

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO			
RAD: 11-74834- -13	FECHA: 2014-04-29 14:50:49		
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 378 FASE NACIONAL INCLUIDO CAPITULO I		
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 7		
ACT: 519 PRESENTACION			

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
CARLOS REINALDO OLARTE GARCIA

Asunto: Radicación: 11-74834- -13
 Trámite: 11
 Evento: 378
 Actuación: 519
 Oficio: 5177
 Folios: 7

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/EP2009/067049				
Fecha de Presentación Internacional	14/12/2009				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Rad. 0000	N°11-74834-00000-	15/Junio/2011
Reivindicaciones:	Rad. 0000	N°11-74834-00000-	15/Junio/2011
	Rad. 0000	N°11-74834-00002-	23/Septiembre/2011
Dibujos:	Rad. 0000	N°11-74834-00000-	15/Junio/2011
Oposición: Laboratorio Synthesis			11/Marzo/2011
Prioridad: EP 08171712.6	Rad. 0000	N°11-74834-00000-	15/Diciembre/2008

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso se estudiará la totalidad del capítulo reivindicatorio (reivindicaciones 1-12).

Título de la solicitud: "FORMULACIÓN DE DEPÓSITO DE OCTREOTIDA CON NIVELES DE EXPOSICIÓN CONSTANTEMENTE ALTOS".

El problema planteado en esta solicitud consiste en la necesidad de tener composiciones de depósito para la terapia de mantenimiento a largo plazo en los pacientes acromegálicos, con diarrea grave asociada a tumores malignos intestinales que garantice un nivel de exposición constantemente alto del fármaco.

Para resolver este problema técnico la solicitud en estudio proporciona una formulación de depósito que comprende octreotida y polímeros lineales de poli-lactido-co-glicólido (PLGAs).

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): A61K 9/52.

4. De la solicitud de patente

Titulo

El título no corresponde con el objeto de la solicitud porque no se refiere a todos los componentes de la formulación reclamada, por lo cual se sugiere al solicitante el título: formulación de depósito de octreotida y polímeros lineales de poli-lactido-co-glicólido.

Reivindicaciones (Art 30 D 486, Art 22 PCT)

La reivindicación 1 no es clara ni concisa porque La definición de los "polímeros lineales de poli-lactido-co-glicólido" se realiza por la proporción molar L: G, lo cual es confuso e impreciso, teniendo en cuenta que la definición enseñada en el capítulo descriptivo se refiere a la proporción de comonomeros de lactido: glicólido (L: G). Adicionalmente, el capítulo descriptivo enseña que el polímero de poli-lactido-co-glicólido se obtiene por la mezcla de dos ingredientes que no son definidos.

La definición de los "polímeros lineales de poli-lactido-co-glicólido" se realiza por su viscosidad y definir un ingrediente por un parámetro de este no lo precisa inequívocamente. Esta observación también se realiza sobre las reivindicaciones 2, 6 y 7.

Por tal motivo se requiere al solicitante delimitar el alcance de la reivindicación teniendo en cuenta que la evidencia técnica que se proporciona el capítulo descriptivo se encuentra relacionada cuando el derivado de gliceril diestearato de polietilenglicol.

La reivindicación 3 no es concisa por cuanto el término "diferentes grupo terminales" es genérico y configura ilimitadas opciones que no encuentran soporte en el capítulo descriptivo. Por tal motivo se requiere al solicitante delimitar el alcance de su

reivindicación teniendo en cuenta que la evidencia técnica que se proporciona el capítulo descriptivo se encuentra relacionada a polímeros que tiene como grupo terminal éster y ácido carboxílico y no se podría extrapolar el comportamiento de dos grupos terminales a todas las ilimitadas alternativas que se reclaman en la reivindicación 3.

La reivindicación 10 no es concisa por cuanto el término "agente contra la aglomeración" es genérico y configura ilimitadas opciones que no encuentran soporte en el capítulo descriptivo. Por tal motivo se requiere al solicitante delimitar el alcance de su reivindicación teniendo en cuenta que la evidencia técnica que se proporciona el capítulo descriptivo se encuentra relacionada a que este agente antiaglomeración corresponde a manitol y no se podría extrapolar el comportamiento de un ingrediente a todas las ilimitadas alternativas que se reclaman en la reivindicación 10.

La reivindicación 11 no es clara porque siendo una reivindicación de producto, se refiere a una característica de proceso (esterilización mediante irradiación gamma). Se recuerda al solicitante que sólo pueden ser definido en términos de su proceso de fabricación si: el producto es nuevo e inventivo y no se puede definir por sus características estructurales, lo cual no es el presente caso. Se sugiere definirlo por sus características esenciales, estructurales o funcionales.

La reivindicación 12 enseña un kit que comprende la composición de las reivindicaciones 1 a 11 en un frasco, junto con un vehículo a base de agua en una ampolla, frasco o jeringa previamente llenada, o como micropartículas y el vehículo separado en una jeringa de doble cámara, sin embargo esta reivindicación no cumple con lo requerido para una solicitud mediante patente de un kit-de-partes ya que esta es elegible para estudio de patentabilidad *"... cuando el centro de la invención es una combinación nueva e inventiva de dos o más compuestos conocidos que provienen de preparaciones independientes para una finalidad terapéutica específica, en la que el producto será comercializado en forma de kit-de-partes"*, adicionalmente se debe tener en cuenta que *"un kit-de-partes es elegible para estudio de patentabilidad cuando se encuentre caracterizado por: Una preparación farmacéutica o medicamento A en una cantidad o proporción definida y Una preparación farmacéutica o medicamento B en una cantidad o proporción definido"*. De acuerdo con lo anterior esta reivindicación no tienen soporte en la descripción.

Descripción (Art 28 D 486)

La descripción no es clara porque la información que contiene no permite que la persona del oficio normalmente versada en la materia pueda comprender la naturaleza del polímero de poli-lactido-co-glicólido por la mezcla de dos ingredientes. Adicionalmente estos dos ingredientes que se mezclan para obtener el polímero no son definidos inequívocamente.

