UNIVERSITI PUTRA MALAYSIA

EFFECTS OF DIETARY PROTEIN LEVELS ON ABDOMINAL OBESITY SYNDROME AND BODY COMPOSITION IN RATS

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By

TAY SOOK HUI

Thesis Submitted to the School of Graduate Studies of Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree Master of Science

October 2007

Dedicated to

My Ever-Faithful God,

Beloved husband, parents, sisters, in-laws and

All the kith and kin in Healthilite Beautilite Consultation and Services

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

EFFECTS OF DIETARY PROTEIN LEVELS ON ABDOMINAL OBESITY SYNDROME AND BODY COMPOSITION IN RATS

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TAY SOOK HUI

October 2007

Chairman : Goh Yong Meng, PhD

Faculty : Veterinary Medicine

It has been hypothesized that restricting carbohydrates with protein intake was found to improve body composition by means of preservation of lean mass, higher satiety and increase thermogenesis. This would eventually improve lipid profile and glucose homeostasis either via a cause-effect of weight and fat loss or protein diet itself. The metabolic syndrome (MetS) or abdominal obesity syndrome generally consists of central/visceral obesity, impaired glucose homeostasis and dyslipidemia, has recently drawn widespread of attention. If the hypothesis of restricting carbohydrate to protein is correct, this intervention would prevent or reverse the development of the MetS.

In this study, the rats were fed with varying level of protein for 12 weeks based on the dietary protein levels classification by American Heart Association (AHA), namely CTRL group (n=7, control, 18% dietary protein), HP group (n=7, high protein, 28% dietary protein), VHP group (n=7, very high protein, 35% dietary protein). In general, between the three diet groups, the HP group had demonstrated the significantly lesser body weight and fats [total fat (TotF), abdominal fat (AF), subcutaneous fat (SF) and renal fat (RF)] followed by the VHP group ($P < 0.05$). The CTRL group, as expected exhibited higher rodent equivalent of the abdominal obesity ($P < 0.05$) and significantly higher growth in body weight and in fat (TotF, SF, and VF) ($P < 0.05$). The CTRL group also had poorer blood lipid picture and glucose tolerance that could predisposed them to the development of MetS.

Generally, blood lipids, glucose and GTT levels were found to be different across treatment groups, and the disparity becoming more apparent at 12^{th} week compared to week-6. The HP group started to exhibit significantly higher HDL-C ($P < 0.05$) compare to the CTRL group at the sixth week. At the end of the 12^{th} week, the CTRL group demonstrated notably higher amount of the TotC, LDL-C, TAG/HDL and FGlu compared to the HP and VHP groups ($P < 0.05$) but significantly lower amount of the HDL-C compared to the VHP group ($P < 0.05$). The CTRL group also displayed a significantly higher GTT compare to HP group ($P < 0.05$). After twelve weeks of dietary intervention, the LDL-C in CTRL groups was found to be

significantly higher compared to the baseline values ($P < 0.05$). Conversely VHP groups were found to be significantly reduced ($P< 0.05$).

High protein diet was found to be protective against derangement of blood lipids and blood glucose. Prolonged supplementation of a high protein diet was not shown to cause adverse effects to the bone status, liver and renal functions. Higher enzyme and metabolite values which are within normal ranges are necessary adjustments to adapt to a high protein diet. However, HP group with 28% of dietary protein appeared to be the most attractive macronutrient diet intervention, capable of inducing a favorable outcome against MetS without compromising the liver and renal functions in the rodent model.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN ARAS PEMAKANAN PROTIEN KE ATAS SINDROM ABDOMEN OBESITI DAN KOMPOSISI BADAN TIKUS

Oleh

TAY SOOK HUI

October 2007

Pengerusi : Goh Yong Meng, PhD

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Terdapat hipotesis bahawa pengurangan karbohidrat pada protien dapat membaiki komposisi badan melalui pengkekalan juzuk otot, peningkatan kekenyangan dan termogenesis. Hal ini dapat memperbaiki profil lipid dan homeostasis glukosa sama ada melalui kesan menurun berat badan atau protien secara langsung. Bagaimana sindrom metabolik atau sindrom obesiti abdomen yang biasanya merangkumi obesiti viseral, perencatan glukosa homeostasis dan dislipidemia, menarik perhation ramai. Jika hipotesis mengenai pengawalan karbohidrat terhadap protien benar, kaedah ini boleh menghindari atau merencatkan pembangunan sindrom metabolik.

