EFFECTS OF ADIPONECTIN EXTRACTS ON BLOOD PARAMETERS, HISTOLOGICAL & MOLECULAR CHANGES IN INDUCED HYPERGLYCAEMIC RATS

BY

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in Health Sciences

Kulliyyah of Allied Health Sciences
International Islamic University Malaysia

SEPTEMBER 2018

ABSTRACT

Given the beneficial roles of adiponectin protein on body metabolism and its profound protective effects against metabolic diseases, a better understanding of the protein's secretion and regulation is very important. The objectives of this study were to extract, detect and quantify the total adiponectin in trimmed off abdominal adipose tissues from meat sources, namely chicken, beef and lamb, and test it on STZ-induced hyperglycaemic rats. Abdominal adipose tissues were isolated from the aforementioned sources and delipidation of the tissues were performed through chloroform/methanol extractions. Afterwards, the protein concentration was determined by using Protein Assay Bicinchoninate Kit method. This was followed by quantification of the adiponectin using ELISA assay kit. Followed by tests in STZ-induced hyperglycaemic rats on blood glucose and blood lipid, hormone measurement and lastly quantification of PPAR-α mRNA, AMPK mRNA, AdipoR1 mRNA, AdipoR2 mRNA and AdipoQ mRNA in the hepatocytes. The experiment was conducted in triplicates and the results were presented as mean ± SD. The data was statistically analyzed by using SPSS statistical software version IBM 21.0. One-way analysis of variance (ANOVA) was used and the data were considered statistically different at 95% confidence interval. Results indicated that the extraction of 10-gram subcutaneous adipose tissues from chicken, beef and lamb yielded 0.10-gram, 0.15 gram and 0.15 gram of protein, respectively, which were 1 - 1.5 % from the total tissue mass. The protein concentration in the abdominal adipose tissues from chicken, beef and lamb were 1.25 ± 0.05, 1.75 ± 0.05 & 2.53 ± 0.07 mg/ml, respectively. The isolated adiponectin concentration in chicken, beef and lamb was 158 ± 0.05 ng/ml, 24240 ± 0.05 ng/ml and 37 ± 0.08 ng/ml, respectively. Adiponectin concentration in beef abdominal adipose tissues was significantly (p<0.001) higher compared to chicken and lamb. In the animal study, the normal, insulin-treated, PCCA-treated (protein containing chicken adiponectin), PCBA-treated (protein containing beef adiponectin) and PCLA-treated (protein containing lamb adiponectin) hyperglycaemic groups exhibited significantly (p<0.05) reduced blood glucose levels after the treatment period when compared with the NT (control diabetic) group. Similarly, the normal, insulin-treated, PCBA-treated and PCLA-treated diabetic groups exhibited significantly (p<0.05) reduced in blood cholesterol and triglycerides levels after the treatment period when compared to the NT group. Moreover, serum adiponectin concentration was significantly (p<0.05) higher in the normal, insulin-treated, PCBA-treated, PCCA-treated and PCLA-treated diabetic groups as compared to the NT group. Nevertheless, serum insulin concentration was significantly (p<0.05) higher in the normal, insulin-treated and PCBA-treated groups as compared to the NT group. Lastly, AMPK, AdipoR1 and AdipoR2 mRNAs were significantly (p<0.05) upregulated in the normal, insulin-treated, PCBA-treated, PCCA-treated and PCLA-treated diabetic groups as compared to the NT group. However, there was no significant difference among groups for PPAR-α mRNA and AdipoQ mRNA. In conclusion, the present study suggested that the beef protein had the highest amount of adiponectin protein and gave the highest positive effects on STZ-induced hyperglycaemic rats. Thus, adiponectin proteins extracted from these cheaper sources of wasted adipose tissues in meat can be one of the promising target for future novel pharmacological and therapeutic treatments/preventions for insulin resistance and metabolic diseases.

