

# **STUDY OF GLUCAGON LIKE PEPTIDE -1 IN PATIENTS WITH ISCHEMIC HEART DISEASE**

*Thesis*

Submitted for Partial Fulfillment of Master Degree  
in Internal Medicine

*By*

**Bolis Azmy Bolis Khames**  
M.B., B.CH

*Supervised by*

**Prof. Dr. Nermin Ahmed Sheriba**  
*Professor of Internal Medicine & Endocrinology*  
*Faculty of Medicine – Ain Shams University*

**Dr. Salwa Sedik Hosny**

*Assistant Professor of Internal Medicine & Endocrinology*  
*Faculty of Medicine – Ain Shams University*

**Dr. Matta Makram Anees**

*Lecturer of Internal Medicine & Endocrinology*  
*Faculty of Medicine – Ain Shams University*

**Faculty of Medicine**  
**Ain Shams University**

**2011**

# دراسة البروتين المشابه لهرمون الجلوكاجون ١- في مرضى قصور الشريان التاجي

## رسالة

مقدمة من

الطبيب/ بولس عزمى بولس  
بكالوريوس الطب والجراحة

توطئه للحصول علي درجة الماجستير في الباطنة العامة

تحت إشراف

الأستاذ الدكتور / نرمين أحمد شريفة

أستاذ الباطنة العامة والغدد الصماء

كلية الطب – جامعة عين شمس

الأستاذ الدكتور / سلوى صديق حسنى

أستاذ مساعد الباطنة العامة والغدد الصماء

كلية الطب – جامعة عين شمس

الدكتور / متى مكرم أنيس

مدرس الباطنة العامة والغدد الصماء

كلية الطب – جامعة عين شمس

كلية الطب

جامعة عين شمس

٢٠١١

Glucagon-like peptide-1 (GLP-1) is an incretin hormone secreted by L cells of the GIT in response to nutrient intake and plays a major role in glucose homeostasis by stimulating insulin secretion, suppressing glucagon secretion, inhibiting gastric emptying, and reducing appetite and food intake. This hormone exerts its effects through interaction with G-protein coupled receptors. The GLP-1 receptor is widely expressed throughout the body ( $\alpha$ - and P-cells in the islets of pancreas, other parts of the gastrointestinal tract, central and peripheral nervous systems, heart, kidney, and the lung).

In patients with type 2 diabetes mellitus, the incretin effect is reduced or even absent due to significant reduction in meal-stimulated levels of GLP-1. Therapeutic efforts have been focused on the development of GLP-1 receptor agonists, which would appear to be ideal therapeutic agents for use in patient with type 2 diabetes. Incretin mimetics, exenatide and liraglutide, which are GLP-1 receptor agonists resistant to DPP-IV degradation, and the DPP-IV inhibitors (the gliptins), which potentiate the effect of the endogenously secreted incretin hormones by competitively inhibiting the enzyme responsible for their degradation

The incretin mimetics have effects extending beyond their

## ACKNOWLEDGMENTS

*First and above all, thanks to **ALLAH**, the source of all knowledge and wisdom who enabled me to complete this work.*

*I wish to express my deep and sincere gratitude to **Prof. Dr. Nermin Ahmed Sheriba**, Professor of Internal Medicine and Endocrinology, Faculty of Medicine-Ain Shams University. Her wide knowledge and logical way of thinking have been of great value for me. Her understanding, encouraging and personal guidance have provided a good basis for the present study. No words of thanks could ever express my feelings towards her extreme support.*

*I am deeply grateful to **Prof. Dr. Salwa Sedik Hosny**, Assistant Professor of Internal Medicine and Endocrinology, Faculty of Medicine-Ain Shams University, for her inspiration, sincere care, follow up, encouragement and important support throughout this work., her sincere directions were of great value in this research.*