Se requiere al solicitante hacer las aclaraciones necesarias.

5. Análisis de la Oposición

Argumentaciones de Synthesis

Dice el opositor que esta solicitud pretende obtener patente para el uso y no solo a la composición farmacológica o método de fabricación mediante la cual se obtenga un producto tangible, lo cual no sería materia patentable a la luz del artículo 14 de la Decisión 486.

Argumenta el opositor que según la enseñanza del documento WO2005046645, publicado el 26 de mayo de 2005, la presente solicitud no es patentable por cuanto el documento describe macropartículas que comprenden un análogo de la somastatina incrustado en una matriz de polímero biocompatible (que puede comprender una

mezcla de polímeros que comprende dos polímeros lineales de poli-lactido-glicólido) farmacológicamente aceptable para una acción de liberación prolongada y composiciones farmacéuticas que comprenden tales macropartículas, y por lo cual la presente solicitud carece de altura inventiva, teniendo en cuenta que la única diferencia consiste en que el derivado de somastatina es específicamente la octreotida.

Respuestas del Solicitante

La solicitante no presenta respuesta a la oposición presentada por la sociedad Synthesis S.A.S

Respuesta del Examinador Técnico

Teniendo en cuenta el capítulo reivindicatorio modificado presentado por la solicitante el 23 de septiembre de 2011 y visible en los folios 47 y 48, la materia que pretende protección es patentable a la luz de la Decisión 486, por cuanto está dirigida a productos tangibles.

Por otra parte, este Despacho reconoce que, tal como lo argumenta el opositor, el documento WO2005046645 presentado por la oposición enseña características técnicas esenciales de la invención, tales como la utilización de un análogo de somastatina en una mezcla de polímeros que comprende dos polímeros lineales de poli-lactido-glicólido con el objetivo de tener una formulación de liberación prolongada, por lo cual dicho documento desvirtúa el nivel inventivo del objeto de esta solicitud y se considerará dentro del examen de patentabilidad. De manera que la oposición es fundada.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: diciembre 15 de 2008, y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	WO2005046645	microparticles comprising somatostatin analogues	26/mayo/2005
D2	US5639480	Sustained release formulations of wáter soluble peptides	17 de Junio de 1997

D1¹ divulga micropartículas de análogos de somastatina incrustados en un matriz polimérica biocompatible farmacológicamente aceptable con liberación prolongada y composiciones que comprenden las micropartículas (resumen).

D2² divulga una formulación de liberación sostenida que comprende una sal de octreotida en un polímero biodegradable encontrándose que la sal de pamoato es muy estable en este tipo de formulaciones (col. 4, ln 29-35) para el tratamiento de la acromegalia (col. 7, ln 32).

7. Examen de Patentabilidad (Art 14 D486)

Nivel inventivo (Art18 D486)

La reivindicación 1 consiste en una formulación de depósito, la cual comprende como ingrediente activo octreotida o una sal farmacéuticamente aceptable de la misma y dos

1

URL:[http://worldwide.espacenet.com/publicationDetails/biblio?](http://worldwide.espacenet.com/publicationDetails/biblio?DB=worldwide.espacenet.com&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20050526&CC=WO&NR=2005046645A1&KC=A1)

DB=worldwide.espacenet.com&II=0&ND=3&adjacent=true&locale=en_EP&FT=D&date=20050526&CC=WO
&NR=2005046645A1&KC=A1

2[http://worldwide.espacenet.com/publicationDetails/originalDocument?](http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=5639480A&KC=A&FT=D&ND=3&date=19970617&DB=EPODOC&locale=en_EP)

CC=US&NR=5639480A&KC=A&FT=D&ND=3&date=19970617&DB=EPODOC&locale=en_EP

polímeros lineales de poli-láctido-co-glicólido (PLGAs) que tiene una proporción molar de L:G de 75:25 y diferentes viscosidades, según Rad. N°11-74834-00002-0000.

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1	D2
Formulación de depósito de octreotida o una sal farmacéuticamente aceptable	Formulación de depósito de un análogo de somastatina o sus sales farmacéuticamente aceptables (Pág. 1, Parr.1; Pág. 5, Párr. 2 y 4)	formulación de liberación sostenida que comprende la sal pamoato de octreotida
Dos polímeros lineales de poliláctido-co-glicólido (PLGAs) que tiene una proporción molar de L:G de 75:25 y diferentes viscosidades	Polímeros lineales de poliláctido-co-glicólido (PLGAs) que tiene una proporción molar de L:G de 75:25. (Pág. 7, Párr. 1 a 3)	un polímero biodegradable poliláctido-co-glicólido (PLGAs) que tiene una proporción molar de L:G de 50:50,55:45.

Los documentos D1 y D2 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque el documento D1 divulga una formulación de administración parenteral de depósito de un análogo de somastatina o sus sales farmacéuticamente aceptables (Pág. 1, Parr.1; Pág. 5, Párr. 2 y 4, ej. 12), que comprende polímeros lineales de poli-láctido-co-glicólido (PLGAs) que tiene una proporción molar de L: G de 75:25. (Pág. 7, Párr. 1 a 3; Pág. 8, Párr. 3) en forma de micropartículas de 1 a 200 micras (Pág. 12, Párr. 6) que comprenden agentes antiaglomeración como el manitol (Pág. 13, Párr. 3 y 4).

La diferencia entre la invención, definida en la reivindicación 1 y la formulación enseñada en el documento D1 consiste en que se incluye específicamente el análogo de somastatina como octreotida.

No hay evidencia de que la inclusión de octreotida presente un efecto técnico sorprendente.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: "cómo modificar la composición de D1 como una alternativa a la ya conocida también de liberación prolongada?".

