Radionuclide angiocardiographic evaluation of the cardiovascular effects of recombinant human IGF-I in normal adults

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Abstract

Objective: IGF-I possesses specific myocardial receptors and is able to promote cardiac remodelling and even inotropic effects in both humans and other animals. In fact, reduced cardiac mass and performance are present in GH deficiency and these alterations are counteracted by recombinant human (rh) GH replacement, restoring IGF-I levels. Recently, the acute administration of 60 μg/kg rhIGF-I has also been reported to be able to improve cardiac performance evaluated by echocardiography or impedance cardiography in normal subjects. The aim of our study was to verify the effects of a subcutaneous low dose of rhIGF-I (20 μg/kg) on cardiac performance in humans.

Methods: In six healthy male adults (mean ± s.l.m.: 35.7 ± 4.3 years of age), the effects of rhIGF-I on left ventricular function evaluated by radionuclide angiocardiography and on circulating IGF-I, GH, insulin, glucose and catecholamines levels were studied.

Results: Administration of rhIGF-I increased circulating IGF-I (peak at +150 min vs baseline: 330.2 ± 9.6 vs 199.7 ± 8.7 μg/l, P < 0.03) to levels which persisted similarly up to +180 min. Neither GH nor catecholamine levels were modified by rhIGF-I administration, while insulin and glucose levels showed a slight but significant decrease. Basal left ventricular ejection fraction (61.8 ± 2.0%) significantly increased at +180 min after rhIGF-I (65.3 ± 2.7%, P < 0.03). No change was recorded in mean blood pressure while a non-significant trend towards a reduction of heart rate was present by +120 min.

Conclusions: These findings indicate that even subcutaneous administration of a low dose of rhIGF-I has acute inotropic effects as evaluated by radionuclide angiography in healthy adults.

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Introduction

Recent evidence indicates that the activity of the growth hormone (GH)/insulin-like growth factor (IGF-I) axis has important influences on myocardial function, which may explain cardiac abnormalities evidenced in GH hyper- and hyposecretory states in both humans and other animals (1).

IGF-I, as well as GH, possesses specific receptors at the myocardial level (1–3). IGF-I synthesis in and release from myocardial tissue have been demonstrated and they are probably involved in the auto/paracrine actions of the peptide (4–11). In addition to growth-promoting and metabolic actions, IGF-I has specific cardiovascular effects. It specifically stimulates heart, but not skeletal muscle growth, induces mRNA expression for specific contractile proteins, myocardial hypertrophy and contractility and has cardioprotective effects (11–21). In fact, reduced cardiac mass and performance are present in GH deficiency but are counteracted by a recombinant human (rh) GH replacement therapy restoring IGF-I levels (1, 22–26). More recently, in keeping with animal data (15, 16, 27, 28), acute intravenous infusion or subcutaneous rhIGF-I administration to healthy subjects has also been reported to be capable of improving cardiac performance evaluated by impedance cardiography or echocardiography respectively (29, 30). The intravenous infusion of 60 μg/kg IGF-I over 3 h induced overt systemic side-effects in addition to metabolic changes (29) while the same rhIGF-I dose injected subcutaneously did not induce side-effects or modify glucose but inhibited insulin levels as well as the GH response to physical exercise (30).

Based on the foregoing, the aim of our study was to evaluate the effects of a low rhIGF-I dose (20 μg/kg s.c.)
on cardiac performance in healthy adults using radio-
nuclide angiocardiography. Radionuclide imaging was
chosen because of its high reliability in evaluating
cardiac performances (31, 32).
The effects of rhIGF-I on circulating IGF-I, GH, insulin,
glucose and catecholamine levels were also studied.

Materials and methods

Study design and protocol

Six healthy male volunteers (mean ± s.i.m. : age,
35.7 ± 4.3 years; body mass index, 22.4 ± 0.5 kg/m²)
gave their informed consent to the study which had
been approved by the local, independent ethical
committee. No subject had any history of hypertension,
cardiovascular, renal, respiratory, hepatic or metabolic
diseases and none was taking medication. Physical
examination, blood pressure, electrocardiographic and
echocardiographic findings were also normal.

