLA, 2010).

7. Geno Valgo

Um dos desvios que mais acometem crianças e jovens é o Geno Valgo. Esse desalinhamento do joelho é definido como um afastamento dos joelhos em relação ao eixo proximal do corpo, sendo mais prevalente em meninas e em casos com graus elevados podem influenciar diretamente o desempenho de aptidão física. Existem algumas hipóteses sugerindo que indivíduos com excesso de peso e obesidade (SOUZA *et al.*, 2013) teriam maiores probabilidades de desvios posturais (WEARING *et al.*, 2006; CICCA *et al.*, 2007).

8. Função e características articulares na deficiência genética do receptor do GH

A Síndrome de Laron (Figura 2), descrita em 1966, também denominada Síndrome de Insensibilidade ao GH primária, é uma doença herdada, com traço autossômico recessivo, caracterizada por baixa estatura extrema, fronte proeminente, hipoplasia nasal, cabelos escassos, sinal do sol poente nos olhos durante a infância, escleras azuladas, atraso da primeira dentição, alterações metabólicas e musculoesqueléticas, com redução da massa magra, mãos e pés pequenos, hipoglicemia espontânea e micropênis em meninos, resultando de defeitos moleculares no receptor do GH (LARON; BLUM, CHATELAIN, 1993; SAVAGE *et al.*, 1993; ROSENFELD, R.G.; ROSENBLOOM, A.L.; GUEVARA-AGUIRRE, J., 1994; LARON, 2002).

Na síndrome de Laron, o defeito ocorre no gene do receptor do GH ou na sinalização pós-receptor, resultando num defeito no sinal de transmissão desse hormônio e causando uma diminuição da produção de IGF-I. As características clínicas dessa síndrome são parecidas às DGH não tratada. Entretanto, possui níveis altos de GH e níveis indetectáveis de IGF-I (KORNREICH *et al.*, 2002).

A identificação dessa síndrome no sul do Equador permitiu ampliar a avaliação dos efeitos do IGF-I independente dos efeitos do GH (LARON, 2002). Pacientes com síndrome de Laron possuem estreitamento do canal espinhal e alterações degenerativas precoces na articulação atlanto-odontóidea coluna cervical associado a "*os odontoideum"* (anomalia óssea que a porção mais distal do processo odontóide da segunda vértebra cervical se separa do corpo da mesma) (KORNREICH *et al*., 2002). Também foi descrito a presença de osteófitos vertebrais e geno valgo nesses indivíduos (LARON; KOPCHICK, 2011).

Figura 2: Dr. Zvi Laron e pacientes com resistência ao GH.

9. Função e características articulares na deficiência genética do receptor do GHRH

Não há descrições da função e estrutura articular neste modelo, provavelmente devido a fato de que apenas quatro indivíduos jovens do sexo masculino (23 a 30 anos) com DIGH devido a uma mutação inativadora do gene do receptor do hormônio liberador de GH (*GHRHR*) foram estudados do ponto de vista da DMO, mas não do ponto de vista articular (MAHESHWARI *et al*., 2003). Esta constatação nos motivou a proceder o estudo articular na

maior coorte de indivíduos vivos com DIGH congênita e vitalícia sem tratamento (Figura 3).

Figura 3: Dr. Manuel Aguiar-Oliveira e pacientes com DIGH de Itabaianinha.

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III. NORMAS DE PUBLICAÇÃO DO ARTIGO

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The Endocrine Society's mission is to advance excellence in endocrinology and be an integrative force in scientific research and medical practice. Such progress depends on integrity in the conduct of scientific research and truthful representation of findings. Specific guidelines regarding the Society's expectations for ethical conduct can be found in the Code of Ethics of The Endocrine Society and the Ethical Guidelines for Publications of Research.

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- **Original Articles** should be no longer than 3600 words and include no more than six figures and tables and 40 references. The Journal has a special interest in publishing results of major prospective randomized clinical trials which may be eligible for submission through Endocrine Trials Express, a pathway for expedited manuscript review that aims to provide an initial editorial decision within two weeks. Authors who wish to request consideration by **Endocrine Trials Express** should contact the Managing Editor by e-mail (sherman@endo-society.org) before submitting their paper.
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- **Images in Endocrinology** are to be comprised of a single figure or two closely related figures that illustrate the value of visual information in clinical diagnosis of endocrine and metabolic disorders, with a caption that is 50 words or less, an accompanying commentary that is 250 words or less, and five or fewer references.
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- Completed Disclosure Summary on the title page. For instructions on preparing the summary, see the following page (http://jcem.endojournals.org/site/author/RequiredForms.pdf).
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- All text should be double-spaced with 1-inch margins on both sides using 11-point type in Times Roman font.
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- All tables and figures must be placed after the text and must be labeled. Submitted papers must be complete, including the title page, abstract, figures, and tables. Papers submitted without all of these components will be placed on hold until the manuscript is complete.
- Authors are encouraged to cite primary literature rather than review articles in order to give credit to those who have done the original work.
- Any supplemental data intended for publication must meet the same criteria for originality as the data presented in the manuscript.

Title Page

The title page should include the following:

- Full title (a concise statement of the article's major contents).