*I wish to express my warm and sincere thanks to **Dr. Matta Makram Anees**, Lecturer of Internal Medicine and Endocrinology, Faculty of Medicine-Ain Shams University, for his kind help in this research.*

*Also I wish to express many thanks to **Dr. Maram Mohammed Maher** Lecturer of Internal Medicine and Endocrinology, Faculty of Medicine-Ain Shams University, for her kind support, instructions and help.*

*Lastly I would like to extend my warm thanks to **Dr. Magdy Abbas Abdel Aziz** Assistant consultant of biochemistry Ain Shams University Hospitals, for his hard work in laboratory investigations of this work.*

## CONTENTS

---

|                                             | <b>Page</b> |
|---------------------------------------------|-------------|
| Acknowledgment .....                        | <b>I</b>    |
| List of abbreviations .....                 | <b>III</b>  |
| List of figures .....                       | <b>VII</b>  |
| List of tables .....                        | <b>IX</b>   |
| Protocol .....                              | <b>XI</b>   |
| <b>Review of literature</b>                 |             |
| Chapter 1:                                  |             |
| Glucagons like peptide – 1 .....            | <b>1</b>    |
| Chapter 2:                                  |             |
| Incretins and cardiovascular diseases ..... | <b>40</b>   |
| Chapter 3:                                  |             |
| Ischemic heart disease risk factors .....   | <b>80</b>   |
| Subjects and methods .....                  | <b>111</b>  |
| Results .....                               | <b>121</b>  |
| Discussion .....                            | <b>142</b>  |
| Summary and conclusions .....               | <b>152</b>  |
| Recommendations .....                       | <b>155</b>  |
| References .....                            | <b>156</b>  |
| Arabic summary .....                        | <b>—</b>    |

# List of Abbreviations

---

|                   |                                                       |
|-------------------|-------------------------------------------------------|
| <b>ACTH</b>       | Adrenocorticotropin hormone.                          |
| <b>ACCORD</b>     | Action to control cardiovascular risk in diabetes     |
| <b>ADA</b>        | American diabetes association.                        |
| <b>AGEs</b>       | Advanced glycation end products                       |
| <b>AHA</b>        | American heart association                            |
| <b>AMI</b>        | Acute myocardial infarction.                          |
| <b>AMP</b>        | Adenosine monophosphate.                              |
| <b>AMPK</b>       | AMP-activated protein kinase.                         |
| <b>Ach</b>        | Acetylcholine                                         |
| <b>ANOVA</b>      | Analysis of variance                                  |
| <b>ATP</b>        | Adenosine triphosphate.                               |
| <b>BMI</b>        | Body mass index.                                      |
| <b>CABG</b>       | Coronary artery bypass graft.                         |
| <b>CAD</b>        | Coronary artery disease.                              |
| <b>cAMP</b>       | Cyclic AMP.                                           |
| <b>cAMP-GEFII</b> | cAMP-regulated guanine nucleotide exchange factor II. |
| <b>CHF</b>        | Congestive heart failure.                             |
| <b>CD26</b>       | Dipeptidyl peptidase IV.                              |
| <b>CVA</b>        | Cerebrovascular accident.                             |
| <b>CHF</b>        | Congestive heart failure.                             |
| <b>Chol.</b>      | Cholesterol.                                          |
| <b>CRH</b>        | Corticotrophin-releasing hormone.                     |
| <b>CRP</b>        | C-reactive protein                                    |
| <b>CYP</b>        | Cytochrome pathway                                    |
| <b>CV</b>         | Cardiovascular.                                       |
| <b>CVD</b>        | Cardiovascular disease.                               |