On the day of the study, subjects had breakfast
at 0800 h and were admitted to the study room 2 h
before the beginning of the testing session (1600 h).
The study room was maintained in constant conditions
of temperature and light, and in the absence of noise.

An antecubital vein was cannulated for blood
sampling and kept patent by slow infusion of isotonic
saline. ECG was continuously monitored in lead II.

Hormonal parameters: analytical methods

Serum IGF-I levels (µg/l) were measured in duplicate
by RIA (Nicholls Institute Diagnostics, San Juan
Capistrano, CA, USA) after acid–ethanol extraction to
avoid the interference of binding proteins. The sensitiv-
ity of the assay was 0.2 µg/l. The inter- and intra-assay
coefficients of variation were 8.8–10.8% and 5.0–9.5%
respectively. In our laboratory, the 3rd and 97th centile
limits of normal IGF-I in adulthood are 65 and 385
µg/l. The inter- and the intra-assay coefficients of
variation were 8.8–10.8% and 5.0–9.5% respectively.

Serum GH levels (µg/l) were measured in duplicate
by RIA (INSIK-5; Diasorin Biomedica, Saluggia,
Italy). The sensitivity of the assay was 0.15 µg/l. The
inter- and the intra-assay coefficients of variation were
2.9–4.5% and 2.4–4.0% respectively.

Serum insulin levels (µU/ml) were measured in duplicate
by RIA (INSIK-5; Diasorin Biomedica). The sensitivity
of the assay was 4.0 µU/ml. The inter- and intra-assay
coefficients of variation were 5.9–6.3% and 3.5–8.7%
respectively.

Plasma glucose levels (mg/dl) were measured by a
gluco-oxidase colorimetric method (Menarini Diagnos-
tics, Florence, Italy).

Plasma epinephrine (E) and norepinephrine (NE)
levels (ng/l) were assayed after extraction with alumina
using high-performance liquid chromatography with
electrochemical detection. The sensitivity of the assay
was 5.0 ng/l. The inter- and the intra-assay coefficients
of variation were 8.5% and 4% respectively for E, and 7%
and 3% respectively for NE.

Radionuclide imaging methods

Equilibrium radionuclide angiocardiography was per-
formed after in vitro labelling of red blood cells with
925 MBq (25 mCi) of 99mTc.

Subjects were imaged supine in the best septal
anterior oblique projection. Acquisition and processing
were made using a 400 T GE scintillation camera,
equipped with a low energy, all purpose parallel hole
collimator, using 24 frames/cycle, 64 × 64 matrices and
a × 1.6 zoom factor. Data processing was performed
using a standard, highly reproducible, validated semi-
automatic procedure, involving multiple regions of
interest and background subtraction.

Left ventricular ejection fraction (LVEF) was calcu-
lated from the left ventricular curve. Right ventricle
ejection fraction was estimated using a method of two
regions of interest. Absolute left ventricular end-systolic
volume and end-diastolic volume (LVEDV) were calcu-
lated using a validated non-geometric method (33).

Mean (MBP), systolic, and diastolic blood pressure
as well as heart rate (HR) were measured at each time-
point. Stroke volume (SV), cardiac output (CO) and
systemic vascular resistance (SVR) were derived from
the other measured parameters.

Eight-minute repeated acquisitions were made
basally and for the first 8 min of each 30-min period
from +90 to +180 min. For each acquisition, a
minimum of 800000 counts was acquired.

Statistical analysis

Results are expressed as mean ± s.i.m. Haemodynamic
parameters are expressed as absolute values or as
percent changes from baseline. Hormonal parameters
are expressed either as absolute values or as areas
under curves, calculated by trapezoidal integration.
Data were analyzed using ANOVA for repeated mea-
surement (Friedman two-way), followed by Wilcoxon
signed rank test where appropriate.

Results

Hormonal parameters

Administration of rhIGF-I increased circulating
IGF-I (peak at +150 min vs baseline: 330.2 ± 9.6 vs
199.7 ± 8.7 µg/l, P<0.03) to levels which remained similar up to +180 min (Fig. 1). Following s.c. rhIGF-I administration GH levels showed no significant changes (+150 min: 0.1 ± 0.1 vs baseline, 0.3 ± 0.1 µg/l) (Fig. 1).