Sin embargo, la utilización de la octreotida como un análogo de la somastatina está divulgada en el documento D2 que divulga una formulación de liberación sostenida que comprende una sal de octreotida en un polímero biodegradable encontrándose que la sal de pamoato es muy estable en este tipo de formulaciones (col. 4, ln 29-35) para el tratamiento de la acromegalia (col. 7, ln 32), como también ya se conocía que la octreotida es el análogo de somastatina más frecuentemente utilizado en el tratamiento de la acromegalia (Pág. 551, Col. 2, renglones 2 a 4, referencia 3³).

En consecuencia, una persona del oficio normalmente versada en la materia tendría una razonable expectativa de éxito de obtener una composición alternativa de octreotida de liberación prolongada, al combinar la composición enseñada por el documento D1 y la posibilidad de utilizar la octreotida como análogo de la somastatina, según lo enseñado por el documento D2, llegando así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia.

Las reivindicaciones dependientes 2 a 12 no mencionan alguna característica que haga inventiva a la composición de la reivindicación 1, por lo cual se considera que tampoco son inventivas.

8. Conclusión

3 LDL physical properties, lipoprotein and Lp(a) levels in acromegalic patients. Effects of octreotide therapy M. Arosio a,*, G. Sartore b, C.M. Rossi c, G. Casati a, G. Faglia a, E. Manzato b, Italian Multicenter Octreotide Study Group. 151 (2000) 551- 557 Adjunto al concepto

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Item	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO2005046645	1 – 12	Nivel inventivo
D2	US5639480	1 – 12	Nivel inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-05-06

Elaboró: Lyna Del Carmen Mordecay Dunoyer
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LDL physical properties, lipoprotein and Lp(a) levels in acromegalic patients. Effects of octreotide therapy

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Received 11 March 1999; received in revised form 17 September 1999; accepted 4 October 1999

Abstract

High vascular morbidity and mortality is associated with acromegaly. The aim of the present study was to assess the effects of octreotide therapy on several known cardiovascular risk factors and to correlate them with octreotide-induced hormonal changes. Lipid levels, LDL particle size distribution as evaluated by single vertical spin density gradient ultracentrifugation, apolipoproteins AI and B, lipoprotein (a) [Lp(a)] concentrations and apo(a) phenotypes were evaluated in 20 non-diabetic acromegalic patients (6 M, 14 F), with normal thyroid, adrenal and gonadal function, aged 29–66 years. Normal subjects (20), matched for age, sex and BMI served as control for lipid variables. Acromegalic patients were characterized by lower HDL cholesterol (and apoA-I) and by higher Lp(a) concentrations in comparison to controls. Treatment with octreotide (100 µg t.i.d. for 3 months) led to: an increase in HDL cholesterol (median: +22%), a decrease in LDL cholesterol (–14%) and a decrease of the Lp(a) levels (all phenotypes) (–28%). The expected decreases of IGF-I levels (median: –48%) and 7-h AUC of GH (–50%), insulin (–40%), and glucagon (–20%) were observed. Only Lp(a) modifications showed a correlation with GH modifications. The study of LDL physical properties showed that acromegalic patients had smaller and/or more dense LDL particles, in comparison with normal controls (relative flotation rate, Rf: 0.40 ± 0.03 versus 0.42 ± 0.02 $P < 0.05$), an alteration that might contribute to the high vascular risk of acromegalic patients. However, the LDL subfraction distribution remained unmodified during octreotide therapy (Rf 0.39 ± 0.03). In conclusion, this study shows that in acromegalic patients octreotide treatment is indeed associated with an amelioration of some lipoprotein parameters, i.e. LDL, HDL, and Lp(a) concentrations. However, this treatment has no effect on the small and/or dense LDL particles present in these patients. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Acromegaly; Octreotide; Somatostatin-analogs; Lipoproteins; Small dense low density lipoprotein; Lipoprotein(a); Apo(a)isoform

1. Introduction

Acromegaly is a rare and serious disease associated with an increased cardiovascular mortality [1,2]. For this reason the effects on the cardiovascular risk factors

induced by the medical therapy of this disease must be thoroughly evaluated. Octreotide is the most widely investigated and most frequently used somatostatin analogue in the treatment of acromegaly.

Our previous experience, involving a large number of acromegalic patients from all over Italy, seemed to indicate that octreotide, besides decreasing GH and IGF-I concentrations, had also beneficial effects on lipid levels [3]. In fact, a significant decrease of both total and LDL cholesterol was observed after 3 months of octreotide treatment. However, besides LDL cholesterol concentrations, it is now well recognized that the LDL physical characteristics are important, a predominance of small, dense LDL particles being associated

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with increased vascular risk [4,5]. Recent data suggest that GH excess has a direct effect on LDL subfraction distribution [6]; however, no data exist about the possible effects of therapy, and in particular of octreotide, on LDL size and density.

Increased circulating concentrations of lipoprotein(a) [Lp(a)] may represent an independent risk factor for cardiovascular disease in acromegaly. In fact, although the mechanism by which Lp(a) influences cardiovascular morbidity is largely unknown, several epidemiological studies have shown an association between coronary heart disease incidence and Lp(a) levels above a threshold concentration of 20–30 mg/dl [7–9]. Lp(a) shows considerable size variation (from ≈ 300 to ≈ 800 KDa) among individuals owing to different numbers of cysteine-rich sequences (homologous to plasminogen kringle 4) in the apolipoprotein(a) [apo(a)], resulting in different apo(a) phenotypes. The serum concentrations of Lp(a) are inversely related to its size, which is genetically determined. Although it is widely accepted that GH has important effects in increasing Lp(a) concentrations [10,11], it is not known if all apo(a) phenotypes are equally sensitive to GH modifications.

The aim of the present study was to assess the effects of octreotide therapy on LDL physical properties, and on lipoprotein and Lp(a) levels (in relation also to the apo(a) phenotype) and to correlate them with hormonal and metabolic changes induced by the therapy. A secondary objective was to verify if acromegaly, non-complicated by diabetes, is associated with modifications of these lipid parameters.