|                         |                                                                                |
|-------------------------|--------------------------------------------------------------------------------|
| <b>CPAP</b>             | Continuous positive airway pressure                                            |
| <b>CHD</b>              | Coronary heart disease.                                                        |
| <b>D DM</b>             | Duration of diabetes.                                                          |
| <b>DSE</b>              | Dobutamine stress echocardiography                                             |
| <b>Dias. BP</b>         | Diastolic blood pressure.                                                      |
| <b>DNA</b>              | Deoxyribonucleic acid.                                                         |
| <b>DPP-IV</b>           | Dipeptidyl peptidase IV.                                                       |
| <b>eNOS</b>             | Endogenous nitric oxide synthase                                               |
| <b>ECG</b>              | Electrocardiogram.                                                             |
| <b>ECHO</b>             | Echocardiogram.                                                                |
| <b>EF</b>               | Ejection fraction.                                                             |
| <b>ELIZA</b>            | Enzyme linked immunosorbant assay.                                             |
| <b>Epac2</b>            | cAMP-regulated guanine nucleotide exchange factor II.                          |
| <b>ED</b>               | Endothelial dysfunction.                                                       |
| <b>FBG</b>              | Fasting blood glucose.                                                         |
| <b>FMD</b>              | Flow mediated dilatation.                                                      |
| <b>FDA</b>              | FOOD and drug association                                                      |
| <b>GIP</b>              | Gastric inhibitory polypeptide / Glucose dependent insulinotropic polypeptide. |
| <b>GLP-1</b>            | Glucagon like peptide-1.                                                       |
| <b>GLP-1r</b>           | GLP-1 receptors.                                                               |
| <b>GLP-2</b>            | Glucagon like peptide-2.                                                       |
| <b>GPCR-kinase</b>      | G-protein-coupled receptor kinase.                                             |
| <b>GRP</b>              | Gastrin releasing peptide.                                                     |
| <b>GRPP</b>             | Glicentin related pancreatic polypeptide.                                      |
| <b>GLUT</b>             | Glucose transporter.                                                           |
| <b>HbA<sub>1C</sub></b> | Glycated hemoglobin / hemoglobin A <sub>1C</sub> .                             |
| <b>HDL</b>              | High density lipoprotein.                                                      |
| <b>His.</b>             | Histidine                                                                      |
| <b>HF</b>               | Heart failure                                                                  |
| <b>HR</b>               | Hazard ratio.                                                                  |
| <b>IL-1</b>             | Interleukin-1.                                                                 |

|                  |                                                                                                            |
|------------------|------------------------------------------------------------------------------------------------------------|
| <b>IP-1</b>      | Intervening peptide-1                                                                                      |
| <b>IP-2</b>      | Intervening peptide-2                                                                                      |
| <b>IR</b>        | Insulin resistance.                                                                                        |
| <b>IDF</b>       | International diabetes foundation.                                                                         |
| <b>ICAM</b>      | Intracellular adhesion molecule.                                                                           |
| <b>LDL</b>       | Low density lipoprotein.                                                                                   |
| <b>LV</b>        | Left ventricle.                                                                                            |
| <b>LVEF</b>      | left ventricular ejection fraction.                                                                        |
| <b>MAPK</b>      | Mitogen-activated protein kinase.                                                                          |
| <b>mRNA</b>      | Messenger ribonucleic acid.                                                                                |
| <b>MPGF</b>      | Major proglucagon fragment.                                                                                |
| <b>MDP-1</b>     | Monocyte chemotactic protein-1                                                                             |
| <b>NMR</b>       | Neuclear magnetic resonance.                                                                               |
| <b>NCEP</b>      | National cholesterol education programme                                                                   |
| <b>NO</b>        | Nitric oxide                                                                                               |
| <b>NPV</b>       | Negative peridictive value.                                                                                |
| <b>NYHA</b>      | New York heart association.                                                                                |
| <b>OSA</b>       | Obstructive sleep apnea                                                                                    |
| <b>OGTT</b>      | Oral glucose tolerance test                                                                                |
| <b>P</b>         | P-value / probability                                                                                      |
| <b>PAI</b>       | Plasminogen activator inhibitor                                                                            |
| <b>PG</b>        | proglucagon                                                                                                |
| <b>PCI</b>       | Percautaneous coronary intervention                                                                        |
| <b>PK</b>        | Protein kinase                                                                                             |
| <b>PERISCOPE</b> | Pioglitazone effect on regression of intravascular sonographic coronary obstruction prospective evaluation |
| <b>PC</b>        | Proglucagon convertase                                                                                     |
| <b>PKA</b>       | Protein kinase A.                                                                                          |
| <b>PPAR</b>      | Peroxisome proliferators activator receptor.                                                               |
| <b>PVN</b>       | Paraventricular nucleus.                                                                                   |
| <b>PPV</b>       | Positive peridictive value                                                                                 |
| <b>PYY</b>       | Peptide YY / Peptide Tyrosine Tyrosine.                                                                    |