- Authors' names and institutions. At least one person must be listed as an author; no group authorship without a responsible party is allowed. A group can be listed in the authorship line, but only on behalf of a person or persons. All group members not listed in the authorship line must be listed in the Acknowledgments.
- Abbreviated title of not more than 40 characters for page headings.
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- Disclosure summary (see Disclosure of Potential Conflict of Interest form for instructions).
- Clinical Trial Registration Number, if applicable.

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All Original Articles, Brief Reports, Clinical Reviews, Clinical Case Seminars, Consensus and Position Statements, Controversies in Endocrinology, and Extensive Clinical Experiences should be submitted with structured abstracts of no more than 250 words. All information reported in the abstract must appear in the manuscript. The abstract should not include references. Write the abstract with a general medical audience in mind. Please use complete sentences for all sections of the abstract. Detailed instructions on writing Structured Abstracts are at http://jcem.endojournals.org/site/misc/Structured_Abstracts.xhtml.

Introduction

The article should begin with a brief introductory statement that places the work to follow in historical perspective and explains its intent and significance.

Materials and Methods

These should be described and referenced in sufficient detail for other investigators to repeat the work. The source of hormones, unusual chemicals and reagents, and special pieces of apparatus should be stated. For modified methods, only the modifications need be described.

Results and Discussion

The Results section should briefly present the experimental data in text, tables, and/or figures. For details on preparation of tables and figures, see below. The Discussion should focus on the interpretation and significance of the findings with concise objective comments that describe their relation to other work in that area. The Discussion should not reiterate the Results.

Acknowledgments

The Acknowledgments section should include the names of those people who contributed to a study but did not meet the requirements for authorship. The corresponding author is responsible for informing each person listed in the acknowledgment section that they have been included and providing them with a description of their contribution so they know the activity for which they are considered responsible. Each person listed in the acknowledgments must give permission - in writing, if possible - for the use of his or her name. It is the responsibility of the corresponding author to collect this information.

References

References to the literature should be cited in numerical order (in parentheses) in the text and listed in the same numerical order at the end of the manuscript on a separate page or pages. The author is responsible for the accuracy of references. The number of references cited should be limited, as indicated above for each category of submission. Appropriate recent reviews should be cited whenever possible.

Examples of the reference style that should be used are given below. Further examples will be found in the articles describing the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (Ann Intern Med.1988; 108:258-265, Br Med J. 1988; 296:401-405).

The titles of journals should be abbreviated according to the style used in the **Index Medicus. Journal articles and abstracts:** List all authors. The citation of unpublished observations, of personal communications, and of manuscripts in preparation or submitted for publication is not permitted in the bibliography. Such citations should be inserted at appropriate places in the text, in parentheses and without serial number, or be presented in the footnotes. The citation of manuscripts accepted for publication but not yet in print is permitted in the bibliography provided the DOI (Digital Object Identifier) and the name of the journal in which they appear are supplied. Listing a manuscript as "in press" without a DOI and journal title is not permitted. If references to personal communications are made, authors

are encouraged to keep written proof of the exchange. If it is necessary to cite an abstract because it contains substantive data not published elsewhere, it must be designated at the end of the reference [e.g., 68:313 (Abstract)].

Books: List all authors or editors.

Sample References

1. **Binoux M, Hossenlopp P** 1986 Insulin-like growth factor (IGF) and IGF-binding proteins: comparison of human serum and lymph. J Clin Endocrinol Metab 67:509-514

2**. MacLaughlin DT, Cigarros F, Donahoe PK** 1988 Mechanism of action of Mullerian inhibiting substance. Program of the 70th Annual Meeting of The Endocrine Society, New Orleans, LA, 1988, p 19

3. **Bonneville F, Cattin F, Dietemann J-L** 1986 Computed tomography of the pituitary gland. Heidelberg: Springer-Verlag; 15-16

4. **Burrow GN** 1987 The thyroid: nodules and neoplasia. In: Felig P, Baxter JD, Broadus AE, Frohman LA, eds. Endocrinology and metabolism. 2nd ed. New York: McGraw-Hill; 473-507

For general aid in the preparation of manuscripts, authors should consult: CBE Style Manual: A Guide for Authors, Editors and Publishers. 5th ed. Bethesda, MD: Council of Biology Editors; 1983.

Tables

Tables must be constructed as simply as possible and be intelligible without reference to the text. Each table must have a concise heading. A description of experimental conditions may appear together with footnotes at the foot of the table. Tables must not simply duplicate the text or figures. The width of the table must be designed to occupy one or two journal columns, with no more than four table columns or 8-10 table columns, respectively.

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1 column = 18 picas, 7.5 cm, 3.0 in
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Shading: Avoid the use of shading, but if unavoidable, use a coarse rather than a fine screen setting (80-100 line screen is preferred). Avoid 1-20% and 70-99% shading; make differing shades vary by at least 20%, i.e., 25%, 45%, 65%. Instead of shading, denote variations in graphs or drawings by cross-hatching; solid black; or vertical, horizontal, or diagonal striping. Avoid the use of dots.

