Regulation of cardiac fatty acids metabolism in transgenic mice overexpressing bovine GH

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Abstract

Cardiac energy metabolism depends mainly on fatty acid (FA) oxidation; however, regulation of FA metabolism in acromegalic (Acro) heart is unknown. The aim of the study was to evaluate cardiac expression of key proteins of FA metabolism in young and elder transgenic mice overexpressing bovine GH Acro. Expression of proteins regulating FA entry into the cells, their uptake by mitochondria and β-oxidation were evaluated by western blot, while FA content by Fourier transformed infrared microspectrometry. Regulatory mechanisms of key steps of FA metabolism were also studied. The expression of plasma-membrane FA carriers (fatty acid-binding protein and fatty acid transport protein–1) and acylCoA synthetase was higher in young and lower in elder Acro than in corresponding controls; likewise, expression of cytoplasm to mitochondria-1 (CPT-1), the key enzyme of mitochondrial FA uptake, and that of medium-chain acyl-CoA dehydrogenase and long-chain acyl-CoA dehydrogenase, two regulatory β-oxidation dehydrogenases, followed a similar pattern. FA content was lower in young and higher in elder Acro than in wild-type, suggesting an increased utilisation in young animals. GH regulated expression of key proteins of FA metabolism through changes in peroxisome proliferator-activated receptor α (PPARα) expression, which varied accordingly. GH effect was confirmed by treatment of Acro mice with a receptor antagonist, which abolished changes in key proteins of FA metabolism in young Acro. GH increased phosphorylation of AMP-activated protein kinase and anti-acetyl-CoA-carboxylase, two regulatory kinases, leading to lower CPT-1 inhibition by malonyl-CoA, and intervened in regulating PPARα expression through the ERK 1/2 pathway. In conclusion, chronic GH excess increased FA metabolism in the young age, whereas its action was overwhelmed in elder ages likely by GH-independent mechanisms, leading to reduced expression of key enzyme of FA metabolism.

Journal of Endocrinology (2009) 201, 419–427

Introduction

Acromegaly (Acro) is a clinical syndrome due to GH–insulin-like growth factor (IGF)-1 excess sustained in most cases by a GH-secreting pituitary adenoma (Melmed 2006). Patients with Acro frequently have hypertrophic cardiomyopathy (Lie & Grossman 1980, Saccà et al. 1994, Colao et al. 2004, Melmed 2006, Bogazzi et al. 2008b), considered due to a direct effect of GH/IGF-1 excess (Fazio et al. 1994, Ciulla et al. 1999, Colao et al. 2004). Mice-overexpressing bovine (b) GH have many features of patients with Acro, including heart hypertrophy and impaired energetic metabolism (Bollano et al. 2000, Fu et al. 2000, Omerovic et al. 2000). However, young and elder acromegalic (Acro) mice differed as: i) apoptosis was reduced in the former and increased in the latter (Bogazzi et al. 2008a,b); ii) cardiac hypertrophy was evident, at histology, in transgenic mice aged 6 months but not in younger animals (Bollano et al. 2000, Fu et al. 2000, Bogazzi et al. 2008a,b); iii) hearts of elder Acro mice have reduced expression of adenosine translocase-1, a key mitochondrial protein involved in ATP generation (Bogazzi et al. 2007). Hence, the hearts of young and elder Acro mice might have different energy requirements.

Energy metabolism in normal hearts depends on ATP production from mitochondrial oxidation of glucose and fatty acids (FA; Taegtmeyer 1994, Stanley & Chandler 2002). FA oxidation is the main cardiac energy source accounting for up to 90% of myocardial ATP generation (Shipp et al. 1961). However, storage capability of FA in the heart is limited and FA uptake and oxidation need to be closely linked. The expression of genes encoding proteins for FA transport and mitochondrial β-oxidation is coordinately regulated assuring appropriate utilisation of FA (Kodde et al. 2007). Peroxisome proliferator-activated receptor α (PPARα) is considered the master regulator of FA metabolism either in physiological or pathological conditions (Desvergne et al. 2006). In fact, decrease in mitochondrial oxidative metabolism and increase in anaerobic glycolytic pathway (Sack et al. 1996, Finck 2007)
occur through decreased expression of PPARα and genes encoding proteins of FA transport and oxidation (Depre et al. 1998, Barger et al. 2000). Moreover, PPARα expression or activity is reduced in acquired cardiac disorders, including left ventricular hypertrophy (Finck 2007).