Catecholamine levels were not modified by IGF-I administration (E. peak at +180 min: 55.3 ± 14.2 vs 47.8 ± 9.7 ng/l; NE, peak at +180 min: 248.2 ± 20.8 vs 236.8 ± 16.4 ng/l (Fig. 1 and Table 1). On the other hand, a slight but significant decrease in both glucose (nadir at +150 min: 67.3 ± 3.7 vs baseline: 84.3 ± 1.7 mg/dl, P<0.03) and insulin levels (nadir at +180 min: 5.3 ± 0.2 vs baseline: 12.1 ± 1.3 µU/ml, P<0.03) was observed after rhIGF-I administration.

Figure 1 Mean ± S.E.M. circulating levels of IGF-I, GH, glucose, insulin, epinephrine (E), and norepinephrine (NE) at baseline (bas) and after rhIGF-I administration in six normal male volunteers.
Table 1 Effects of rhIGF-I administration on haemodynamic and hormonal parameters in six normal male volunteers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>90 min</th>
<th>120 min</th>
<th>150 min</th>
<th>180 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVEF (%)</td>
<td>61.8 ± 2.9</td>
<td>63.5 ± 3.1</td>
<td>62.0 ± 3.1</td>
<td>64.3 ± 2.6</td>
<td>65.3 ± 2.7*</td>
</tr>
<tr>
<td>LVEDV (ml)</td>
<td>120.0 ± 6.8</td>
<td>115.8 ± 8.0</td>
<td>119.7 ± 8.2</td>
<td>121.7 ± 7.3</td>
<td>122.5 ± 6.8</td>
</tr>
<tr>
<td>SV (ml)</td>
<td>74.2 ± 5.5</td>
<td>73.5 ± 6.3</td>
<td>74.4 ± 6.6</td>
<td>77.8 ± 4.2</td>
<td>79.6 ± 4.3</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>5.0 ± 0.4</td>
<td>5.0 ± 0.6</td>
<td>4.6 ± 0.5</td>
<td>4.9 ± 0.4</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>90.2 ± 4.6</td>
<td>87.8 ± 3.9</td>
<td>91.1 ± 6.9</td>
<td>86.3 ± 4.3</td>
<td>87.6 ± 5.4</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>67.0 ± 2.3</td>
<td>68.0 ± 5.4</td>
<td>61.7 ± 4.0</td>
<td>63.0 ± 4.1</td>
<td>63.0 ± 4.0</td>
</tr>
<tr>
<td>SVR (dyn.s.cm⁻⁵)</td>
<td>1506.6 ± 145.9</td>
<td>1472.2 ± 138.8</td>
<td>1634.5 ± 120.5</td>
<td>1447.1 ± 108.0</td>
<td>1429.9 ± 113.5</td>
</tr>
<tr>
<td>E (ng/l)</td>
<td>47.8 ± 9.7</td>
<td>49.2 ± 9.3</td>
<td>55.3 ± 11.7</td>
<td>51.2 ± 10.3</td>
<td>55.3 ± 14.2</td>
</tr>
<tr>
<td>NE (ng/l)</td>
<td>236.8 ± 16.4</td>
<td>207.3 ± 25.5</td>
<td>241.3 ± 25.9</td>
<td>218.7 ± 34.3</td>
<td>248.2 ± 20.8</td>
</tr>
</tbody>
</table>

* P < 0.03 vs baseline.  
E, epinephrine; NE, norepinephrine.

Haemodynamic parameter

Basal LVEF was 61.8 ± 2.0%. Following rhIGF-I administration LVEF did not vary up to +120 min but then showed an increase which was significant at +180 min after rhIGF-I (65.3 ± 2.7%, P < 0.03 vs baseline). Individual LVEF responses to rhIGF-I are reported in Fig. 2. No change was observed in any of the other measured haemodynamic parameters, including LVEDV. A trend towards an increase in SV and a decrease in HR was apparent, resulting in no change of CO (Fig. 2 and Table 1). No change was recorded in systolic and diastolic blood pressure.

Side-effects

Transient pain in the injection site was recorded in all subjects after rhIGF-I administration. No clear general side-effects were recorded but, late after rhIGF-I administration, all subjects complained of fatigue and of not being well.