|                      |                                                                                      |
|----------------------|--------------------------------------------------------------------------------------|
| <b>r</b>             | Pearson's correlation coefficient.                                                   |
| <b>RAS</b>           | Renin angiotensin aldosterone system.                                                |
| <b>RECORD</b>        | Rosiglitazone Evaluated for Cardiac Outcomes and Regulation of Glycemia in Diabetes. |
| <b>RNA</b>           | Ribonucleic acid.                                                                    |
| <b>RAGEs</b>         | Receptors of advanced glycation end products                                         |
| <b>ROS</b>           | Reactive oxygen species.                                                             |
| <b>ROC</b>           | Receiver operator characteristic.                                                    |
| <b>Sc</b>            | Subcutaneous.                                                                        |
| <b>SD</b>            | Stander deviation.                                                                   |
| <b>Sig.</b>          | Significance.                                                                        |
| <b>SNS</b>           | Sympathetic nervous system.                                                          |
| <b>SNP</b>           | Single nucleotide polymorphism.                                                      |
| <b>SPSS</b>          | Statistical program for social science.                                              |
| <b>SR</b>            | Sarcoplasmic reticulum.                                                              |
| <b>SS</b>            | Somatostatin.                                                                        |
| <b>SAS</b>           | Sleep apnea syndrome                                                                 |
| <b>Sys. BP</b>       | Systolic blood pressure.                                                             |
| <b>T2DM</b>          | Type 2 diabetes mellitus.                                                            |
| <b>TG</b>            | Triglycerides.                                                                       |
| <b>TF</b>            | Tissue factor                                                                        |
| <b>TNF</b>           | Tumor necrosis factor.                                                               |
| <b>TZD</b>           | Thiazolidinediones                                                                   |
| <b>UKPDS</b>         | United kingdom prospective diabetes study                                            |
| <b>VSMC</b>          | Vascular smooth muscle cells                                                         |
| <b>VLDL</b>          | Very low density lipoproteins                                                        |
| <b>VP</b>            | Valine pyrrolide                                                                     |
| <b>X<sup>2</sup></b> | Chi-square test.                                                                     |

# List of Figures

---

|                                                                                                                              | <b>Page</b> |
|------------------------------------------------------------------------------------------------------------------------------|-------------|
| <b>A. <u>Figures of the Review:</u></b>                                                                                      |             |
| <b>Figure (1)</b> Alternative posttranslational processing of proglucagon and the transcription of proglucagon gene .....    | <b>5</b>    |
| <b>Figure (2)</b> 2D-NMR structure of GLP-1 .....                                                                            | <b>9</b>    |
| <b>Figure (3)</b> Structure of L cell .....                                                                                  | <b>10</b>   |
| <b>Figure (4)</b> Factors involved in the control of the activity of L cells .....                                           | <b>13</b>   |
| <b>Figure (5)</b> Plasma concentrations of insulin, GLP-1 (total) and GIP (total) during the day time in healthy subjects .. | <b>15</b>   |
| <b>Figure (6)</b> GLP-1 and glucose signaling .....                                                                          | <b>19</b>   |
| <b>Figure (7)</b> Ultracellular actions of GLP-1 that lead to stimulation of insulin secretion .....                         | <b>23</b>   |
| <b>Figure (8)</b> The neural pathway of the action of GLP-1 .....                                                            | <b>28</b>   |
| <b>Figure (9)</b> Structure of native GLP-1, exenatide, liraglutide, sitagliptin and vildagliptin .....                      | <b>39</b>   |
| <b>Figure (10)</b> Proposed antihyperglycemic strategy in T2DM and CAD patient .....                                         | <b>60</b>   |
| <b>Figure (11)</b> Propsed antihyperglycemic strategy in T2DM and HF patient .....                                           | <b>64</b>   |
| <b>Figure (12)</b> GLP Curve .....                                                                                           | <b>115</b>  |

