ผลของอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูงร่วมกับการออกกำลังกายต่อเมแทบอลิซึมของคีโตน และการควบคุมระดับน้ำตาลในเลือดในหนูเบาหวาน

นายคุณคง หูชัยภูมิ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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EFFECTS OF HIGH-MONOUNSATURATED FAT DIET IN COMBINATION WITH EXERCISE TRAINING ON KETONE METABOLISM AND GLYCEMIC CONTROL IN DIABETIC RATS

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A Thesis Submitted in Partial Fulfillment of the Requirements
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Thesis Title	EFFECTS OF HIGH-MONOUNSATURATED FAT DIET IN COMBINATION WITH EXERCISE TRAINING ON KETONE METABOLISM AND GLYCEMIC CONTROL IN DIABETIC RATS
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คุณคง หูชัยภูมิ : ผลของอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูงร่วมกับการออกกำลังกายต่อเมแทบอลิซึมของคีโตนและการควบคุมระดับน้ำตาลในเลือดในหนูเบาหวาน (EFFECTS OF HIGH-MONOUNSATURATED FAT DIET IN COMBINATION WITH EXERCISE TRAINING ON KETONE METABOLISM AND GLYCEMIC CONTROL IN DIABETIC RATS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.พญ.จุไรพร สมบุญวงค์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.ดร.พญ.อรอนงค์ กุละพัฒน์, 94 หน้า.

การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาผลของอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูงร่วมกับการออกกำลังกายต่อเมแทบอลิซึมของคีโตนและการควบคุมระดับน้ำตาลในเลือดในหนูเบาหวาน โดยหนูแรทเพศผู้ สายพันธุ์วิสตาร์ จำนวน 38 ตัว ถูกเหนี่ยวนำให้เป็นเบาหวานด้วยการฉีดสเตรปโตโซโตซิน เข้าทางช่องท้อง ขนาด 55 มิลลิกรัมต่อ 1 กิโลกรัมน้ำหนักตัว แบ่งหนูเบาหวานเป็น 5 กลุ่ม ได้แก่ 1) กลุ่มควบคุมที่กินอาหารปกติ (n=7) 2) กลุ่มกินอาหารที่มีไขมันอิ่มตัวสูง (n=8) 3) กลุ่มกินอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูง (n=8) 4) กลุ่มออกกำลังกายที่กินอาหารปกติ (n=7) 5) กลุ่มออกกำลังกายที่กินอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูง (n=8) หนูกลุ่มที่ 4 และ 5 ออกกำลังกายบนลู่วิ่งสายพาน วันละ 2 ครั้ง 5 วันต่อสัปดาห์ ทำการทดลองเป็นเวลา 12 สัปดาห์ เมื่อครบกำหนดเก็บตัวอย่างเลือดจากหัวใจเพื่อตรวจหาระดับกลูโคส ฮีโมโกลบินเอวันซี อินสุลิน กรดไขมันอิสระ และเบต้าไฮดรอกซีบิวทีเรต เก็บตับและกล้ามเนื้อแกสตร็อกเนเมียสเพื่อวัดการทำงานของเอนไซม์เบต้าไฮดรอกซีบิวทีเรตดีไฮโดรจีเนสในวิถีการสร้างคีโตนที่ตับ และเอนไซม์ 3-คีโตเอซิลโคเอทรานสเฟอเรสในวิถีการใช้คีโตนที่กล้ามเนื้อตามลำดับ

ผลการวิจัยพบว่า เมื่อเทียบกับกลุ่มควบคุม กลุ่มที่ได้รับอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูงมีการลดระดับน้ำตาลในเลือดได้อย่างมีนัยสำคัญทางสถิติ แต่ไม่ลดระดับฮีโมโกลบินเอวันซี กรดไขมันอิสระและเบต้าไฮดรอกซีบิวทีเรต อีกทั้งไม่มีผลต่อการทำงานของเอนไซม์ในเมแทบอลิซึมของคีโตน กลุ่มออกกำลังกายที่กินอาหารปกติไม่มีการลดระดับน้ำตาลในเลือด แต่สามารถลดกรดไขมันอิสระได้อย่างมีนัยสำคัญทางสถิติ และมีแนวโน้มลดเบต้าไฮดรอกซีบิวทีเรต ส่วนหนูกลุ่มที่ได้รับอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูงร่วมกับการออกกำลังกายมีการลดระดับน้ำตาลฮีโมโกลบินเอวันซี กรดไขมันอิสระและเบต้าไฮดรอกซีบิวทีเรตในเลือดได้อย่างมีนัยสำคัญทางสถิติ การทำงานของเอนไซม์เบต้าไฮดรอกซีบิวทีเรต ดีไฮโดรจีเนสลดลงและการทำงานของเอนไซม์ 3-คีโตเอซิดโคเอ ทรานสเฟอร์เรสเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติเฉพาะในกลุ่มออกกำลังกายที่กินอาหารปกติ และกลุ่มที่ได้รับอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูงร่วมกับการออกกำลังกาย