Discussion

The results of the present study show that the acute rhIGF-I administration enhances cardiac inotropism in normal humans even after s.c. injection of a low dose. The increase in circulating IGF-I levels that we found in the present study after the subcutaneous administration of 20 μg/kg rhIGF-I overlaps with that reported by other authors (1, 34–37) and it must be emphasized that it remained within the high normal range of basal IGF-I levels in adulthood (38). Thus, this suggests that we were investigating the effects of 'physiological' IGF-I doses.

In our study, rhIGF-I administration did not significantly inhibit spontaneous GH levels in spite of the well-known inhibitory action of IGF-I on somatotroph secretion (36, 39, 40). The lack of a significant inhibitory effect of rhIGF-I on somatotroph secretion in our study could be due to the fact that we did not perform frequent sampling and ultrasensitive GH assays (39).

In agreement with previous studies (29, 30, 34), insulin and glucose levels showed a reduction after rhIGF-I administration. On the other hand, after 20 μg/kg (present study) as well as after 60 μg/kg rhIGF-I s.c. (30) catecholamine levels did not show any significant variation while they have been shown to be increased by higher rhIGF-I doses leading to hypoglycaemia (34).

An inotropic effect of acute rhIGF-I administration had already been reported both in animals (15, 16, 27, 28) and in humans (29, 30). In fact, acute intravenous or subcutaneous rhIGF-I administration has been found to be capable of improving cardiac performance evaluated by impedance cardiography or echocardiography, respectively, in healthy adults (29, 30). The intravenous infusion of 60 μg/kg over 3 h induced systemic side-effects and, in addition, metabolic changes (29), while the same dose injected subcutaneously did not induce side-effects but still inhibited insulin levels and blunted the GH response to physical exercise (30).
Our present findings, although from a non-randomized study, show that acute rhIGF-I administration enhances cardiac inotropism in humans even after subcutaneous injection of a dose so low as to maintain circulating IGF-I levels within the normal range.

The validity of these findings is strengthened by the use of radionuclide angiocardiography, a technique which is much more reliable than echocardiography (31, 32), for measuring cardiac performances.

The increase in LVEF after rhIGF-I could be due to a direct cardiac effect or, alternatively, to a reflection of vasodilatation. Indeed, vasodilatory effects of rhIGF-I in human forearm muscles and kidney have been reported (41–45). However, in humans in vivo after 20 μg/kg (present study) as well as after 60 μg/kg rhIGF-I (29, 30), no significant change in SVR was found. Another explanation for increased LVEF could rely on an enhanced catecholamine secretion; again, no changes were found in epinephrine and norepinephrine levels by us or by others (30).

The hypothesis that IGF-I has a direct positive inotropic effect is supported by the evidence that it has an inotropic effect in neonatal rat cardiomyocytes (27) and in the isolated rat heart (28). It is therefore reasonable to speculate that the increase in ventricular isometric force development caused by IGF-I in man could be linked either to a sensitization of cardiac neurofilaments to Ca²⁺ without modifying high energy phosphate metabolism (46) or the enhancement of peak cytosolic Ca²⁺ concentration on cardiac myocytes (47).

In contrast to another study which was performed during physical exercise (30), we found that the increase in the LVEF was coupled with a non-significant increase in SV; no change was recorded in CO, likely due to the concomitant reduction of HR. In previous studies, an increase in HR occurred after intravenous IGF-I infusion but not after subcutaneous injection (29, 30).

Interestingly, in early stage acromegaly an increased cardiac contractility has been shown while GH-deficient adults have impaired cardiac performances (1–3). These findings could be explained in part by the stimulatory effects of GH on circulating and locally produced IGF-I (1–3, 14, 45, 48, 49) since no direct effect of GH has been found on cultured cardiomyocytes (12–14). Moreover, although acute inotropic effects of rhGH administration have been reported (50) in a study protocol which overlapped with the present one, we found no effect of acute intravenous rhGH on cardiac contractility in normal adults (51).

In conclusion, the present study shows that acute subcutaneous administration of a low rhIGF-I dose leading to an increase of circulating IGF-I levels within the high normal range induced a delayed increase in cardiac inotropism in healthy human subjects.

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