## **List of Figures (Cont.)**

| <b>B. <u>Figures of the Results:</u></b>                                                                     | <b>Page</b> |
|--------------------------------------------------------------------------------------------------------------|-------------|
| <b>Figure (1)</b> Comparison between mean systolic and diastolic blood pressure among different groups ..... | <b>133</b>  |
| <b>Figure (2)</b> Sex distribution in all studied cases .....                                                | <b>134</b>  |
| <b>Figure (3)</b> Comparison of mean and range of GLP-1 in different groups .....                            | <b>134</b>  |
| <b>Figure (4)</b> Comparison of mean and range of EF in different studied groups .....                       | <b>135</b>  |
| <b>Figure (5)</b> Comparison between diabetics and non diabetics as regard GLP-1 .....                       | <b>135</b>  |
| <b>Figure (6)</b> Comparison between cases and controls as regard GLP-1 .....                                | <b>136</b>  |
| <b>Figure (7)</b> Comparison between ischemic cases and controls as regard GLP1 .....                        | <b>136</b>  |
| <b>Figure (8)</b> Correlation between fasting GLP-1 and ejection fraction in group-1 .....                   | <b>137</b>  |
| <b>Figure (9)</b> Correlation between fasting GLP-1 and HbA <sub>1C</sub> in group -1 .....                  | <b>137</b>  |
| <b>Figure (10)</b> Correlation between fasting GLP-1 and LDL in group-1 .....                                | <b>138</b>  |
| <b>Figure (11)</b> Correlation between fasting GLP-1 and duration of diabetes in group 1 .....               | <b>138</b>  |
| <b>Figure (12)</b> Correlation between fasting GLP-1 and 2HPP glucose in group 1 .....                       | <b>139</b>  |
| <b>Figure (13)</b> Correlation between fasting GLP-1 and TG in group 2 .....                                 | <b>139</b>  |
| <b>Figure (14)</b> Correlation between fasting GLP-1 and HBA1c in group 3 .....                              | <b>140</b>  |
| <b>Figure (15)</b> ROC curve for overall predictivity of GLP1 .....                                          | <b>140</b>  |

# List of Tables

---

**Page**

## **A. Tables of the Review:**

|                  |                                                                           |            |
|------------------|---------------------------------------------------------------------------|------------|
| <b>Table (1)</b> | Anti-Hyperglycemic Medications in Type 2 Diabetes .....                   | <b>48</b>  |
| <b>Table (2)</b> | Classification of ischemic heart disease Risk factors .....               | <b>82</b>  |
| <b>Table (3)</b> | Classification of arterial blood pressure according to BHS .....          | <b>86</b>  |
| <b>Table (4)</b> | The risk increases for diseases related to obesity as BMI increases ..... | <b>90</b>  |
| <b>Table (5)</b> | Drug therapy treatment levels goal of LDL in adults .....                 | <b>100</b> |