However, it is unknown whether FA metabolism might be different in the hearts of young and elder mice overexpressing bGH, and whether PPARα might drive changes in the expression of genes encoding key-regulating enzymes of FA metabolism.

The aim of the study was to evaluate the effect of chronic GH excess on FA metabolism in young and elder transgenic mice overexpressing bGH.

Materials and Methods

Study design

Transgenic mice overexpressing bGH (Acro) aged 3 (young) and 9 months (elder) were used as an animal model of Acro; transgenic animals overexpressing GH are widely used for studying the effects of excess GH/IGF-1 (for review, see Bartke et al. 1999, Kopchick et al. 1999). The expression of protein involved in FA uptake from the plasmatic membrane, their entry into the cytoplasm, the mitochondria uptake and key enzymes of β-oxidation in Acro mice were compared to that of wild-type (Wt) animals. Acro mice treated with a GH receptor antagonist were used to further confirm that GH/IGF-1 regulated expression of the above reported proteins. Animals aged 3 and 9 months were chosen as representative of young and elder animals as previously reported (Bollano et al. 2000, Bogazzi et al. 2008a,b).

Animals

Transgenic mice overexpressing a coding sequence of bovine GH gene under the control of metallothionein (MT) promoter in the C57Bl/6J×CBA genetic background were a generous gift of Dr Bohlooly (University of Goteborg, Sweden) and have been described (Bohlooly et al. 2001). C57Bl/6J×CBA mice were purchased from Harlan (Udine, Italy). The identity of transgenic mice was confirmed by PCR analysis of DNA from tail biopsy specimens using PCR primers located in the MT promoter and in the bGH gene, as reported (Fu et al. 2000). Only male animals were used for the experiments. Preliminary experiments were carried out with animals of the following age: 1, 3, 6, 9 and 12 months. Thereafter, we chose 3- and 9-month-old animals as representative of young and elder animals, as previously reported (Bogazzi et al. 2008a,b). The study groups included Wt, Acro and Acro mice treated with pegvisomant (Acro–PEG). Each group consisted of five animals of each age. The environment of the animal rooms was controlled with a 12 h light:12 h darkness cycle, a relative humidity of 45–55% and temperature of 20 °C. Animals had free access to tap water and standard pellet chow. All procedures on animals followed the recommendations reported in the Universities Federation for Animal Welfare (UFAW) handbook on the care and management of laboratory animals (UFAW at the Old School, Brewhouse Hill, UK). The study was approved by the local Board for animal’s experimentation at the University of Pisa.

Treatment

Some Acro animals (see animals) were treated with PEG (Pfizer, Rome, Italy), a specific antagonist of GH receptor, to further confirm that different expression of proteins was due to GH excess; PEG was administered at 0.1 mg/daily, subcutaneously for 15 days, as previously reported (Bogazzi et al. 2007). Effectiveness of PEG was evaluated by measuring serum IGF-1 concentrations at the baseline (before starting therapy) and at the end of treatment.

Serum IGF-1 measurement

Serum IGF-1 concentrations were measured using a commercial kit (Diagnostic System Laboratories, Webster, TE, USA). This is a RIA containing a specific goat anti-mouse/rat IGF-1 serum. Sensitivity was 21 ng/ml; intra-and inter-assay variations were 12 and 9% respectively.