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Graphs: Graphs with axis measures containing very large or small numbers should convert to easily readable notations. **Example:** For an ordinate range of "counts per minute" values from 1,000 to 20,000, the true value may be multiplied by 10-3 (scale would read from 1 to 20) and the ordinate axis display "cpm ($x10^{-3}$)." Similarly, for a Scatchard plot with values ranging from 0.1 to 2 femtomolar (10^{-15} m), the scale may run from 0.1 to 2 with the abscissa labeled "m ($x10^{-15}$)." **Three-dimensional bar graphs will not be published if the information they refer to is only two-dimensional.**

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All nonstandard abbreviations in the text must be defined immediately after the first use of the abbreviation. The list of Standard Abbreviations is given in the link.

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Authorship Criteria

An author should have participated in either the conception, planning, or execution of the work, the interpretation of the results and the writing of the paper. An acknowledgment accompanying the paper is appropriate recognition for others who have contributed to a lesser extent, e.g., provision of clones, antisera or cell lines, or reading and reviewing manuscripts in draft. The signature of each author on the Affirmation of Originality and Copyright Release form that must be submitted with the manuscript indicates that all authors have had a part in the writing and final editing of the report, all have been given a copy of the manuscript, all have approved the final version of the manuscript, and all are prepared to take public responsibility for the work, sharing responsibility and accountability for the results. Medical writers can be legitimate contributors, and their roles, affiliations, and potential conflicts of interest should be described when submitting manuscripts. These writers should be acknowledged on the byline or in the Acknowledgments section in accord with the degree to which they contributed to the work reported in the manuscript. Failure to acknowledge these contributors would mean that the manuscript could have been "ghost-written," which is not allowed.

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The editors of The Endocrine Society's journals appreciate the importance of assuring unbiased authorship of editorials, reviews, and other non-research features involving selection of evidence to be discussed and perspectives to be presented. Consequently, special care is taken in choosing authors for such articles to assure their views are balanced and unencumbered, and that the Society's policies on disclosure of conflicts of interest are implemented.

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The critical and confidential review of manuscripts is an essential element of research publications. Every scientist has an obligation to contribute to the peer review serving as a reviewer. Among the obligations of reviewers is the commitment to providing an expert, critical, and constructive scientific and literary appraisal of research reports in their fields of knowledge, skills, and experience in a fair and unbiased manner. In order to facilitate the prompt sharing of scientific results, it is also the obligation of each reviewer to complete their assignments promptly, within the editor's deadline. Should a delay in their review occur, the reviewer has the obligation to notify the editor at once. Reviewers should not review a

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To ensure rigor in genetic and genome-wide association studies and permit readers to assess their biological and clinical significance, submitted manuscripts describing such work should generally conform to the following study design criteria, which will be applied by the Journal's reviewers and editors in their evaluations.

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Validation Samples: The most rigorous association studies should include both a testing (or training) sample set and an independent validation series.

Functional Data: Functional data strengthen association data if the functional assay(s) have demonstrable relevance to the associated phenotype. In some instances, association

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Negative Association Studies: Well-designed and executed association studies that demonstrate significant negative findings will be considered if the gene in question has clear relevance to disease pathogenesis or has been implicated in prior published association studies.

Microarray Expression Studies

Genome-wide expression studies require both technical validation and an independent validation series. Technical validation entails application of a different technique (e.g., RT-PCR of single genes or immunohistochemistry) to confirm the differential expression detected by genome-wide expression. An independent validation series of samples should be utilized to confirm the differential expression noted by genome-wide analysis of the initial testing sample set.

Nomenclature and Technical Requirements

The value of study data is enhanced if, where relevant, manuscripts:

- Use standard terminology for variants, providing rs numbers for all variants reported. These can be easily derived for novel variants uncovered by the study. Where rs numbers are provided, the details of the assay (primer sequences, PCR conditions, etc.) should be described very concisely.
- Describe measures taken to ensure genotyping accuracy, e.g., percentage of genotype calls, number of duplicate samples that were genotyped, and percentage concordance.
- Provide approved GDB/HUGO approved gene names, in the appropriate cases and italics.
- Provide linkage disequilibrium (LD) relationships between typed variants.

- Provide information and a discussion of departures from Hardy-Weinberg equilibrium (HWE). The calculation of HWE may help uncover genotyping errors and impact on downstream analytical methods that assume HWE.
- Provide raw genotype frequencies in addition to allele frequencies. It is also desirable to provide haplotype frequencies.
- Provide the criteria they have used to select tagSNPs.
- Denote the boundaries considered when studying SNPs within a gene of interest. For example, "gene X and 100 kb upstream of the first translational start site and 150 kb downstream of the stop codon."

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Manuscripts reporting amino acid or nucleotide sequences of proteins with sequences already known from other tissues or species will be considered only if they provide new biological insight. Manuscripts dealing with partial sequence data are not likely to be considered. The Endocrine Society has established policy that deals with submission of new protein or nucleic acid sequences. When a manuscript is accepted that contains novel sequences, such sequences must be deposited in the appropriate database (such as GenBank) and an accession number obtained before the manuscript is sent to the printer. It is recommended that the following statement containing the assigned accession number be inserted as a footnote: "These sequence data have been submitted to the DDBJ/EMBL/GenBank databases under accession number Ul2345."