2. Materials and methods

2.1. Design

Open, multicentric prospective study. The study was approved by the local ethics committees and written informed consent was obtained from all patients.

2.2. Subjects

Patients (20) with active acromegaly (as shown by elevated serum IGF-I levels and serum GH not suppressible by glucose load) (14 women, six men), aged 29–66 years (mean $\pm$ SD = 46 ± 10 years), were recruited. Ten patients had been previously submitted to unsuccessful adenomectomy, followed in one case by conventional pituitary radiotherapy and in seven cases by medical therapy with bromocriptine (three patients) and/or somatostatin analogs (seven patients) that was discontinued 1 or more months prior to the start of this study.

Only patients with normal thyroid, adrenal and gonadal functions were admitted to the study, and none of the patients was taking drugs known to influence lipid metabolism, including estrogen-containing drugs or hypoglycaemic agents. Patients with known diabetes mellitus were excluded, and all patients had fasting glucose levels below 110 mg/dl and normal glycated hemoglobin levels. When considering glucose response to OGTT, however, four patients showed an impaired glucose tolerance according to the criteria of the Expert Committee of the American Diabetes Association [12].

A group of 20 subjects, matched for age, sex and BMI (24 ± 2 Kg/m²) served as control for lipid variables.

2.3. Design of the study

Clinical and biochemical evaluations were performed at baseline and after 3 months of therapy with octreotide (Longastatina®, Italfarmaco S.p.A.), 100 μ g t.i.d. sc under controlled dietary conditions. Pre-treatment studies included the following tests: a 4-h OGTT (75 g orally, to confirm active acromegaly) and a 7-h saline infusion, with a light, standardized meal of about 600 kcal given at 4 h, for GH, insulin, glucagon and glucose assay. IGF-I and lipid parameter measurements were done on two basal samples taken on 2 different days. After 3 months of therapy, the same 7-h study giving octreotide 100 μ g sc at time 0 and, on a different day, OGTT, giving glucose 2-h after octreotide sc injection, were carried out. Additionally, serum levels of TSH, free T3, free T4, and, in the women, estrogen and progesterone, together with routine laboratory biochemical analysis, were also evaluated.

2.4. Analytical methods

All lipid assays were performed at the lipoprotein laboratory in the Department of Internal Medicine, University of Padova.

Cholesterol [13], HDL cholesterol [14], and triglycerides [15] were measured by standard methods. The coefficient of variation (CV), intra-assay and inter-assay, for total cholesterol were 1.0 and 1.2%, respectively, for triglycerides 1.5% and 2.5%, for HDL cholesterol 1.2% and 1.5%. LDL cholesterol levels were calculated using the equation of Friedewald [16].

LDL distribution and flotation properties were evaluated by single vertical spin density gradient ultracentrifugation (SVS-DGUC) using a modification of the method described by Chung et al. [17,18]. One aliquot of 1 ml of plasma adjusted to a density of 1.080 g/ml (final volume 4 ml), was layered underneath 9 ml of a 1.006 g/ml NaCl solution, producing a discontinuous salt gradient in a Beckman VTi 65.1 vertical rotor (Beckman Instruments, Inc., Palo Alto, CA, USA).

Samples were centrifuged at 65 000 rpm for 70 min (total $t_2 = 2.36 \times 1011$) at 5°C; the tubes were then fractionated (Fraction Recovery System by Beckman Instruments, Palo Alto, CA, USA) from the bottom at a flow rate of 1.7 ml/min, and 37 0.35-ml fractions were collected. Total cholesterol was measured in each fraction. The relative flotation rate (Rf), which characterizes LDL peak buoyancy, was obtained by dividing the fraction number containing the LDL-cholesterol peak by the total number of fractions collected. The CV for LDL Rf was 1.1% intra-assay and 1.7% inter-assay.

Apolipoproteins AI and B (apo-AI and apo-B) were measured by radial immunodiffusion using standards and plates from Daiichi Pure Chemicals Company. The CV for apo-AI and apo B were 2.4% and 1.9%, respectively, intra-assay, and 3.8 and 4.3%, respectively, inter-assay.

Lp(a), was measured by non competitive sandwich-ELISA method (Immuno AG, Vienna Austria). Lp(a) glycoprotein phenotype was determined by gel electrophoresis according to Utermann et al. [19] (Immuno AG, Vienna, Austria). The CV, intra-assay and inter-assay, was 2.9 and 4.1%, respectively.

Hormone measurements were also centralized. Serum GH assay was carried out by IFMA (Delfia, Pharmacia®) at the University of Milano. Plasma IGF-I was measured by RIA after acid-ethanol extraction at the University of Genova [20]. The upper limit of normal IGF-I levels (mean $\pm$ 3SD) was 366 μ g/l for age-matched adults. Insulin and glucagon levels were measured by commercial RIA kits (provided by Medgenix Diagnostics, Belgium and DPC, USA, respectively) at the University 'La Sapienza' of Rome. Intra-assay and inter-assay CV were below 5 and 8%, respectively, for all hormone measurements.

Table 1
Effects of 3 months of octreotide therapy (100 μ g t.i.d., sc) on hormonal and metabolic parameters (mean $\pm$ SD and AUC $\pm$ SD)

Parameters	Before therapy	Octreotide	P
IGF-I (ng/ml)	665.5 $\pm$ 155	354.3 $\pm$ 206	<0.01
GH (μ g/l) ^a	14.3 $\pm$ 31	7.5 $\pm$ 22	<0.01
GH, 7-h AUC (μ g*min/l)	6021 $\pm$ 13283	3002 $\pm$ 9089	<0.01
Insulin (μ U/ml) ^a	47.2 $\pm$ 26	29.9 $\pm$ 21	<0.01
Insulin, 7-h AUC (μ U*min/ml)	19 718 $\pm$ 11137	11 904 $\pm$ 8086	<0.01
Glucagon (pg/ml) ^a	72.2 $\pm$ 24	59.6 $\pm$ 25	<0.05
Glucagon, 7-h AUC (pg*min/ml)	30 018 $\pm$ 10494	24 120 $\pm$ 10370	<0.05
Glucose (mg/dl) ^a	100.4 $\pm$ 10	112.0 $\pm$ 16	<0.01
Glucose, 7-h AUC (mg*min/dl)	42 184 $\pm$ 4480	47 003 $\pm$ 6686	<0.01

^a Mean of eight samples taken hourly during 7-h saline infusion.