Tissue samples

At killing time, hearts were rinsed with saline buffer. Left ventricles were dissected and immediately frozen in liquid nitrogen until further examination.
Histology

The hearts were fixed in 10% formalin, embedded in paraffin and then subjected to light-microscopic examinations. Serial 4 μm tissue sections were deparaffinised and stained with haematoxylin and eosin or reticulin. Fibre diameter was determined by calculating the mean of the shortest and longest diameters as reported (Lund & Tomanek 1978) using a binocular microscope connected to a computer and analysed with a morphometric software. Left ventricle’s hypertrophy was defined as increased cardiac fibre diameter and size as reported (Fu et al. 2000). For quantitative measurements, four transverse measurements were made along the length of the fibre and the cross sectional area was calculated. Two hundred fibres were counted for each sample.

Tissue extracts

Tissue extracts were obtained homogenising hearts with lysis buffer (150 mM NaCl, 10 mM Tris–HCl pH 7.4, 1 mM EGTA, 1 mM EDTA, 1% Triton-X 100 and protease inhibitor). After incubation on ice for 30 min and subsequent centrifugation, supernatants were stored at −80 °C. Protein concentration was measured by Bradford assay using the Bio-Rad reagent (Bio-Rad Laboratories).

Antibodies

Rabbit polyclonal anti-PPARα (s.c., 9000), anti-CPTI-M (s.c., 20670), p38(C-20) and extracellular signal-regulated kinase 3 (MAPK3, Erk1 K-23), goat polyclonal anti-A-fatty acid-binding protein (FABP) (s.c., 18661), anti-fatty acid transport protein (FATP)-1 (s.c., 14497) and anti-acylCoA synthetase (ACS) (s.c., 49008) were obtained from (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Rabbit polyclonal anti-medium-chain acyl-CoA dehydrogenase (MCAD) (cat. 101730) was obtained from Cayman Chemical (Ann Arbor, MI, USA). Rabbit polyclonal anti-AMP-activated protein kinase (AMPK)α (cat. 2532), anti-phospho-AMPKα (Thr172) (cat. 2531), anti-acetyl-CoA-carboxylase (ACC) (cat. 3662) and anti-phospho-ACC (Ser79) (cat. 3661) were obtained from (Cell Signaling Technology Inc., Beverly, MA, USA). Rabbit polyclonal anti-long-chain acyl-CoA dehydrogenase (LCAD) (cat. 59005-1-Ig) was obtained from (Acris Antibodies GmbH, Herford, Germany). Mouse monoclonal anti-α-sarcomeric actin was obtained from (Sigma–Aldrich). Goat anti-rabbit and chicken anti-goat IgG HRP-conjugated secondary antibodies were obtained from Bio-Rad Laboratories and used for revealing PPARα, MCAD, CPTI-M, AMPKα, pAMPKα, ACC, pACC, LCAD, p38 and ERK 1/2.

Figure 2 Expression of carriers for fatty acid entry into the cells. Fatty acids binding protein (FABP), fatty acids transport protein (FATP-1) and acyl-CoA synthetase (ACS) expressions were higher in young acromegalic (Acro) mice than in littermate controls (Wt). Elder Acro mice had lower FABP and FATP-1 degree than that of Wt, whereas expression of ACS did not change. Pegvisomant (PEG) treatment abolished GH-dependent variations of FABP, FATP-1 and ACS in young Acro mice but not in elder Acro. Data are expressed as percentage of optical density (OD). Results represent mean ± s.d. obtained in five animals for each group after correction for α-sarcomeric actin (α-actin).
respectively. Donkey anti-mouse IgG HRP-conjugated secondary antibody was obtained from Santa Cruz Biotechnology and used for revealing α-sarcomeric actin.

**Western blotting**

Proteins (50 μg) were resolved by 12% SDS–PAGE, transferred onto nitrocellulose membrane and stained with red Ponceau to verify the amount of proteins per lane. Transferred proteins were incubated overnight at 4 °C in 50% TBS (200 mM Tris–HCl, pH 7.6 and 1.4 M NaCl) and 50% TTBS (TBS, 0.05% Tween-20), containing 5% non-fat dry milk, and subsequently incubated with the appropriate primary antibody for 1 h at RT. After TTBS washing, an IgG HRP-conjugated secondary antibody was added for 1 h at RT; positive proteins were detected using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech). Intensity of bands of interest was quantified by densitometry (Interfocus GmbH, Sonnenblumenring, Mering, Germany). α-Sarcomeric actin was used as internal control as reported (Bogazzi et al. 2008a,b). Degree of PPARα, cytoplasm to mitochondria-I (CPT-I), MCAD, FABP, p38, ERK 1/2, AMPKα, phospho-AMPKα, ACC, phospho-ACC, LCAD, FATP-1 and ACS was expressed as percentage of optical density (OD) measured by densitometry and corrected by OD of α-sarcomeric actin.