สรุปได้ว่า อาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูงไม่มีผลต่อเมแทบอลิซึมของคีโตน ส่วนอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูงร่วมกับการออกกำลังกายสามารถควบคุมระดับน้ำตาล ลดระดับกรดไขมันอิสระและคีโตนในเลือดได้ดีกว่าอาหารที่มีไขมันไม่อิ่มตัวเชิงเดี่ยวสูงอย่างเดียวหรือการออกกำลังกายอย่างเดียว การลดคีโตนผ่านกลไกที่ลดระดับการทำงานของเอนไซม์ในวิถีการสร้างคีโตนจากตับและเพิ่มระดับการทำงานของเอนไซม์ในวิถีการใช้คีโตนที่กล้ามเนื้อ

สาขาวิชา วิทยาศาสตร์การแพทย์ ลายมือชื่อนิสิต คุณคง หูชัยภูมิ

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KHUNKHONG HUCHAIYAPHUM: EFFECTS OF HIGH-MONOUNSATURATED FAT DIET IN COMBINATION WITH EXERCISE TRAINING ON KETONE METABOLISM AND GLYCEMIC CONTROL IN DIABETIC RATS. THESIS ADVISOR: ASSOC. PROF. JURAIPORN SOMBOONWONG, M.D., M.Sc., THESIS CO-ADVISOR: ASSOC. PROF. ONANONG KULAPUTANA, M.D., Ph.D. 94 pp.

The present study aimed to investigate the effects of high-monounsaturated fatty acid (MUFA) plus exercise on ketone metabolism and glycemic control in rats with diabetes mellitus (DM). Diabetes was induced in male Wistar rats (n=38) by intraperitoneal injection with streptozotocin (55 mg/kg BW). The diabetic rats were divided into five groups: 1) Control: DM + regular diet (n=7) 2) DM + high-saturated fatty acid (SFA) diet (n=8) 3) DM + high-MUFA diet (n=8) 4) DM + exercise (Ex) + regular diet (n=7) 5) DM + high-MUFA + Ex (n=8). Exercise training program was carried out progressively on a treadmill twice daily, 5 days/wk for twelve weeks. At the end of experiment, cardiac blood was rapidly taken for measuring plasma glucose, HbA_{1C}, insulin, serum free fatty acid (FFA) and serum β-hydroxybutyrate. The liver and gastrocnemius muscle were collected to determine enzymatic activity of β-hydroxybutyrate dehydrogenase used in hepatic ketone body synthesis pathway and 3-ketoacyl-CoA transferase used in muscle ketone body utilization pathway, respectively.

The results revealed that when compared with Control, DM+MUFA group showed a significant decrease ($p<0.05$) in plasma glucose but not HbA_{1C}, FFA and β-hydroxybutyrate levels. In DM+Ex group, plasma glucose and HbA_{1C} were unchanged while FFA was significantly decreased ($p<0.05$) and β-hydroxybutyrate tended to decrease. In DM+MUFA+Ex group there was a significant decrease ($p<0.05$) in plasma glucose, HbA_{1C}, serum FFA and serum β-hydroxybutyrate levels. The activity of β-hydroxybutyrate dehydrogenase was significantly decreased ($p<0.05$) and that of 3-ketoacyl-CoA transferase was significantly increased only in DM+Ex and DM+MUFA+Ex groups.

In conclusion, MUFA alone did not affect ketone metabolism. A combination of MUFA and exercise was more effective than either MUFA or exercise alone for improvement of glycemic control and ketone metabolism. The decrease in ketone bodies level was mediated by decreasing the activity of hepatic ketone synthesis and increasing the activity of muscle ketone utilization pathway.