Standards for Steroid Nomenclature

The 3 major classes of mammalian sex hormones - androgens, estrogens, and progestins (or progestagens or gestagens) - are defined by their biological activities, which are mediated via the well-defined androgen, estrogen and progesterone (or progestin) receptors. The principal bioactive sex steroid and natural ligand for each class is testosterone (or 5α-dihydrotestosterone), estradiol and progesterone, respectively. Androgen(s), estrogen(s) and progestin(s) are classes of compounds with hormonal activity, and not the names of individual steroids. Synthetic steroids or extracts can be considered as members of a generic steroid class (androgens, estrogens, progestins), but are distinct from the natural cognate ligand itself. Synthetic hormones or extracts of biological origin of each class may also have agonist, antagonist or mixed bioactivity in one or more classes. Therefore, the terms androgens,

estrogens and progestins (or progestagens or gestagens) should be used when referring to the class of hormones, whereas when a specific natural or synthetic steroid is being used or assayed, the particular compound must be specified.

Apart from accepted trivial names, steroids should be named according to the systematic nomenclature of the IUPAC convention on Nomenclature of Steroids (Moss et al Pure & Applied Chemistry 61:1783-1822, 1989) at first mention in a single footnote defining all letter abbreviations. Subsequently, generic or trivial names or letter abbreviations, but not trade-names, should be used.

Examples of accepted trivial names include: cholesterol, estrone, 17α and 17β estradiol (estradiol is also acceptably used as the trivial name for 17β estradiol), estriol, aldosterone, androsterone, etiocholanolone, dehydroepiandrosterone, testosterone, 5α dihydrotestosterone, 5β dihydrotestosterone, androstenedione, pregnenolone, progesterone, corticosterone, deoxycorticosterone, cortisone, and cortisol.

Trivial names may be modified by prefixes or suffixes indicating substituents (as in 17-hydroxyprogesterone for 17-hydroxy-4-pregnene-3, 20-dione), double bonds (as in 7-dehydrocholesterol for 5,7-cholestadien-3-ol) and epimeric configurations of functional groups provided the locus of epimerization is indicated (as in 11-epicortisol for 11α21-trihydroxypregn-4-en-3-one).

Manuscripts Reporting Novel Compounds

Manuscripts describing experiments with new compounds must provide their chemical structures. For known compounds, the source and/or literature reference to the chemical structure and characterization must be provided.

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Pulse analysis: Data from studies of pulsatile hormone secretion should be analyzed using a validated, objective pulse detection algorithm. The algorithm used should require that false-positive rates of pulse detection be defined in relation to the measurement error of the data set being analyzed, and the methods used to determine the measurement error should be described. The author(s) also should describe the methods used: 1) to deal with missing or undetectable values; 2) to determine peak frequency, interpeak interval, and pulse amplitude; and 3) for statistical comparisons of peak parameters.

Data analysis: It is the author's responsibility to document that the results are reproducible and that the differences found are not due to random variation. No absolute rules can be applied, but in general quantitative data should be from no fewer than three replicate experiments. Appropriate statistical methods should be used to test the significance of differences in results. The term "significant" should not be used unless statistical analysis was performed, and the probability value used to identify significance (e.g., $P > 0.05$) should be specified.

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In presenting results of linear regression analyses, it is desirable to show 95% confidence limits. When data points are fitted with lines (as in Scatchard or Lineweaver-Burk plots), the method used for fitting (graphical, least squares, computer program) should be

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[Please see paper of Rossner and Yamada (Journal of Cell Biology, 2004, 166:11-15), which was consulted in developing these policy issues, for additional discussion, and the CSE's White Paper on Promoting Integrity in Scientific Journal Publications, published by the Council of Science Editors, 2006.]

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IV. ARTIGO CIENTÍFICO

ISOLATED GH DEFICIENCY DUE TO A GHRH RECEPTOR MUTATION CAUSES HIP JOINT PROBLEMS AND GENU VALGUM, AND REDUCES SIZE BUT NOT DENSITY OF TRABECULAR AND MIXED BONE

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Key words: Isolated GH deficiency; osteoarthritis; bone density.

Word count: 2829

Disclosure statement: The authors have nothing to disclose.

ABSTRACT

Context: The GH/IGF-I axis is important for bone growth, but its effects on joint function are not completely understood. Adult onset GH deficient (GHD) individuals have often reduced bone mineral density (BMD). However, there are limited data on BMD in adult patients with untreated congenital isolated GHD (IGHD). We have shown that adult IGHD individuals from the Itabaianinha, homozygous for the c.57+1G>A *GHRHR* mutation, have reduced bone stiffness, but BMD and joint status in this cohort are unknown.

Objective: To study BMD, joint function, and osteoarthritis score in previously untreated IGHD adults harboring the c.57+1G > A *GHRHR* mutation.

Design: Cross-sectional.

Methods: Areal BMD by dual-energy X-ray absorptiometry was measured in 25 IGHD and 23 controls (CO). Volumetric BMD (vBMD) was calculated at the lumbar spine and total hip. Joint function was assessed by goniometry of elbow, hips and knees. X rays were used to measure the anatomic axis of knee and the severity of osteoarthritis, using a classification for osteophytes (OP) and joint space narrowing (JSN).