**Fourier transform infrared microspectrometry**

Four-micrometer tissue slices were used for evaluating the presence of α, β unsaturated and aril-conjugated acids, saturated aliphatic acids, α, β unsaturated and aril esters and normal saturated esters in heart samples, as reported (Albuquerque et al. 2003). The Fourier transform infrared microspectrometry (FTIR-M) analysis was carried out as reported (D’Alessio et al. 2005, Bogazzi et al. 2007). An autoimage microscope connected with a Perkin–Elmer 2000 spectrophotometer was employed, and measurements were carried out using 15X Reflachromat lenses in the 4000–600/cm region with a 2/cm resolution with 100 scans signal on average.

**Statistics analysis**

Results were expressed as mean ± s.d.; comparison of parameters among the study groups was performed by Kruskal–Wallis analysis; comparison of parameters

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*Figure 3* Expression and regulation of CPT-1 in the heart of normal or acromegalic mice. Carnitine palmytoiltransferase-1 (CPT-1) expression was higher in young acromegalic (Acro) mice than in littermate controls (Wt), whereas elder Acro mice had lower CPT-1 degree. Pegvisomant (PEG) treatment abolished GH-dependent variations of CPT-1 in young Acro mice only. Regulation of CPT-1 is mediated by malonyl-CoA, exerting a negative feedback. The cellular level of malonyl-CoA is regulated by the phosphorylated (p) AMP-activated protein kinase (AMPK), which, through phosphorylation (p) of acetyl-CoA carboxylase (ACC), finally leads to lowering malonyl-CoA. Data are expressed as percentage of optical density (OD). Results represent mean ± s.d. obtained in five animals for each group after correction for α sarcomeric actin (α-actin).
between two study groups was performed by the Mann–Whitney U test. A P value <0.05 was considered statistically significant.

Results
The study was conducted for evaluating cardiac FA metabolism (expression of proteins regulating FA entry into the cells, their uptake by mitochondria and β-oxidation) in young and elder transgenic mice overexpressing bGH. Preliminary experiments were carried out using animals aged 1, 3, 6, 9 and 12 months; animals aged 3 and 9 months were chosen as representative of young and elder animals respectively, in keeping with previous reports (Bogazzi et al. 2008a,b).

As expected and in keeping with previous reports (Bollano et al. 2000, Bogazzi et al. 2007), mean body weight and heart weight of Acro were greater than that of Wt, and increased during life span (data not shown). Cardiac hypertrophy was revealed in elder Acro, at histology (Fig. 1), confirming previous data (Bollano et al. 2000, Fu et al. 2000). Mean serum IGF-1 concentrations were higher in Acro than in Wt at any age as previously reported (Bogazzi et al. 2008a,b); 297±49 ng/ml in Wt mice, 667±51 ng/ml in Acro mice and 290±64 ng/ml in Acropeg mice (P<0.05); and 291±59 ng/ml in Wt mice, 763±71 ng/ml in Acro mice and 304±67 ng/ml in Acropeg mice in 3- and 9-month-old animals respectively.