2.5. Statistical analysis

Data with multiple observations for each time (GH, insulin) were quantified by measuring the area under the curves (AUC) using the trapezoidal method. Areas and the mean hormonal and lipid levels were compared using non-parametric statistics (Wilcoxon's signed rank test or Mann–Whitney *U*, as appropriate). Correlation between continuous variables was investigated by regression analysis. All results are expressed as mean $\pm$ SD, unless otherwise stated. Significance is taken as $P < 0.05$.

3. Results

3.1. Hormonal and metabolic changes induced by octreotide

Basal serum GH and IGF-I were elevated in all patients. During octreotide therapy, serum GH levels (mean of eight samples taken hourly during 7-h saline infusion) decreased from 14.3 ± 31 to 7.5 ± 22 μ g/l (median: – 60%) ($P < 0.01$). GH AUC (7-h) was halved ($P < 0.01$). Plasma IGF-I levels (mean of two samples) decreased from 665 ± 155 to 354 ± 206 ng/ml (median: – 48%); ($P < 0.01$). Nine patients showed a decrease of mean serum GH levels to below 2 μ g/l and normalized IGF-I levels, while one patient normalized IGF-I levels even if mean serum GH concentration was 5.5 μ g/l.

During octreotide therapy mean insulin and glucagon concentrations significantly decreased, while glucose levels (both pre-prandial and post-prandial), significantly increased (Table 1). Expressed as 7-h AUC, a mean insulin decrease of about 40%, a mean glucagon decrease of about 20% and a mean glucose increase of 11% were observed.

As far as the response to a glucose load is concerned, during treatment the blood glucose peak at 2 h (4 h after octreotide administration) was increased in comparison with the baseline test (glucose peak: 194.1 ± 50 versus 162.4 ± 42 mg/dl, $P < 0.01$). Insulin release after glucose load was significantly reduced during octreotide therapy with respect to baseline (129 ± 89 versus 155 ± 243 μ U/ml, $P < 0.01$).

3.2. Lipids

Before treatment, fasting total cholesterol was 207 ± 47 mg/dl, ranging from 128 to 291 mg/dl, and being higher than 200 mg/dl in ten patients. After treatment, it slightly decreased to 196 ± 47 (range from 137 to 305 mg/dl, median: – 5%), being higher than 200 mg/dl (5.2 mol/l) in seven patients.

Table 2

Fasting lipid, lipoprotein levels and the relative flotation rate, an index of LDL peak buoyancy, in 20 acromegalic patients before and after 3 months of octreotide therapy (100 µg t.i.d., sc) in respect to 20 normal controls^a

Parameters	Controls	Acromegalics	
		Before therapy	Octreotide
Total cholesterol (mg/dl)	207 ± 42	207 ± 47	196 ± 47
Total triglycerides (mg/dl)	84 ± 30	98 ± 94	99 ± 38
LDL cholesterol (mg/dl)	125 ± 40	146 ± 43**	125 ± 40
HDL cholesterol (mg/dl)	65 ± 13	42 ± 10* **	51 ± 15*
Apo-AI (mg/dl)	175 ± 25	131 ± 17*	140 ± 18*
Apo-B (mg/dl)	89 ± 15	84 ± 27	79 ± 26
Lp(a) (mg/dl)	13 ± 13	19 ± 19**	14 ± 13
Relative flotation rate (Rf)	0.42 ± 0.02	0.40 ± 0.03*	0.39 ± 0.03*

^a Data are given as mean ± SD.

* $P < 0.05$ versus controls.

** $P < 0.05$ versus octreotide.

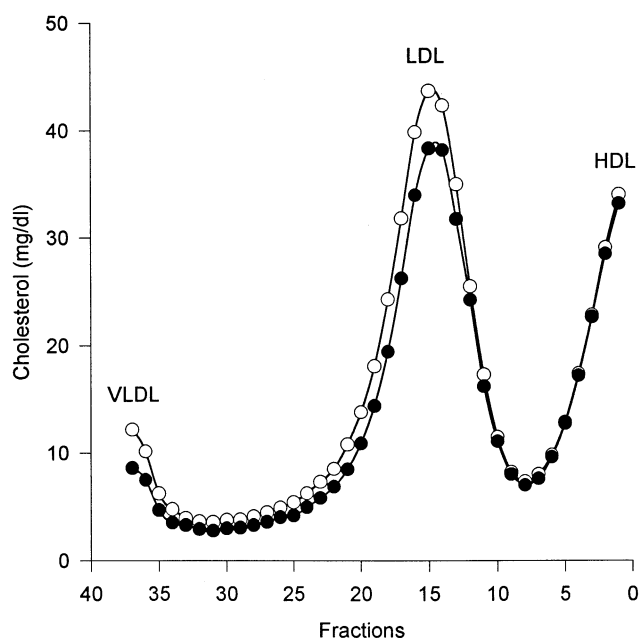


Fig. 1. Vertical spin density gradient ultracentrifugation of plasma lipoproteins in acromegalic patients before (open circles) and during (closed circles) octreotide therapy.