Results: Genu valgum was more prevalent in IGHD than CO. The osteoarthritis knees OP score was similar in both groups, and knees JSN score showed a trend to be higher in IGHD. The hips OP score, and JSN score were higher in IGHD. Areal BMD was lower in IGHD than CO, but vBMD was similar in the two groups. Range of motion was similar in elbow, knee and hip in IGHD and CO.

Conclusions: Untreated congenital IGHD due to a *GHRHR* mutation causes hip joint problems and genu valgum, without apparent clinical significance, reduces bone size, but does not reduce vBMD of the lumbar spine and hip.

INTRODUCTION

Growth hormone (GH) and its principal mediator IGF-I are crucial for bone growth and development due to complex actions on osteoblasts, osteoclasts and chondrocytes (1, 2). Reduction of activity of the GH/IGF-I axis can be related to osteoporosis (3, 4) and osteoarthritis, important causes of disability (5). Furthermore, there is a positive correlation between bone mineral density (BMD) and serum IGF-I in men with idiopathic osteoporosis and in postmenopausal women (6-9). Although the liver is the principal source of circulating IGF-I, bone contribution to the circulating IGF-I cannot be neglected. Moreover, it has been suggested that in idiopathic osteoporosis, low bone mass *per se* can cause low plasma IGF-I (8). The GH/IGF-I axis has also important effects in the formation and function of cartilages, being IGF-I critical for the matrix synthesis and chondrocytes growth (10-14). Indeed, acromegalic individuals have exuberant osteoarthritis, and pre-treatment serum IGF-I concentrations predict the degree of radiographic osteoarthritis (15). Conversely, there is evidence that chronic low IGF-I levels (16) or hyporesponsiveness of chondrocytes to stimulation by IGF-I may play a role in the pathophysiology of osteoarthritis (15).

While adult-onset GH deficient (AOGHD) individuals have reduced BMD, and increased risk of fractures (1, 2) childhood onset isolated GHD (IGHD) individuals have normal BMD (16). AOGHD individuals have often other pituitary deficits, with secondary gonadal, glucocorticoid, and thyroid hormone deficits, often with inadequate replacements. Hypogonadism and either the lack or the excess glucocorticoids and thyroid hormone replacement could impact BMD (17-21). Also, in one report AOGHD subjects were found to have low prevalence of radiographic osteoarthritis features (22). Therefore, it would be useful to have a model to study if untreated IGHD has consequence on BMD and joint status. One report described reduced areal BMD but normal volumetric BMD (vBMD) in 4 young men (23 to 30 yrs) with congenital untreated IGHD due to an inactivating GH releasing hormone receptor gene (*GHRH-R)* mutation (23). Similarly, in 11 adult with congenital GH insensitivity (Laron syndrome, LS), vBMD was reported to be normal (24), but early osteoarthritis and orthopedic problems (limitation of elbow extension and genu valgum) were observed (25).

We have described an extended kindred with nearly 100 individuals with congenital IGHD caused by the c.57+1G>A *GHRHR* mutation (26, 27). These individuals present severe short stature, increased fat mass percentage, and reduction in lean mass (28). We have previously shown that these subjects have decreased bone stiffness (29), as measured by quantitative heel ultrasound. However, an influence of bone size on the evaluation of skeletal properties by bone ultrasonography cannot be excluded. The purposes of this work were to study BMD by dual-energy X-ray absorptiometry, joint function and the occurrence of osteoarthritis in a group of IGHD adults of both genders.

SUBJECTS AND METHODS

Subjects

In a cross-sectional study, performed in Itabaianinha County, in the Northeastern Brazilian state of Sergipe, IGHD and control subjects were recruited by advertising placed in the local Dwarfs Association building and by word of mouth. Inclusion criteria for IGHD are individuals genotyped to be homozygous for the c.57+1G>A *GHRHR* mutation, whereas controls (CO) were normal statured individuals proven to be homozygous for the wild-type *GHRHR* allele. Exclusion criteria were: age less than 20 or more than 59 yr, known history of chronic disease (DM, rheumatologic disease) and use of glucocorticoids, anticonvulsants, GH and thyroid hormone. Twenty-five IGHD subjects and 23 CO were enrolled.

Both the Federal University of Sergipe and the Johns Hopkins University Institutional Review Boards approved these studies, and all subjects gave written informed consent.

Study protocol

Anthropometric measurement

The subjects' height and body weight were measured (with light clothes) using a wall mounted stadiometer, (Digital Wall Mounted Stadiometer, Model HM210D, Charder Medical Weighing and Measuring Products) and scale (Charder MS2510 Platform Scale, Taichung City, Taiwan). Height was measured, with the head at Frankfurt plane, by the same person (G.M.F.S.). As we include individuals of both genders, height, weight and body mass index (BMI) were converted to standard deviation scores (SDS) using the site http://www.phsim.man.ac.uk/SDSCalculator/SDSCalculator.aspx.