Uptake and activation of FA into the cells
The main transporters of FA entry into the cells are FABP and FATP-1. The expression of FABP was higher in Acro aged 3 months than in Wt; on the opposite, 9-month-old Acro had lower expression of FABP than littermate controls (Fig. 2); a GH receptor antagonist lowered FABP expression in 3-month-old Acro, whereas it was without effect in elder animals. Likewise, FATP-1 expression was higher in 3-month-old Acro than in Wt animals, and increased during life span. Acropeg mice had lower expression of FABP than Wt, and PEG was effective only in young Acro mice aged 9 months had lower CPT-1 expression than that of Wt; PEG was effective only in young Acro but not in elder animals (Fig. 3). Next, we evaluated the expression and phosphorylation (i.e. activity) degree of AMPK; from a physiological point of view, pAMPK phosphorylates ACC, which, in turn, regulates malonyl-CoA availability into the cells; finally, malonyl-CoA exerts an inhibitory effect on CPT-1 (hence, higher malonyl-CoA levels are associated with lower CPT-1 expression). Young Acro had higher phosphorylated levels of AMPK than those of Wt animals, which reduced after PEG treatment. Likewise, pACC increased in Acro aged 3 months. On the opposite, pAMPK and pACC expressions were lower in elder Acro than in Wt; in addition, PEG was without effect in animals of this age (Fig. 3).

Regulation of mitochondrial β-oxidation
Because key steps of FA mitochondrial β-oxidation cycle are regulated by dehydrogenases, we evaluated the expression of MCAD and LCAD. MCAD expression was higher in 3-month-old Acro than in Wt animals, and reduced in elder Acro when compared with littermate controls (Fig. 4); in addition, it showed a GH sensitivity only in young animals. Likewise, LCAD showed a superimposable pattern of expression to that of MCAD in young and elder animals (Fig. 4).

Entry of acyl groups into mitochondria
Carnitine palmitoyltransferase-1 catalyses the important step of FA flux to mitochondrial β-oxidation. Expression of CPT-1 was higher in young Acro than in littermate controls; on the contrary, Acro mice aged 9 months had lower CPT-1 expression than that of Wt; PEG was effective only in young Acro but not in elder animals (Fig. 3). Next, we evaluated the expression and phosphorylation (i.e. activity) degree of AMPK; from a physiological point of view, pAMPK phosphorylates ACC, which, in turn, regulates malonyl-CoA availability into the cells; finally, malonyl-CoA exerts an inhibitory effect on CPT-1 (hence, higher malonyl-CoA levels are associated with lower CPT-1 expression). Young Acro had higher phosphorylated levels of AMPK than those of Wt animals, which reduced after PEG treatment. Likewise, pACC increased in Acro aged 3 months. On the opposite, pAMPK and pACC expressions were lower in elder Acro than in Wt; in addition, PEG was without effect in animals of this age (Fig. 3).

Figure 4 Expression of key dehydrogenases of fatty acid β-oxidation. Medium-chain acyl-CoA dehydrogenase (MCAD) and long-chain acyl-CoA dehydrogenase (LCAD) expressions were higher in young acromegalic (Acro) mice than in littermate controls (Wt). Elder Acro mice had lower MCAD and LCAD degree. Pegvisomant (PEG) treatment abolished GH-dependent variations of MCAD and LCAD in young Acro mice only. Data are expressed as percentage of optical density (OD). Results represent mean ± S.D. obtained in five animals for each group after correction for α sarcomeric actin (α-actin).
Cardiac FA content

The above-mentioned changes in the expression of key proteins of FA metabolism may lead to different cardiac content in FA. Cardiac FA content was evaluated by FTIR-M. Overall, FA content was lower in the hearts of young Acro mice than that of the corresponding littermate controls (P<0.005; Fig. 5); in addition, Acro mice treated with PEG had cardiac FA content indistinguishable from that of Wt animals. On the contrary, elder Acro had higher cardiac FA content than that of corresponding Wt (Fig. 5), which was not modified by PEG treatment (Fig. 5).