Dalam kajian ini, tikus diberi makan aras protien yang berbeza berasaskan aras klasifikasi daripada ‘American Heart Association, AHA). Kumpulan CTRL (n=7, kawalan, 18% kandungan protien), kumpulan HP (n=7, protien tinggi, 28% kandungan protien), kumpulan VHP (n=7, protien lebih tinggi, 35% kandungan protien). Antara tiga kumpulan tikus, kumpulan HP menunjukkan berat badan dan lemak [jumlah lemak (TotF), lemak abdomen (AF), lemak subkutaneus (SF) dan lemak ginjal (RF) yang lebih rendah diikuti oleh kumpulan VHP ($P< 0.05$). Kumpulan CTRL menunjukkan AF yang lebih ketara ($P< 0.05$) dan lebih peningkatan berat badan dan lemak ($P< 0.05$). Kumpulan CTRL juga mempunyai profil kolesterol dan toleransi glukosa yang kurang baik. Hal ini boleh meningkatkan risiko sindrom metabolik.

Secara keselurahannya, profil lipid, glukosa dan ujian toleransi glukosa (GTT) didapati berbeza antara kumpulan kajian dan lebih ketara pada minggu ke 12 dibandingkan dengan minggu ke 6. Kumpulan HP mula menunjukkan HDL-C yang lebih tinggi dibandingkan dengan kumpulan CTRL pada minggu ke 6. Pada akhir minggu ke 12, kumpulan CTRL ternyata menunjukkan TotF, LDL-C, trialglycerol (TAG)/ HDL-C, dan aras glukosa semasa puasa (FGlu) yang lebih tinggi dibandingkan dengan kumpulan HP and VHP ($P <0.05$) tetapi HDL-C lebih rendah dibandingkan dengan kumpulan VHP ($P< 0.05$). Kumpulan CTRL juga menunjukkan GTT yang ternyata lebih tinggi dibandingkan dengan kumpulan HP ($P< 0.05$). Kumpulan CTRL menunjukkan LDL-C yang lebih tinggi

dibandingkan pada aras permulaan (P< 0.05), manakala kumpulan VHP mempunyai aras yang lebih rendah (P< 0.05).

Makanan berprotien tinggi dapat melindungi masalah ketinggian kandungan lipid dan glukosa darah. Penambahan protien tidak menunjukkan kesan sampingan ke atas status tulang, fungsi hati dan ginjal. Ketinggian aras enzim dan metabolit masih dalam lingkungan yang normal sebagai keperluan untuk menyesuaikan dengan makanan berprotien tinggi. Kumpulan HP dengan 28% kandungan protien menunjukkan keputusan yang paling menarik, berkebolehan untuk melindungi daripada sindrom metabolik dengan tidak memberikan kesan sampingan kepada fungsi hati dan ginjal dalam model roden.

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I certify that an Examination Committee has met on _____ to conduct the final examination of Tay Sook Hui on her Master of Science thesis entitled "Effects of Dietary Protein Levels on Abdominal Obesity Syndrome and Body Composition in Rats" in accordance with Universiti Pertanian Malaysia (Higher Degree) act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the revelant degree. Members of the Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Date : 21 February 2008

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

TAY SOOK HUI

Date : 9 Nov 2007

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LIST OF ABBREVIATIONS

AA	amino acid
AF	abdominal fat
AHA	American Heart Association
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
BCAAs	branched-chain amino acids
BM	bone mass
BMI	body mass index
BW	body weight
BUN	blood urea nitrogen
CCK	cholecystokinin
CLM	cumulative lean mass
CTRL	control (diet intervention group)
CVD	cardiovascular disease
DM	dry matter
FFA	free fatty acid
FGlu	fasting glucose
GGT	gamma –glutamyltransferase
GI	glycemic index

GLP-1	glucagon-like peptide-1
HC	high carbohydrate
HDL-C	high density lipoprotein cholesterol
HP	high protein (diet intervention group)
IR	insulin resistance/ resistant
IRS-1	insulin receptor substrate-1
LDL-C	low density lipoprotein cholesterol
MetS	metabolic syndrome
NCEP	National Cholesterol Education Panel
NPY	neuropeptide Y
PDCAAS	protein digestibility–corrected amino acid score
PYY	peptide YY
REE	resting energy expenditure
SF	subcutaneous Fat
T2D	type 2 diabetes
TAG	triacylglycerol
THP	total high protein groups (HP group + VHP group)
TNF	tumor necrosis factor
TotC	total cholesterol
TotF	total fat
VHP	very high protein (diet intervention group)
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Obesity, the Pathological Feature of Metabolic Syndrome and Macronutrient Diet Intervention