Bone mineral density (BMD) measurement

Bone mineral density was evaluated by dual-energy X-ray absorptiometry using the GE Lunar Prodigy apparatus (GE Healthcare, Madison, WI). BMD was measured at lumbar spine, femoral neck

and total hip, and expressed in absolute values (g/cm^2), and at Z-scores, as 78 % of all individuals are younger than 50 years old. The exams were performed by the same operator according to rigid positioning criteria standardized for each target site. Because areal BMD can be misleading when used to compare bones of different sizes, volumetric density of the lumbar spine (vBMDL14), total hip (vBMDTH) and total body (vBMDTB) were mathematically estimated. For vBMD we used L1-L4, instead of L2-L4 proposed by Carter et al at (30), and based on the current recommendation by the International Society for Clinical Densitometry. The following equations were used (23, 30, 31):

Lumbar spine: vBMDL14 (g/cm^3) = BMD (g/cm^2) of the lumbar spine (L1-L4) / square root of the bone area (cm^2) of the lumbar spine (L1-L4);

Total hip: vBMDTH (g/cm^3) = BMDTF (g/cm^2)/ square root of the bone area (cm^2) of the total femur;

Total body: vBMDTB (g/cm^3) = BMDTB (g/cm^2)/ height (m).

The coefficient of variation for the BMD measurements in normal subjects was 1% at both lumbar spine and femur neck.

Joint function

X rays were obtained in 23 IGHD and 19 controls to measure the anatomic axis of knee and the severity of osteoarthritis, defined by an adaptation of the Osteoarthritis Research Society International (ORSI) classification that accounts for joint space narrowing or osteophytes scores in the knees and hip (32). Joint status was assessed by goniometry of elbow and knees (measure of the amplitude from maximal extension to maximal flexion), and of the hips (measure of the amplitude of the external and internal rotation). All these physical maneuvers were done by a single observer (C.C.E-P). X-ray films were obtained in anterior-posterior (AP) and profile position of both knees, and AP supine of the pelvis. The anatomical axis of the lower limb was measured by radiography of the knee. Genu valgum was defined as the angle between the femur and tibia of 7 or more degrees.

Assessment of radiographic osteoarthritis

For the assessment of radiographic exams it was used an adaptation of a validated classification of osteoarthritis, recently used in acromegalic patients (33). The radiographs were graded on a scale of 0 to 3 for osteophytes (OP), defined by bony projections formed along joint margins, and joint space narrowing (JSN), defined by the loss of cartilage in the joints, for a semi-quantitative assessment of radiographic osteoarthritis. In the knees and hips, medial and lateral compartments were analyzed for JSN. In knees, medial and lateral osteophytes in femur and in tibia were analyzed. In hip, medial and lateral osteophytes in acetabulum, upper and lower femur were graded. The OP minimal and maximum individual total scores ranged from 0 to 24 for knees and from 0 to 18 for hips. The JSN minimal and maximum individual total scores ranged from 0 to 12 for knees and from 0 to 12 for hips. Total scores were calculated by adding left plus right scores in each site.

Statistical analysis

Values for continuous variables were expressed as mean ± standard deviation. Statistical analysis was performed by independent samples t test and Fisher's exact test by using the statistical software SPSS/PC 11.5 (Statistical Packet for Social Science, Inc., Chicago, IL). *P* values of 0.05 or less were considered to be statistically significant.

RESULTS

Table 1 shows the demographics of the two groups. There was no difference in age and gender distribution between the two groups. As expected, height and weight SDS were lower in IGHD than CO. BMI standard deviation scores were lower in IGHD.

Areal BMD (g/cm^2) in femoral neck was lower in IGHD than CO: 0.79 (0.14) vs. 1.01 (0.13), $p < 0.0001$. Table 2 shows the other areal bone mineral density (BMD) measurements, and the calculated volumetric BMD (vBMD). IGHD group had areal BMD at the lumbar spine and in total hip significantly lower than CO. However, vBMD at L1-L4 and total hip were similar in the two groups. Areal BMD in total body was lower, but vBMD of total body was higher in IGHD than CO.

Knee OP were found in 1 of 23 IGHD individuals, and 1 of 19 CO (NS), while hip OP were found in 15 of 23 IGHD individuals and in 6 of 19 CO ($p=0.003$). JSN of the knee was found in 6 of 23 IGHD individuals and in 1 of 19 CO ($p=0.025$), while JSN of the hip was found in 5 of 23 IGHD individuals and in none of 19 CO ($p=0.05$). Table 3 shows the data on genu valgum, osteoarthritis and goniometry. Genu valgum was more prevalent in IGHD than CO. 77% of the individuals with genu valgum had also osteophytosis in the hips. The osteoarthritis knees OP score was similar between groups, and knees JSN score had a non-significant trend to be higher in IGHD. The hips OP score and JSN score were higher in IGHD than CO. Range of motion was similar in elbow, knee and hip. Limitation of extension of the elbow was found in 1 IGHD individual and in none of controls.