Cardiac expression and regulation of PPARα

PPARα is considered the master regulator of FA metabolism in several organs, including heart; in fact, degree of expression of key proteins of FA metabolism is regulated by PPARα (Desvergne et al. 2006). Therefore, we evaluated the expression of PPARα in the hearts of young and elder Acro mice. PPARα degree of expression was higher in young Acro than in the corresponding controls (P<0.0001; Fig. 6). On the contrary, in elder Acro, the degree of PPARα expression was lower than that of littermate controls (Fig. 6; P<0.003). These changes were due to increased expression of PPARα in Wt and reduced degree in Acro. In addition, changes in cardiac PPARα expression in Acro were confirmed by treatment with a selective GH receptor competitor, which significantly counteracted GH effect. In young Acro, PEG restored PPARα expression to levels indistinguishable to those found in Wt (Fig. 6), whereas it was without effect in elder Acro. Variations of PPARα expression in hypertrophic hearts had been linked to p38 or ERK 1/2 activation (Barger et al. 2000, 2001). Hence, we evaluated whether changes in the expression of p38 or ERK 1/2, which are affected by GH (Yamauchi et al. 1997, Bogazzi et al. 2008a,b), were associated with PPARα variations. Expression of ERK 1/2 and p38 was lower in young Acro mice than in littermate controls (P<0.0001). These changes were abolished by treatment with a GH receptor antagonist (Fig. 6), on the contrary, p38 expression did not differ in Acro mice and in controls aged 9 months, whereas ERK 1/2 expression was higher in Acro than in controls (P<0.005). However, a GH receptor antagonist was without effect in elder Acro mice.

To summarise data, expression of PPARα and key proteins of FA metabolism were higher in young Acro than in corresponding littermate, whereas it was lower in elder Acro than in Wt.

Discussion

Acro cardiomyopathy is characterised by biventricular hypertrophy, impaired diastolic filling eventually leading to systolic dysfunction and heart failure (Fazio et al. 1994, Colao et al. 2004), which are common features of either patients or animal models (Bartke et al. 1999, Kopckick et al. 1999, Bollano et al. 2000, Fu et al. 2000, Colao et al. 2004, Melmed 2006). However, FA metabolism, which provides the substrate for the energetic demands of hearts, still remains largely unknown in Acro.

Our data showed changes in FA metabolism during lifespan of mice overexpressing bGH. GH excess was linked to increased expression of PPARα, which drove a coordinate increased expression of plasma membrane carriers for FA (FABP, FATP-1 and ACS), of proteins transferring acyl-CoA from CPT-I and dehydrogenases regulating β-oxidation of FA (MCAD and LCAD) in young Acro mice. The increased expression of key-regulatory proteins of FA oxidation led to the increased utilisation of FA in young Acro animals. This was further supported by the reduced cellular content of FA in young Acro mice when compared with littermate controls. GH specificity of this coordinate variation in the expression of PPARα and key enzymes of FA metabolism along with increased FA utilisation were demonstrated by its reversal by treatment with a specific GH receptor antagonist. However, it cannot be excluded that production of IGF1 might partially contribute to the effects of GH; in fact, Acropeg had serum IGF1 levels indistinguishable from those of Wt due to the competitive action of PEG on GH receptor. Thus, blocking of the GH receptor was associated with reduced GH action, including IGF1 production: the two aspects could not be separated in the present study. Several studies have shown that expression of genes encoding for the enzymes of mitochondrial FA β-oxidation is regulated at the transcriptional level in parallel with FA utilisation rates (Sack et al. 1996, Huss & Kelly 2004, Finck 2007). In fact, they are down-regulated in the hypertrophied heart that preferentially uses glucose and induced in the diabetic state, where hearts mainly utilise lipids owing to insulin resistance and high circulating FA levels (Sack et al. 1996, Depre et al. 1998, Finck et al. 2002).
Studies using gain- and loss-of-function mutations have shown that PPARα is the main regulatory key of FA metabolism in the heart, including FA uptake, thioesterification of acyl-CoA, transport into the mitochondria and β-oxidation. In fact, expression of MCAD, CPT-I and other genes of FA metabolism decreased in the hearts of PPARα knockout (K) mice (Djouadi et al. 1999, Watanabe et al. 2000). Likewise, FA uptake and utilisation were impaired in PPARα knockout hearts (Watanabe et al. 2000, Campbell et al. 2002).