خلاصة البحث

نَظراً للدورِ المفيد لبروتينِ الاديبونكتينِ على الافعالِ الحيويةِ للجسمِ و اثارهِ الوقائيةِ ضدَ الامراضِ الايضيةِ، مِنَ المهمِ أخذِ نظرةٍ اوسع على عملياتِ إفرازِ وَ تَنظيمِ البروتينِ. الهدفُ مِنْ هذهِ الِدراسةِ هوَ عَزلِ ،كَشفِ وَ تَحديدِ الكَميةِ الكُليَةِ للأَديبونكتين في الانسجةِ الدهنيةِ المهدبةِ في االبطنِ منْ مَصادرِ اللحومِ الحلالِ وَ هيَ الدجاجِ ،البقرِ وَالضأنِ المختبرةِ على الجرذان المصابةِ تحريضياً بِمَرَضِ السُكري. تَمَ عزلُ الانسجةِ الدهنيةِ في البطنِ مِنَ المصادرِ المذكورةِ انفاً وَتمت عملياتِ هَدمِ الأنسجةِ من خِلالِ إستخلآصِ الكلوروفورم \ميثانول . بعدَ ذلكَ تَمَ تَحديدُ تَركيزَ البروتين باستعمالِ طريقةِ فَحصِ البروتين بسينشونات كيت. وَأعقبَ ذلكَ تَحديدُ كميةِ الاديبونكتين بواسطةِ استعمالِ مَجموعة فحص ELISA . تَليها إختبارات في الفِئرانِ المصابةِ بالسُكري في كلكوزِ الدمِ وَالدّهونِ في الدمِ، وَقياسِ الهرمونات وَأخيراً تحديد الكَمياتِ التاليَةِ ,PPAR-α mRNA, AMPK mRNA AdipoR1 mRNA, AdipoR2 mRNA and AdipoQ mRNA في خلايا الكَبِدِ. لَقد أُجرِيَتْ التجربةٌ على ثلاثِ مراحلٍ وَ قُدِمَتْ النتائجُ كَمتوسط وانحراف معياري. تَمَ تَحليلُ البَياناتِ إحصائياً بِواسطةِ برنامجِ SPSS الاصدار IBM 21.0.تَمَ إستخدامٌ تحليلِ التباينِ الأُحاديANOVA وَ أُعتُبِرَتْ البَياناتُ مُختلفةً إحصائياً على اساسِ فاصلِ الثقَةِ بِنسبةِ 95% . وَأظهرَتْ النَتائجُ إنَ إستخلآصَ 10 غم مِنَ الأنسجةِ الدهنيةِ التي تَحتَ الجلدِ مِنَ الدَجاجِ وَ لِحمِ البَقرِ وَ الضأنِ وَ أُنْتِجَتْ تِباعاً 0.10 غم، 0.15غم ،0.15 غم مِنْ كَميةِ البروتين، وَ التي كانَتْ 1 -1.5 % مِنْ كُتلةِ الأنسجةِ الكُليةِ. إنَ تَركيزَ البروتين في الأنسجةِ الدهنيةِ البَطنيةِ مِنْ الدَجاجِ وَ لَحمِ البَقرِ وَ الضأنِ كانَ 1.25 ± 0.05 ، 1.75 ± 0.05 وَ 2.53 ± 0.05 ± 0.07 ملغم \ملل تباعا. وَكانَ تَركيز الأديبونكتين المعزول من الدجاجِ وَلحم البقر وَالضأنِ 158± 0.05 ng/ml وَ 24± 0.05 µg/mlوَ 37± 0.08 ng/ml تِباعاً. لَقد كانَ تَركيزُ الأديبونكين في الأنسجة الدهنية البطنية لِلَحمِ البقرجَيد بشكل ملحوظ ($p<0.001$) وَاعلى مُقارنةً بالدَجاجِ . المعالجةٌ الطبيعيةُ العاديةٌ للانسولينِ فَي الدراساتِ الحيوانيةِ، معالجةِ PCCA(البروتين الذي يَحتوي على أديبونكتينِ الدجاجِ)، معالجةِ PCBA(البروتين الذي يَحتوي على أديبونكتين لحمِ البقرِ) وَ معالجةِ PCLA (البروتين الذي يَحتوي على أديبونكتين لحمِ الضأنِ)، وَمجموعةٌ السكري سَجِلِو بِشكلٍ ملحوظٍ $p<0.05$ انخفاضاً بمعدلِ سكرِ الدَمِ بَعدَ فَترةٍ مِنَ العلاجِ مقارنةً مَعَ NT (مجموعة السيطرة). وَعلى نحوٍ مماثلاً، اظهرتْ دراسةٌ مُعالجةِ الأنسولينُ، معالجة PCBA، وَمعالجة PCCA و مجموعة معالجة PCLA السكري نَتائِجاً جيدةً بشكلٍ ملحوظٍ بِمعدلِ $p<0.05$ وَانخفاضٌ في مستوياتِ الكوليسترولِ في الدمِ بَعد فترةٍ مِنَ العلاجِ بالمقارَنةِ مَعَ مجموعةِ السيطرةِ وَعلاوةً على ذلكَ، كانَ تركيزُ مَصلَ الأديبونكتين أعلى بشكلٍ ملحوظٍ في المعالجاتِ العاديةِ ($p<0.05$)، لَقدْ قارنا معالجةَ الأنسولين، معالجة PCBA، معالجة PCCA، معالجة PCLA، مَعَ مَجموعةِ السيطرة. بِالإضافةِ الى ذلكَ، كانَ تركيزُ مَصلَ الأُولنسولين في المجموعةِ العاديةِ وَمجموعةِ الأنسولين المعالجة أعلى بِشكلٍ ملحوظٍ ($p<0.05$) مقارنةً بِمجموعةِ السيطرةِ . وَاخيراًAMPK, AdipoR1 and AdipoR2 mRNAs كانو منظمين بِشكل اعلى وَمَلحوظٍ ($p<0.05$) في كُلاً مِنَ المجموعةِ العاديةِ، مجموعةِ معالجةِ الأنسولين، معالجة PCBA، معالجةِPCCA ، وَ معالجة السكريPCLA مقارنةً بمجموعةِ السيطرةِ. -PPAR. عَلى الَرغمِ مِن ذلكَ، لم يَكنْ اي فرق جيد بين مجموعَتيّ AdipoQ, PPAR-α mRNA. على ان بروتين اللحم له كميه اعلى من بروتين الأديبونكتين وَكذلكَ اعطى أعلى الاثار الأيجابية على الفئرانِ المصابة تحريضيا بالسكري. بِذلِكَ، تَم إستخراجُ بروتيناتِ الأديبونكتين مِنَ الأنسجَةِ الدهنيةِ اللحميةِ المهدَرَةِ من مصادرٍ رَخيصةٍ ،التي ممكنْ أنْ تَكونَ واحِدَةً منَ الأهدافِ الواعِدَةِ المستقبليةِ للعِلاجاتِ الدوائيةِ وَالعِلاجاتِ الوقائيةِ من مرضِ السُكري والأمراضِ الأيضيةِ.