Octreotide therapy induced a significant decrease of LDL cholesterol from 142 ± 41.1 to 125 ± 40 mg/dl (median: -14% , $P < 0.05$) and a significant increase of HDL cholesterol from 41.7 ± 9.7 to 50.6 ± 15.2 mg/dl (median: $+22\%$, $P < 0.05$). The number of patients with HDL-cholesterol above 50 mg/dl increased from 4 to 9 and the number of patients with LDL-cholesterol below 130 mg/dl increased from 8 to 13. Triglyceride

levels were not modified by octreotide administration. Slight hypertriglyceridemia was present in two patients only, and in both triglyceride levels decreased (from 228 to 206 mg/dl, and from 161 to 115 mg/dl).

In respect to normal subjects, there was no significant difference in the pre-treatment or post-treatment levels of total cholesterol and triglycerides. HDL cholesterol levels of untreated acromegalic patients were significantly lower than HDL cholesterol levels of normal subjects (Table 2). The statistical significance was still present when considering post-treatment HDL values.

Single vertical spin-LDL ultracentrifugation showed that the physical characteristics of LDL before and during therapy remained unmodified (Fig. 1). In fact the relative flotation rate, Rf, was 0.40 ± 0.03 before octreotide treatment and 0.39 ± 0.03 after therapy. In comparison with controls, the LDL particles (both before and during therapy) in acromegalic patients appeared different, with a significantly lower Rf (Table 2).

During therapy apo-AI increased and apo-B decreased in parallel with HDL and LDL changes (Table 2), but the differences failed to reach statistical significance. Just like HDL, apo-AI were significantly lower in acromegalic patients (both before and after therapy) in comparison to normal subjects.

No significant correlations were found between these lipid modifications and GH, IGF-I, insulin, and glucagon changes. Indeed, a greater decrease of LDL was observed in patients who did not normalize GH and IGF-I concentrations in comparison with patients who did (-19 ± 13 versus $1 \pm 28\%$, $P < 0.05$).

Lp(a) levels were significantly reduced from 19 ± 19 to 14 ± 13 mg/dl (median: -28%) (Table 2). Apo(a) phenotype distribution in these acromegalic patients is reported in Table 3. All phenotypes were affected by octreotide. Both pre-treatment and post-treatment Lp(a) concentrations were significantly correlated with pre-treatment ($r = 0.6$, $P < 0.01$) and post-treatment ($r = 0.5$, $P < 0.05$) GH concentrations respectively, but not with IGF-I or insulin levels. Lp(a) percent decrease observed during therapy, was significantly correlated with GH percent decrease, (Fig. 2). However, this correlation was not statistically significant when one patient who had major modifications of both GH and Lp(a) is excluded.

3.3. Other findings

Thyroid function tests remained within the normal range during the entire study period in all patients. Serum TSH, free T3 and free T4 were unchanged (TSH: 1.1 ± 0.7 versus 1.0 ± 0.8 µU/ml; free T4: 8.4 ± 5 versus 8.4 ± 6 ; free T3 3.5 ± 1.5 versus 3.4 ± 1.4). In 12 women, mean 17 β-estradiol levels were 38.9 ± 68 on the day of pre-treatment saline infusion, and 40.7 ± 57

Table 3

Phenotypes of Lp(a): distribution and levels before and after octreotide (mean $\pm$ SD) in acromegalic patients

Phenotype of Apo(a)	No of patients (% of total)	Before therapy mg/dl	Octreotide mg/dl
S2	2 (10)	36.1 $\pm$ 28	22.1 $\pm$ 17
S3	7 (35)	32.8 $\pm$ 20	25.4 $\pm$ 11
S4	8 (40)	2.9 $\pm$ 1	2.3 $\pm$ 1
S2/S3	2 (10)	16.2 $\pm$ 10	11.2 $\pm$ 10
S2/S4	1 (5)	30.5	25.0

the day of control in therapy (P : NS), progesterone levels non-significantly changed from 3.1 ± 8.9 to 0.3 ± 0.3 .

4. Discussion

Acromegalic patients were characterized by lower HDL cholesterol levels (and apo-AI) in comparison to controls. Treatment with octreotide led to remarkable changes of the plasma lipoprotein parameters with beneficial effects on several known cardiovascular risk factors: an increase in HDL cholesterol (+22%), a decrease in LDL cholesterol (−14%) and a decrease of the Lp(a) levels (all phenotypes) being observed. The study of LDL particles confirmed that acromegalic patients had smaller and/or more dense LDL particles in comparison with normal controls. However, the LDL subfraction distribution remained unmodified during octreotide therapy, as we have shown for the first time.

The results on the effects of octreotide on total, LDL, and HDL cholesterol and on triglyceride concentrations are in agreement with the observations previously made in another multicenter Italian study [3]. In fact in that study we found a significant decrease of both total (−9%) and LDL cholesterol (−18%), and a slight, but not significant increase of HDL (+4%) cholesterol. In both studies triglyceride levels decreased only in patients with pre-treatment hypertriglyceridemia (only two cases in the present series).

Only a few other studies report on serum lipid changes in acromegalic patients treated with octreotide [10,21–25]. A decrease of triglyceride levels has been reported [21–24], mostly in patients who had hypertriglyceridemia [22]. The different results with our study may be explained by the facts that in all the mentioned series, about one third of patients had diabetes mellitus (that we have excluded) and also that about 50% of their patients had moderate hypertriglyceridemia before treatment. No or minor modifications of triglycerides, a significant increase of HDL cholesterol, and a signifi-

cant decrease of Lp(a) were observed by Lam et al. [10] and by Wildbrett et al. [25], in agreement with us, after 6 months of octreotide therapy. No significant change in LDL cholesterol levels has been previously reported. As in our study, Lp(a) apart, no correlations between lipid, and hormonal changes have been reported.

It has been shown that GH is able to increase hepatic LDL receptor expression, resulting in an accelerated elimination rate of the LDL particles and subsequent lowering of LDL cholesterol [26]. The lack of a correlation between hormonal parameters and LDL reduction, suggests that this modification is not due to the GH metabolic effects. Other factors, including octreotide, might be responsible for the LDL reduction here observed.