DISCUSSION

It is generally accepted that GH and IGF-I have a positive long-term effect on bone apposition and bone density. Indeed, AOGHD individuals have invariably reduced BMD (16). These data are however collected mostly in hypopituitary patients, and it is difficult to tease out the influences of each pituitary axis deficit or replacement therapy. Subjects with IGHD or GH resistance represent an ideal model to determine the influence of the GH-IGF-I axis. In agreement with previous literature, we found here that adult individuals with congenital lifetime untreated IGHD, despite reduced areal BMD in the spine and femur, do not present reduction of calculated vBMD in lumbar spine and femur. Volumetric BMD of total body was actually higher in IGHD than controls. These data demonstrate that reduction in areal BMD reflects the lack of correction of bone size. Accordingly, these individuals do not seem to be prone to fractures. Animal and human studies in severe GHD or IGF-I deficiency suggest a deficit in periosteal bone formation (cortical bone) to some extent but preservation of cancellous bone **(**34). In our study, BMD was assessed in sites composed predominantly by trabecular bone (L1-L4), and mixed trabecular and cortical bone (total hip). BMD assessed by dual energy X-ray absorptiometry does not allow a specific analysis of each one of different bone components separately. However, a previous study of four IGHD individuals with a different *GHRHR* mutation showed reduced areal BMD in the distal third of the forearm (composed predominantly by cortical bone), while forearm vBMD was similar to vBMD of femoral neck, suggesting a similar consequence of GHD in these sites (23). Furthermore, one study in 11 adult patients with GH receptor deficiency reported normal cortical thickness, bone formation rate and trabecular bone volume, but poor trabecular connectivity (24).

The "mechanostat theory" proposes that bones adapt their strength to keep the strain caused by physiological loads close to a set point (35). This could explain why the bones of these IGHD individuals, despite being smaller, may be well adapted to their body size and to the much reduced muscle mass (28), thereby maintaining normal resistance to fractures.

We had previously shown in this cohort that GH therapy has important metabolic and body composition effects, increasing lean mass (36). Beneficial effects of GH treatment have been also

reported in other types of childhood-onset growth hormone deficiency (34), including increasing cortical thickness, probably leading to higher peak bone mass.

The finding of normal vBMD in congenital IGHD confirm the previous reports generated in a small group of young male individuals with IGHD due to another *GHRHR* mutation (23), and in GH resistant individuals (24) and extends other reports (16, 37). These data highlight the need for caution in the assessment of BMD in individuals with severe short stature without correction for bone size.

The reason for the discrepancy in BMD findings between AO and congenital IGHD is unclear. As previously mentioned, it may be due in part to lack of gonadal hormone or excessive replacement of glucocorticoid and thyroid hormones in panhypopituitary patients (1). It is worth noticing that in this population we have found normal free serum testosterone in males and estradiol in women (38). It is also possible that the lack of GH from birth trigger unknown compensatory mechanisms that cannot be activated in AOGHD. In addition to reduced BMD, several factors (absent in IGHD) can contribute to the high prevalence of fractures observed in AOGHD. Hypopituitary patients have frequently undergone pituitary surgery or radiotherapy, and may have neurological disabilities that together to reduction of muscle size and function may lead to frailty and falls. As adult onset GHD have attained normal adult height and bone dimensions, the loss of GH and IGF-I stimulus to muscles and bones may produce muscle weaken and cortical bone loss (22).

The present study probably includes the largest cohort of congenital naïve GHD individuals, with known etiology, and probably these findings represent the natural history of bone and joint disease in congenital childhood-onset GHD due to a *GHRHR* mutation. We found that IGHD individuals present increased osteoarthritis hips OP score and JSN score. We have utilized an adaptation of OARSI classification used in acromegalic patients (33), instead of the Kellgren and Lawrence criteria, frequently used in primary osteoarthritis, which uses a global score system (39). The reason of this choice was that in secondary osteoarthritis, dissociation may occur between OP and JSN. For instance, in active acromegaly the typical aspect is severe osteophytosis, with extremely wide joint spaces and JSN progression can be due to a degenerative osteoarthritic feature or to the normalization of hypertrophied cartilage after remission (33). Osteoarthritis is characterized by progressive degradation of articular cartilage and bone remodeling, resulting from an imbalance

between synthesis and breakdown of cartilage components, with the rate of loss of proteoglycans and other matrix components eventually exceeding their formation rate (3). Our main findings are increased osteophytosis in hips, and cartilage hypotrophy in hips and possible in the knee. This fits with an effect of GH on cartilage proliferation, as acromegaly patients show cartilage hypertrophy. The higher hip osteoarthritis in our IGHD cohort is in agreement with similar reports in GH resistant individuals (25), suggesting that deficiency of IGF-I can compromise the growth or repair of the cartilage (14-15). Our IGHD individuals have very low serum IGF-I levels, but the molar ratio of total IGF (IGF-I + IGF-II) bound to IGF binding protein type 3 (IGFBP3) is higher than in CO (27). We hypothesize that this increased IGF/IGF BP3 ratio can contribute to the increased hip osteophytes formation and osteoarthritis score. Interestingly, GHD rats, despite advanced cartilage lesions, do not exhibit osteophytes formation (40). In this species IGF-II disappear after birth (41), and IGF-I deficiency cannot be compensated by a residual but significant IGF-II secretion. When compared with acromegaly patients (33), the severity of osteophytosis in IGHD was lower in knees and hips, and JSN was lower in knees and higher in hips. Globally, in Acromegaly, osteoarthritis is more severe in knee whereas in IGHD, osteoarthritis seems to affect more frequently the hip. Although the statistical difference between IGHD and CO in OP and JSN scores in the hip is unquestionable, the clinical relevance of this osteoarthritis in IGHD individuals seems small, as these IGHD individuals apparently do not exhibit disabilities and limitations with work and sports activities.