## **B. Tables of the results:**

|                  |                                                                          |            |
|------------------|--------------------------------------------------------------------------|------------|
| <b>Table (1)</b> | Comparison between the studied groups as regard clinical data .....      | <b>123</b> |
| <b>Table (2)</b> | Comparison between the studied groups as regard laboratory data .....    | <b>124</b> |
| <b>Table (3)</b> | Comparison between the studied groups as regard GLP1 .....               | <b>125</b> |
| <b>Table (4)</b> | Comparison between the studied groups as regard Echo (EF) .....          | <b>126</b> |
| <b>Table (5)</b> | Comparison between diabetic and non diabetic group as regard GLP-1 ..... | <b>126</b> |

## **List of Tables (Cont.)**

---

|                   |                                                                                           | <b>Page</b> |
|-------------------|-------------------------------------------------------------------------------------------|-------------|
| <b>Table (6)</b>  | Comparison between total cases and controls as regard GLP1 .....                          | <b>127</b>  |
| <b>Table (7)</b>  | Comparison between ischemic cases and controls as regard GLP1 .....                       | <b>127</b>  |
| <b>Table (8)</b>  | Correlation between GLP1 versus other variables among group- 1 .....                      | <b>128</b>  |
| <b>Table (9)</b>  | Correlation between GLP1 versus other variables among group 2 .....                       | <b>129</b>  |
| <b>Table (10)</b> | Correlation between GLP1 versus other variables among group 3 .....                       | <b>130</b>  |
| <b>Table (11)</b> | Correlation between GLP1 versus other variables among group 4 .....                       | <b>131</b>  |
| <b>Table (12)</b> | Comparison between GLP1 versus different variables by linear regression among cases ..... | <b>132</b>  |
| <b>Table (13)</b> | Best cut off, sensitivity, specificity, PPV and NPV of GLP1 in ischemic cases .....       | <b>133</b>  |



## **Introduction**

Glucagon-like peptide-1 (GLP-1), is a gut incretin hormone that stimulates insulin secretion and also activates anti apoptotic signaling pathways in pancreatic cells (**Bose et al., 2005**). Glucagon-like peptide-1 has been shown to exert a direct cytoprotective effect via inhibition of apoptosis directly in target cells expressing the GLP-1 receptors (**Redondo et al., 2003**). It has been demonstrated that GLP-1 has receptors in the heart through which it exerts physiologically important effects on cardiac function (**Holst, 2007**). In the resting state, GLP-1 may inhibit the myocardial contractility, but after cardiac injury GLP-1 has constantly increased myocardial performance. It enhances insulin secretion and may enhance myocardial performance via the combined effects of enhanced insulin secretion and stimulation of p70s6 kinase pathway (**Zarich, 2005**).

GLP-1 has been shown to markedly reduce post prandial levels of glucose, triglycerides and free fatty acids and increase HDL levels in diabetics and non diabetic subjects. With this ability to reduce postprandial glucose excursion, it could be a promising agent for improving cardiovascular prognosis (**Riddle and Drucker, 2006**).



It is important to demonstrate that the effects of GLP-1 are not restricted to the pancreas and the gastrointestinal tract. In patients with heart failure, GLP-1 when given intravenously has been shown to improve the ejection fraction and regional wall scores through its cardiac receptors, dilate the pulmonary artery and has a diuretic effect (*Sokos et al., 2006*). Patients with type 2 diabetes mellitus are considered at high risk for cardiovascular disease. (*Song and Hardisty, 2008*) and are characterized by a deficiency of GLP-1 (*Nauck et al., 2004*).

Whether the GLP-1 deficiency is the link between type 2 diabetes mellitus and the development of ischaemic heart disease in need for further study.

## Aim of the work

To measure the plasma levels of GLP-1 in patients with ischemic heart disease, both those with type 2 diabetes mellitus and those who are non-diabetic and to assess the role of GLP-1 in the development of ischemic heart disease.

## Subjects and Methods

### ♦ Subjects

This study will be conducted on 54 subjects who will be selected from the outpatient clinic of cardiology department and the endocrinology unit of internal medicine department, Ain