The data regarding LDL physical characteristics are of interest. In fact, the LDL physical properties have recently attracted a great deal of attention because of an increased risk of vascular complications associated to the presence of small and/or dense LDL particles in both diabetic and non diabetic patients [4,5].

To our knowledge, only a previous paper by Tan et al. [6] dealt with LDL subfractions distribution in acromegaly, obtaining similar results. According to this author, an increased activity of plasma cholesteryl ester transfer protein (CETP) could contribute to the enhanced formation of small dense LDL particles. Other factors might be involved in modifying the LDL physical properties in acromegalic patients. Both hyperinsulinism and reduced post-heparin lipase activity might be involved in the development of hyperlipidemia in acromegaly [6,27–29]. Hyperinsulinism and insulin resistance are associated with the presence of small and/or dense LDL particles in diabetic patients [30].

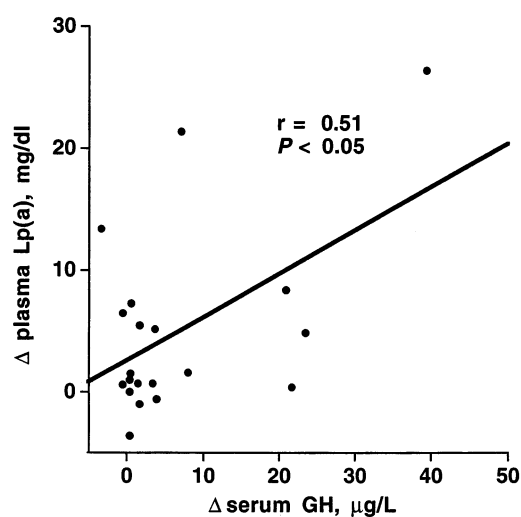


Fig. 2. Correlation between GH and Lp(a) decreases observed during octreotide therapy.

LDL physical characteristics might also be affected by the hepatic lipase activity. In fact it has been demonstrated that LDL size is inversely related to the hepatic lipase activity [31]. Hepatic lipase is low in acromegalic patients [6,27]. Therefore, the presence of small and/or dense LDL particles in acromegalic patients must be determined by factors other than hepatic lipase. Tan et al. [6] indicated that in acromegalic patients GH and HDL are the main determinants of the LDL subclass distribution. The effects of octreotide on CETP and lipases are not known. However, during octreotide treatment we did observe a reduction of GH and an increase of HDL, without any significant effect on LDL physical properties. Octreotide seems to improve insulin sensitivity [21,32,33]. A reduced insulin sensitivity is associated with the presence of small and/or dense LDL particles [30]. The lack of modifications of the LDL physical properties during octreotide therapy is therefore probably due to the balanced results of the modifications in the CETP and hepatic lipase activities, GH levels, HDL levels and insulin sensitivity.

Lp(a) appears to be clearly modulated by GH. The Lp(a) concentrations in acromegalic patients are usually increased [10] and octreotide treatment resulted in a significant reduction of these concentrations, apparently without any regard to the apo(a) phenotype. We have found, in agreement with Lam [10], a good correlation between Lp(a) and GH, but not IGF-I, modifications. This lack of a correlation between Lp(a) and IGF-I levels is consistent with the hypothesis that GH and IGF-I have different, probably opposing effects on Lp(a) levels [34], but also suggests a most important role for GH in the physiological regulation of Lp(a) expression in respect to IGF-I.

In conclusion, Lp(a) concentrations are high and directly correlated with GH levels in acromegalic patients. Octreotide therapy decreases Lp(a) levels by decreasing GH concentrations. Low HDL cholesterol levels and a predominance of small and/or dense LDL particles are also present in acromegalic patients and could contribute to the high vascular risk of these patients. Treatment with octreotide is associated with an increase of HDL levels and a decrease of total concentrations of LDL. However, this treatment has no effect on the LDL physical properties.

Acknowledgements

Octreotide, Longastatina[®], was kindly supplied by Italfarmaco SpA, Milan, Italy. Italfarmaco also covered the financial burden deriving from hormone assays. We are indebted to Dr B. China for statistical advice, to Prof G Tamburrano (University 'La Sapienza' of Rome) for insulin and glucagon assays and to dr A. Barreca (University of Genova) for IGF-I

assay. We gratefully acknowledge Raffaella Marin (University of Padova) for her expert technical assistance in lipid and lipoprotein measurements.