APPROVAL PAGE

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Signature Date

This project is dedicated to Allah S.W.T and His Beloved Prophet Muhammad S.A.W. I also dedicate this work to my beloved husband; Muhammad Hafrizan Bin Hassan, his loving contribution has no boundary and kept me going at difficult times. To my loving parents; Abah (Azahari Bin Mahasan) and Ummi (Che Hamidah Binti Abdullah), parents in law and siblings, who had provided me with spiritual and emotional support throughout this long journey.

ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim,

In the Name of Allah S.W.T, the Most Gracious, the Most Merciful, who has given me the strength and ability to complete my research. With Allah's willing, this work can finally be completed. All perfect praise belongs to Allah S.W.T, Lord of the Universe.

I would like to express my utmost appreciation and special thanks to my main supervisor; Associate Professor Dr. Muhammad Muzaffar Ali Khan Khattak and co-supervisor; Assistant Professor Dr. Nor Azwani Mohd Shukri for their guidance, encouragement and supervision throughout the course of this research. Their patience and hard work will always be admired.

My extended thanks go to my dear loving husband, Muhammad Hafrizan Bin Hassan for his love and patience during my study. I would also like to convey my deepest gratitude to my dear beloved parents, my brothers and sisters for their invaluable moral supports. It is my utmost pleasure to dedicate this work to all of you who have granted me the gift of your unwavering belief in my ability to accomplish this goal: thank you for your support and patience.

I would like to acknowledge the Ministry of Higher Education (MOHE) Malaysia for funding this research through the Myra Incentive Research Grant Scheme (MIRGS 13-01-002-0001). I am also thankful to the Department of Nutrition Sciences and Department of Biomedical Sciences, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia for giving me the opportunity to conduct and complete my research studies here.

Last but not least, thank you to all of my friends and everyone who were involved in helping me to complete this thesis. I wish to express my appreciation and thanks to those who provided their time, effort and support for this project.