Field of Study : Medical Science Student's Signature Khunkhong Huchaiyaphum

Academic Year : 2010 Advisor's Signature Juraiporn Somboonwong

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LIST OF ABBREVIATIONS

AChE	Acetylcholinesterase
ACOD	Acyl-CoA oxidase
ACS	Acyl-CoA synthetase
ANOVA	Analysis of variance
ATP	Adenosine-5'-triphosphate
Bo	Maximum binding
BG	Blood glucose
BHB	β-hydroxybutyrate
BI	Reagent blank
BW	Body weight
CHO	Carbohydrate
Chol	Cholesterol
CoA	Co-enzyme A
CSF	Cerebrospinal fluid
DKA	Diabetic ketoacidosis
DM	Diabetes mellitus
EDTA	Ethylene diamine tetraacetic acid
EIA	Enzyme immunoassay
Ex	Exercise training
FFA	Free fatty acid
G6PDH	Glucose-6-phosphate dehydrogenase
GDM	Gestational diabetes mellitus
GLUT	Glucose transporter
HbA_{1c}	Glycated hemoglobin
HDL	High density lipoprotein
HK	Hexokinase
HMG-CoA	Hydroxy-methylglutaryl-CoA

IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
i.p.	Intra-peritoneal
KOH	Potassium hydroxide
LDL	Low density lipoprotein
MEHA	3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline
$MgCl_2$	Magnesium chloride
MOPS	3-(N-morpholino) propanesulfonic acid
MUFA	Monounsaturated fatty acid
NADH	Nicotinamide adenine dinucleotide
NaF	Sodium fluoride
NEFA	Non-esterified fatty acid
NSB	Non-specific binding
O.D.	Optical Density
POD	Peroxidase
pTA	Phosphotransacetylase
PUFA	Polyunsaturated fatty acid
S	Sample
SBI	Sample blank
SD	Standard deviation
SFA	Saturated fatty acid
Std	Standard
STZ	Streptozotocin
TG	Triglyceride
TTAB	Tetradecyl trimethylammonium bromide
VLDL	Very low density lipoprotein

CHAPTER I

INTRODUCTION

Background and Rationale

Nowadays, more people are living sedentary lifestyle and consuming energy-rich diets. This leads to lifestyle related diseases such as diabetes and other non-communicable diseases that share common risk factors. It is estimated by the International Diabetes Federation (2010) that 285 million people around the world have diabetes. This total is expected to increase up to 438 million within 20 years.

Diabetes mellitus is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. As a result, there is an increased concentration of glucose in the blood, often leading to various complications (WHO, 2009). There are principally three types of diabetes mellitus: type 1, type 2 and gestational diabetes. Type 1 diabetes results from the body's failure to produce insulin. This type of diabetes accounts for about 5 percent of all diabetic patients. Type 2 diabetes is the most common form of diabetes that is characterized by high blood glucose due to insulin resistance and relative insulin deficiency. Gestational diabetes is a condition in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy (Current Medical Diagnosis & Treatment, 2002).

Diabetes mellitus increases risk for many serious health problems and complications. Acute complications include hypoglycemia, hyperglycemia, ketoacidosis and hyper-osmolar syndrome. Chronic complications include heart disease, kidney disease, neuropathy, disease of the eyes and peripheral vascular disease (WHO, 2009). Diabetic ketoacidosis (DKA) is a potentially life-threatening complication in patients with diabetes mellitus. It happens predominantly in those with type 1 diabetes, but it can occur in those with type 2 diabetes under certain circumstances. DKA results from a deficiency of insulin, so the body cannot utilize glucose as a source of energy. In

response the body switches to burning fatty acids and producing acidic ketone bodies that cause excess morbidity and mortality (The American Diabetes Association, 2006). Studies in streptozotocin (STZ)-induced diabetic rat model have shown that the elevation of ketone bodies can be explained in part by an increase in serum free fatty acids and in part by a decreased activity of skeletal muscle 3-ketoacyl-CoA transferase (Midaoui *et al*., 2005) as well as an increased activity of the hepatic ketone body synthesis pathway (Midaoui *et al*., 2006).

A treatment of diabetes requires a strict regimen that typically includes home blood glucose testing several times a day, dietary control, drug therapy, multiple daily insulin injections, and planned exercise activity. Carefully controlled diet includes consumption of low carbohydrate diet, low fat diet or daily caloric intake control (LeRoith, 2004). Current dietary recommendations for diabetic patients have recently prescribed replacement of saturated fatty acid (SFA) with higher consumption of monounsaturated fatty acid (MUFA) (American Diabetes Association, 2000).