Another interesting finding of our study is the high prevalence of genu valgum, which is in agreement with findings in GH-resistant individuals (25). It is well known that there is a correlation between presence of osteoarthritis or genu valgum and BMI. Obesity and reduced muscle mass were related to genu valgum in Laron syndrome (25), but the degree of obesity in our patients is lower than Laron patients (may be related to a minimal residual GH secretion and its lipolytic action). Indeed, our IGHD individuals have lower SDS BMI than the controls (42). Therefore BMI cannot be responsible for the high frequency of osteoarthritis and genu valgum.

Most individuals with genu valgum have osteophytes in the hips, suggesting a relationship in the two conditions. Genu valgum is a frequent finding in rickets, and GH therapy has been proposed for familial X-linked hypophosphatemic rickets (43). It is possible that the lack of stimulatory GH

effect on the production of 1, 25 dihydroxyvitamin D3 (44) may cause deficiency of the active form of vitamin D, causing the knee abnormality. Accordingly, also in thalassaemic patients, genu valgum seems to be associated to GHD (45) or impairment IGF-I production (46).

In conclusion, congenital untreated IGHD due to an inactivating *GHRHR* mutation causes hip joint problems and genu valgum, with low clinical significance, and reduces bone size but does not reduce vBMD of the lumbar spine and hip.

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TABLE.1: Demographics characteristics of the 25 Isolated Growth Hormone Deficiency (IGHD) individuals and 23 controls. Means (SD)

	IGHD	**CONTROLS**	**p**
Age (years)	38.16 (12.17)	39.21 (10.16)	0.747
Sex (Male)	13	9	0.382
Height (cm)	129 (10.73)	163 (9.55)	< 0.0001
SDS Height	-6.47 (1.52)	-0.95 (0.89)	< 0.0001
Weight (Kg)	38.86 (8.15)	68.28 (13.18)	< 0.0001
SDS Weight	-5.26 (3.05)	0.37 (1.03)	< 0.0001
BMI (Kg/m^2)	23.31 (5.29)	25.50 (4.30)	0.125
SDS BMI	-0.29 (2.00)	0.71 (1.10)	0.035

SDS: Standard Deviation Scores

TABLE. 2: Areal bone mineral density (BMD) measurements and calculated volumetric BMD (vBMD) in IGHD individuals and controls.

	IGHD	**CONTROLS**	**p**
BMD L1 (g/cm^2)	0.90 (0.12)	1.12 (0.14)	< 0.0001
BMD L2 (g/cm^2)	0.95 (0.13)	1.20 (0.13)	< 0.0001
BMD L3 (g/cm^2)	0.98 (0.14)	1.25 (0.11)	< 0.0001
BMD L4 (g/cm^2)	0.92 (0.15)	1.23 (0.11)	< 0.0001
BMD L1-4 (g/cm^2)	0.94 (0.13)	1.20 (0.11)	< 0.0001
Z SCORE L14	-0.86 (0.92)	0.25 (0.95)	0.0001
Area L1-4 (cm^2)	34.14 (6.22)	55.14 (7.12)	< 0.0001
vBMD L1-4 (g/cm^3)	0.16 (0.02)	0.16 (0.01)	0.894
BMDTH (g/cm^2)	0.91 (0.09)	1.04 (0.14)	< 0.0001
Z SCORE TH	-0.75 (0.55)	0.18 (0.87)	0.273
TH bone area (cm^2)	22.41 (3.04)	30.16 (3.88)	< 0.0001
vBMDTH (g/cm^3)	0.19 (0.01)	0.19 (0.02)	0.778
BMDTB (g/cm^2)	0.982 (0.005)	1.173 (0.09)	< 0.0001
vBMDTB (g/cm^3)	0.75 (0.03)	0.71 (0.05)	0.006

TH: Total hip; **TB:** Total body.

TABLE. 3: Presence of genu valgum, radiologic osteoarthritis scores (adapted ORSI classification) in knees and hips and goniometry results in IGHD individuals and controls.

		IGHD	CONTROLS	p
Genu Valgum		9/23	2/19	< 0.0001
Knees	**OP**	0.09 (0.41)	0.16 (0.68)	0.697
	JSN	0.57 (1.08)	0.11 (0.45)	0.074
Hips	**OP**	1.35 (1.11)	0.42 (0.69)	0.002
	JSN	0.57 (1.23)	0.00 (0.00)	0.039
Extension of right elbow		147.38 (4.79)	147.54 (3.92)	0.912
Extension of left elbow		147.38 (4.79)	147.62 (3.82)	0.869
Extension of right knee		143.96 (6.72)	143.15 (7.36)	0.747
Extension of left knee		143.96 (6.72)	143.15 (7.36)	0.747
Right hip internal rotation		38.46 (11.19)	42.85 (11.67)	0.279
Left hip internal rotation		50.71 (12.69)	48.62 (11.75)	0.620
Right hip external rotation		38.67 (11.66)	41.77 (10.06)	0.405
Left hip external rotation		49.08 (12.63)	50.85 (16.12)	0.736

OP: Osteophytes; **JSN:** Joint space narrowing.

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