References

- [1] Bates AS, Van't Hoff W, Jones JM, Clayton RN. An audit of outcome of treatment in acromegaly. *Qrt J Med* 1993;86:293–9.
- [2] Rajasoorya C, Holdaway IM, Wrightson P, Scott DJ, Ibbertson HK. Determinants of clinical outcome and survival in acromegaly. *Clin Endocrinol* 1994;41:95–102.
- [3] Arosio M, Macchelli S, Rossi CM, et al. Effects of treatment with octreotide in acromegalic patients—a multicenter Italian study. *Eur J Endocrinol* 1995;133:430–9.
- [4] Lamarche B, Tchernof A, Moorjani S, et al. Small, dense low-density lipoprotein particles as predictor of the risk of ischemic heart disease in men. Prospective results from the Québec Cardiovascular Study. *Circulation* 1997;95:69–75.
- [5] Nosadini R, Manzato E, Solini A, et al. Peripheral, rather than hepatic, insulin resistance and atherogenic lipoprotein phenotype predict cardiovascular complications in NIDDM. *Eur J Clin Invest* 1994;24:258–66.
- [6] Tan KCB, Shiu SWM, Janus ED, Lam KSL. LDL subfractions in acromegaly: relation to growth hormone and insulin-like growth factor-I. *Atherosclerosis* 1997;129:59–65.
- [7] Kostner GM, Avogaro P, Cazzolato G, Marth E, Bittolo-Bon G, Quinci GB. Lipoprotein Lp(a) and the risk for myocardial infarction. *Atherosclerosis* 1981;38:51–61.
- [8] Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H. Lipoprotein (a) and coronary heart disease in a general population sample of middle-aged men. *Brit Med J* 1990;301:1248–50.
- [9] Bostom AG, Cupples LA, Jenner JL, et al. Elevated plasma lipoprotein (a) and coronary heart disease in men aged 55 years and younger. A prospective study. *JAMA* 1996;276:544–8.
- [10] Lam KSL, Pang RWC, Janus ED, Kung AWC, Wang CCL. Serum apolipoprotein(a) correlates with growth hormone levels in Chinese patients with acromegaly. *Atherosclerosis* 1993;104:183–8.
- [11] Mooser V, Hobbs HH. Lipoprotein(a) and growth hormone: is the puzzle solved? *Eur J Endocrinol* 1997;137:450–2.
- [12] The Expert Committee on the diagnosis and classification of diabetes mellitus. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care* 1997;20:1183–1197.
- [13] Roschlau VP, Bernt E, Gruber W. Enzymatische Bestimmung des Gesamt Cholesterins in Serum. *Klin Chem Klin Biochem* 1974;12:403–7.
- [14] Lipid Research Clinics Program. Lipid and lipoprotein analysis. In: US Department of Health and Human Service, editor. *Manual of Laboratory Operations*, 2nd edition, Washington DC, 1982: 63–77.
- [15] Wahlefeld AW. Triglycerides determination after enzymatic hydrolysis. In: Bergmeyer HU, editor. *Methods of Enzymatic Analysis*. New York: Academic Press, 1976, 1976:1831–5.
- [16] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- [17] Chung BH, Wilkinson T, Geer JC, Segrest JP. Preparative and quantitative isolation of plasma lipoproteins: rapid, single continuous density gradient ultracentrifugation in a vertical rotor. *J Lipid Res* 1980;21:284–91.

- [18] Chung BH, Segrest JP, Ray MJ, et al. Single vertical spin density gradient ultracentrifugation. In: Segrest JP, Albers JJ, editors. *Methods in Enzymology*, vol 128. Plasma Lipoproteins: Preparation, Structure and Molecular Biology. Orlando, FL: Academic Press, 1986:181–209.
- [19] Utermann G, Menzel HJ, Kraft HG, Duba HC, Kemmer HG, Seitz C. Lp(a) glycoprotein phenotypes. Inheritance and relation to Lp(a)-lipoprotein concentrations in plasma. *J Clin Invest* 1987;80:458–65.
- [20] Barreca A, Ciccarelli E, Minuto F, Bruzzi P, Giordano G, Camanni F. Insulin-like growth factor I and daily growth hormone profile in the assessment of active acromegaly. *Acta Endocrinol* 1989;120:635–9.
- [21] James RA, Møller N, Chatterjee S, White M, Kendall-Taylor P. Carbohydrate tolerance and serum lipids in acromegaly before and during treatment with high dose octreotide. *Diabetic Med* 1991;8:517–23.
- [22] Sassolas G, Harris AG, James-Deidier A. Long term effect of incremental doses of the somatostatin analog SMS 201-995 in 58 acromegalic patients. *J Clin Endocrinol Metab* 1990;71:391–7.
- [23] Cohen R, Chanson P, Bruckert E, et al. Effects of octreotide on lipid metabolism in acromegaly. *Horm Metab Res* 1992;24:397–400.
- [24] Simsolo RB, Ezzat S, Ong JM, Saghizadeh M, Kern PA. Effects of acromegaly treatment and growth hormone on adipose tissue lipoprotein lipase. *J Clin Endocrinol Metab* 1995;80:3233–8.
- [25] Wildbrett J, Hanefeld M, Fucker K, et al. Anomalies of lipoprotein pattern and fibrinolysis in acromegalic patients: relation to growth hormone levels and insulin-like growth factor I. *Exp Clin Endocrinol Diabetes* 1997;105:331–5.
- [26] Rudling M, Norstedt G, Olivecrona H, Reihner E, Gustafsson J-Å, Angelin B. Importance of growth hormone for the induction of hepatic low density lipoprotein receptors. *Proc Natl Acad Sci USA* 1992;89:6983–7.
- [27] Murase T, Yamada N, Ohsawa N, Kosaka K, Morita S, Yoshida S. Decline of postheparin plasma lipoprotein lipase in acromegalic patients. *Metabolism* 1980;29:666–72.
- [28] Fineberg SE, Merimee TJ, Rabinowitz D, Edgar PJ. Insulin secretion in acromegaly. *J Clin Endocrinol Metab* 1970;30:288–92.
- [29] Hansen I, Tsalikian E, Beaufriere B, Gerich J, Haymond M, Rizza R. Insulin resistance in acromegaly: defects in both hepatic and extra-hepatic insulin action. *Am J Physiol* 1986;250:269–73.
- [30] Zambon S, Manzato E, Solini A, et al. Lipoprotein abnormalities in non-insulin-dependent diabetic patients with impaired extra-hepatic insulin sensitivity, hypertension, and microalbuminuria. *Arterioscler Thromb* 1994;14:911–7.
- [31] Zambon A, Austin MA, Brown BG, Hokanson JE, Brunzell JD. Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. *Arterioscler Thromb* 1993;13:147–52.
- [32] Ho KK, Jenkins AB, Furler SM, Borkman M, Chisholm DJ. Impact of octreotide, a long-acting somatostatin analogue, on glucose tolerance and insulin sensitivity in acromegaly. *Clin Endocrinol* 1992;36:271–9.
- [33] Sato K, Takamatsu K, Hashimoto K. Short-term effects of octreotide on glucose tolerance in patients with acromegaly. *Endocrinol J* 1995;42:739–45.
- [34] Laron Z, Wang XL, Klinger B, Silbergeld A, Wilcken DEL. Insulin-like growth factor-I decreases serum lipoprotein (a) during long-term treatment of patients with Laron syndrome. *Metabolism* 1996;45:1263–6.