Nuraniza Binti Azahari

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACC	Acetyl Coenzyme A carboxylase
ACC1	Acetyl-CoA carboxylase 1
ACC2	Acetyl-CoA carboxylase 2
ACO	Acetyl CoA oxidase
Acrp 30	Adipocyte complement-related protein of 30kDa
AdipoR	Adiponectin receptor
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
ANOVA	One-way analysis of variance
apMI	Adipose most abundant gene transcript 1
APPL1	Adaptor protein
AST	Aspartate transaminase
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
BCA	Bicinchoninic acid
cDNA	Complement DNA
Cl^-	Chloride
CRP	C-Reactive Protein
CVD	Cardiovascular disease
DALY	Disability adjusted life-years
DM	Diabetes Mellitus
EDTA	Ethylenediaminetetraacetate acid

ELISA	Enzyme Linked Immunosorbent Assay
eNOS	Endothelial NOS
ER	Endoplasmic reticulum
FAS	Fatty acid synthase
FBG	Fasting blood glucose
FBG	Full blood count
FFA	Free fatty acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GDM	Gestational diabetes mellitus
GDP	Gross Domestic Product
GLUT 2	Glucose transporter 2
GLUT 4	Glucose transporter 4
GSIS	Glucose-stimulated insulin secretion
H & E	Haematoxylin and eosin
Hb	Haemoglobin
HDL	High-density lipoprotein
IACUC	Institutional Animal Care and Use Committee
IDF	International Diabetes Federation
IFG	Impaired fasting glycemic
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
JNK	c-Jun N-terminal Kinase
K^+	Potassium
LDL	Low-density lipoprotein
MCH	Mean content of haemoglobin
MCHC	Mean concentration of haemoglobin in erythrocytes

MCV	Mean concentration of erythrocytes
mRNA	Messenger DNA
Na^+	Sodium
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
NCD	Non-communicable disease
NF-kB	Nuclear Factor-kB
NHMS	National Health Morbidity Survey
NPD	Normal pellet diet
p38MAPK	P38 mitogen-activated protein kinases
PA-1	Plasminogen activator inhibitor
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV	Haematocrit
PPAR-α	Peroxisome proliferator-activated receptor alpha
qRT-PCR	Quantitative Real-time polymerase chain reaction
Rab5	Ras-related protein
RBC	Red blood cell
RDW	Read cell distribution width
RT-PCR	Real-time polymerase chain reaction
SD	Standard deviation
SPSS	Statistical package social science
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TCA	Trichloro acetic acid
TG	Triglycerides

TNF-α	Tumor Necrosis Factor alpha
TZD	Thiazolidinedione
UV	Ultra-violet
WAT	White adipose tissue
WBC	White blood cell
WHO	World Health Organization
β-cell	Beta cell

LIST OF SYMBOLS

>	Greater than
$	US Dollar
%	Percentage
<	Less than
±	Plus or minus
°C	Degree Celsius
µg/ml	Microgram per milliliter
µl	Microliter
µm	Micromole
µM	Micromole
et al.,	(et alia): and others
g	Gram
g	Gravity
g/day	Gram per day
Kg	Kilogram
M	Meter
Mg	Milligram
mg/kg	Milligram per kilogram
ml	Milliliter
ml/kg	Milliliter per kilogram
mM	Millimole
mmol/L	Millimole per liter
ng/ml	Nanogram per milliliter
nm	Nanometre

RM	Ringgit Malaysia
rpm	Revolution per minute
™	Trademark
U/L	Activity unit per liter
®	Registered trademark
β	Beta
α	Alpha

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Over the recent years, there is worldwide rising prevalence of obesity, diabetes mellitus (DM) and cardiovascular diseases (CVD). One of the factors that contributes to the continuing increase of these diseases is the changing lifestyle among the population of the world. Currently, mortality is not only the main issue, but the associated co-morbidities are concerning the healthcare providers and has also negatively influenced the economy around the world. According to the World Heart Federation, risk factors for CVD include diabetes, being overweight, smoking, hypertension, hyperlipidaemia, lifestyle (sedentary lifestyle and unhealthy eating), family history and previous cardiovascular history. Diabetes, obesity and lifestyle factors have been shown to be interrelated with one another, which could then contribute to the occurrence of CVD (Mattu & Randeva, 2013).

Many evidence suggest that obesity is associated with an increased risk of insulin resistance, diabetes and CVD which are known as metabolic diseases. Obesity results from an imbalance of food intake and energy expenditure which could lead to an excessive accumulation of adipose tissue in the body. In humans, adipose tissue is composed of white and brown tissues consisting of specialized adipocytes that are primarily involved in fat storage and body insulation. Nowadays, adipose tissues are known as an active organ that not only acts as an energy storage depot, but also as a regulator for energy homeostasis. White adipose tissue is known to secrete various kinds of bioactive proteins or hormones known as adipokines. Although the mechanism is still unclear, dysregulated production or secretion of these adipokines, either by excess