The prevalence of obesity and its associated metabolic abnormalities has become a global pandemic and increased markedly over the past two decades, affecting billion worldwide (Flegal, 1998; Must *et al.*, 1999; Kimm and Obarzanek, 2002; WHO, 2003). The consequences of health complication due to obesity and its related health complications cause an economic burden approximating $100 billion (Thompson *et al.*, 1999), and the preventable deaths attributed to obesity exceed 300,000 heads per year (Allison *et al.*, 1999). Obesity, particularly abdominal obesity, often predisposes to a cluster of pathologies including cardiovascular and metabolic disorders like impaired glucose regulation or Impaired Glucose Tolerance or type 2 diabetes (T2D), insulin resistance (IR), hyperinsulinemia, hypertriglycemia, low high density lipoprotein (HDL) cholesterol concentrations and hypertension; collectively referred to as the features of metabolic syndrome (MetS) (Reaven, 1988, DeFronza, 1997; Balkau and Charles, 1999; WHO, 1999; Reaven, 2000; NCEP 2001). The prevalence of the MetS is approximately 25% of the general human population and may vary with an individual's genetic background (Ford *et al*., 2002). Obesity and the features of MetS arise in parallel from

disruptions of insulin metabolism leading to insulin resistance (IR). The IR together with compensatory hyperinsulinemia have been shown to be independent predictors of clinical syndromes of T2D and cardiovascular disease (Kissebah, 1997; Yip *et al.*, 1998; Zimmet *et al.*, 2001; Reaven, 2001; Facchini *et al.*, 2001).

The MetS, also referred to as "Diabesity" (Astrup and Finer, 2000) describes the increasing incidence of diabetes in combination with obesity particularly abdominal obesity. Numerous studies have provided evidences of MetS as a major cause for T2D. Firstly, MetS is usually present ten-twenty years before the onset of T2D (Warram *et al.*, 1990; Lillioja, *et al.*, 1988). Secondly, cross-sectional studies demonstrate that MetS particularly via IR occur consistently in patients with T2D (Lillioja, *et al.*, 1988; Reaven *et al.*, 1976; DeFronza, 1988). Finally, prospective studies demonstrate that the features of MetS are the best predictor of whether or not an individual will become diabetic in the future (Warram *et al.*, 1990; Lillioja, *et al.*, 1988). Currently, the prevalence of T2D has reached epidemic proportions worldwide, and is projected to increase dramatically (Zimmet *et al.*, 2001). It is estimated that by the year 2020, approximately 250 million people will be affected by Type 2 diabetes mellitus worldwide (O'Rahilly, 1997; Zimmet *et al.*, 2001).

Cardiovascular disease (CVD) remain the leading causes of death in most industrialized countries, and their importance as a public health problem is

increasing in developing countries like Malaysia (Thom *et al.*, 1992; Anonymous, 1991). It has become increasingly apparent that elevated low density lipoprotein cholesterol (LDL-C) concentrations are not the only and, possibly not even the major risk factor for CVD. The IR, as can be indicated by it's subset metabolic marker (high ratio of triacylglycerol (TAG: HDL), appear to represent CVD risk factors that are at least as powerful as a high LDL-C concentration (Reaven, 1988, Jeppesen *et al*., 1997a; McLaughlin *et al*., 2000; McLaughlin *et al*., 2003; McLaughlin *et al*., 2005).

The optimal diet for improving MetS has been the focus of much research, and there remains no consensus on macronutrient composition apart from recommendations that saturated fats be kept low and caloric restriction (ADA, 2000; St Jeor *et al.*, 2001). Although the Malaysian urban diet is describe as low in fat and cholesterol (Ng, 1995) complying to the American Heart Association (Poleman and Peckenpaugh, 1991) and World Health Organization (1986, 1990) dietary guidelines, the high risk of population suffering from the progression of MetS into T2D exists. Alberti and Zimmet (1998a; 1998b), representing WHO, envisaged that by the year 2010, the number of T2D will reach 221 million, with the greatest increases in Asia including countries like Malaysia and Africa. Weight-reduction efforts have been made as shown by an overall decline in the proportion of total fat intake to approximately 34% of kilocalories per day (Anonymous, 1998). However, there has been an apparent concomitant increase in total energy intake and significant weight gains have been observed over time