In addition, cardiac-selective PPARα overexpression led to increased expression of genes regulating FA oxidation, whereas glucose utilisation was reduced (Finck et al. 2002). Overall, PPARα activation is involved in cardiac FA uptake and oxidation maintaining a tight lipid and energy balance. The results of the present study showed a link between the expression of PPARα, which increased in young and reduced in elder Acro, and that of key proteins of FA metabolism, which changed accordingly. However, mechanisms whereby PPARα is regulated are largely unknown; PPARα activity was increased by p38 mitogen-activated kinase (MAPK; Barger et al. 2001). On the contrary, ERK 1/2 activity was linked to increased PPARα phosphorylation (Barger et al. 2000) leading to reduced transcriptional activity. Our data are in keeping with a reduced inhibitory effect of ERK 1/2 on PPARα, as a matter of fact, ERK 1/2 was lower in young Acro and associated with reduced PPARα expression. On the contrary, elder Acro mice had increased GH-independent expression of ERK 1/2, which might have led to downregulation of PPARα expression. GH may regulate ERK 1/2 expression through the p44/42 kinase pathway (as reported Bogazzi et al. 2008a,b) by both JAK2-dependent and JAK2-independent mechanisms. The JAK2-dependent mechanism involved a cascade activation of Shc, Ras, Raf and the MAP kinase (MEK; Yamauchi et al. 1997); the JAK2-independent mechanism of ERK 1/2 activation might involve activation of Ral and phospholipase D (Zhu et al. 2002). It is worth noting that some key proteins regulating FA metabolism maintained GH sensitivity also in elder Acro mice, suggesting that GH continues its action and other factors (for example, triggered by hypertrophic hearts) superimpose on those regulated by GH (Fig. 7).

We recently reported (Bogazzi et al. 2007) that young mice overexpressing bGH had increased ATP production linked to GH and increased expression of adenine nucleotide

Figure 6 Expression and regulation of PPARα in the hearts of normal or acromegalic mice. Peroxisome proliferators-activated receptor (PPAR)α expression was higher in young acromegalic (Acro) mice than in littermate controls (Wt), whereas elder (9-month-old) Acro mice had a lower PPARα degree. Pegvisomant (PEG) treatment abolished GH-dependent variations of PPARα in young Acro mice only. Degree of expression of p38 was lower in young Acro than in Wt, whereas it did not change in elder animals. ERK 1/2 expression was lower in young Acro and higher in elder Acro than in corresponding Wt. Data are expressed as percentage of optical density (OD). Results represent mean ± s.d. obtained in five animals for each group after correction for α sarcomeric actin (α-actin).
transferase 1. The data reported in the present study fit well with those previously reported suggesting that young Acro animals have increased FA utilisation, likely driven by GH-induced PPARα expression leading to increased energy production. Thus, increased FA uptake and utilisation and energy production seem to be tightly and coordinately regulated in young Acro mice. It is worth noting that young Acro mice had no detectable hypertrophy at histology. On the contrary, elder Acro mice had cardiac hypertrophy, reduced expression of key oxidation enzymes and increased cellular content of FA associated with loss of GH sensitivity. In addition, GH intervened in the regulation of FA metabolism at the level of CPT-1, which is the key step for mitochondrial uptake of acetylCoA. In fact, young Acro mice had activation of pAMPK, likely leading to lower malonyl-CoA levels, however, malonyl-CoA could not be detected at FITR-M (data not shown) likely owing to its very low-tissue concentration.

In conclusion, our data suggest that GH excess was associated with increased or reduced FA metabolism in young and elder Acro animals respectively, through regulation of key-proteins of FA cellular uptake, esterification and oxidation.

**Declaration of interest**

There is no conflict of interest that would prejudice impartiality of reported data.

**Funding**

This work was partially supported by Grants from the University of Pisa (Fondi d’Ateneo) to F B, from Ministry of Education, University and Research (M I U R, Rome) to E M.
Acknowledgements

We thank Professor A Pinchera (University of Pisa) for his continued encouragement and advice.

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Received in final form 21 February 2009
Accepted 1 April 2009
Made available online as an Accepted Preprint 1 April 2009

Journal of Endocrinology (2009) 201, 419–427

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