MUFAs are fatty acids that have a single double bond in the fatty acid chain and all of the remainder of the carbon atoms in the chain are single-bonded. MUFA was demonstrated to be more effective than low-fat, high-CHO diet of similar fiber content diets for efficient glycemic control, improving insulin sensitivity, lowering blood triglyceride and VLDL cholesterol levels, and elevating HDL cholesterol levels in diabetic rats (Kotake *et al*., 2004; Ramesh *et al*., 2006,) and type 2 diabetic patients (Garg *et al*., 1988, 1994; Parillo *et al*., 1992; Rasmussen *et al*., 1993; Campbell *et al*., 1994; Wright, 1998; Kris-Etherton, 1999; Rodriguez-Villar *et al.,* 2004; Yokoyama *et al.,* 2008).

Exercise training has also been known to have beneficial effects on diabetes, including reduction of blood glucose, blood pressure, body weight, body fat, and more specifically mobilization of abdominal and visceral adipose tissue as well as increased insulin sensitivity, improvement of atherogenic lipoprotein profile and inflammatory elements (Li *et al*., 2003; Despres, 2005). There is evidence that exercise training can improve glucose and ketone metabolism in diabetic rats. These include decreased blood glucose level (Dall'Aglio *et al*., 1983; Goodyear *et al.,* 1988; Chakraphan *et al*.,

2005; Nakai *et al*., 2002; Boule *et al.,* 2005; Ozakaya *et al*., 2007), increased insulin sensitivity, increased glucose homeostasis, lowered free fatty acid and decreased triglyceride synthesis from liver (Beattie and Winder., 1985; Winder *et al*., 1974; Midaoui *et al.,* 1996; Goodyear *et al*., 1988; Chakraphan *et al*., 2002; Nakai *et al.,* 2002; Ozakaya *et al*., 2007). Furthermore, exercise training is reported to reduce β-hydroxybutyric acid level in streptozotocin-induced diabetic rats by increasing 3-ketoacyl-CoA transferase enzyme activity of ketone utilization in gastrocnemius muscle (Midaoui *et al.,* 2005) and decreasing β-hydroxybutyrate dehydrogenase enzyme activity of ketone synthesis pathway in liver (Midaoui *et al*., 2006). However, there are some controversial reports that exercise training does not affect blood glucose level (Midaoui *et al.,* 1996, 2005, 2006).

Since the major effect of MUFA is to improve glucose metabolism which leads to a reduced free fatty acid level, a substrate of ketone body synthesis, MUFA is likely to ameliorate diabetic-induced ketone formation and utilization. However, there is no study regarding effects of high-MUFA on ketone metabolism. There is also no report showing a synergistic effect of high-monounsaturated fat diet in combination with exercise training on ketone metabolism and glycemic control. Therefore, the purpose of the present study is to investigate the effects of high-MUFA diet with/without exercise training on ketone metabolism and glycemic control in diabetic rat model.

Research Questions

1. Does high-monounsaturated fat diet improve ketone metabolism?

2. Do high-monounsaturated fat diet combined with exercise training have a synergistic effect on improvement of glycemic control and ketone metabolism?

3. If so, whether the effects of high-monounsaturated fat diet with/without exercise training on ketone metabolism is mediated by decreasing hepatic ketone synthesis pathway and/or increasing muscle ketone utilization pathway?

Research Objective

1. To examine the effects of high-monounsaturated fat diet on ketone metabolism in diabetic rats.

2. To examine whether high-monounsaturated fat diet in combination with exercise training can synergistically improve glycemic control and ketone metabolism in diabetic rats.

3. To investigate the effects of high-monounsaturated fat diet with/without exercise training on changes of enzymatic activity in hepatic ketone synthesis and muscle ketone utilization pathway.

Hypothesis

1. High-monounsaturated fat diet can decrease serum ketone level in diabetic rats.

2. High-monounsaturated fat diet combined with exercise training can synergistically improve glycemic control and ketone metabolism.

3. The effects of high-monounsaturated fat diet with/without exercise training on ketone metabolism is mediated through a decrease in hepatic ketone synthesis pathway and/or an increase in muscle ketone utilization.

Expected Benefit and Application

The experimental study would give the information on the effects and the possible mechanisms of high-monounsaturated fat diet alone or in combination with exercise training on glycemic control and ketone metabolism. It might be applied for the treatment of type 1 diabetic patients with DKA.

CHAPTER II

LITERATURE REVIEW

DEFINITION AND CLASSIFICATION OF DIABETES MELLITUS

Definition

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Thus, diabetes covers a wide range of heterogeneous diseases. (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus., 2001)

Classification

The classification of diabetes mellitus can be divided into five groups as follows: (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus., 2001)

1. Type 1 Diabetes mellitus

Type 1 diabetes is caused by a destruction of the pancreatic beta cells leading to absolute insulin deficiency. The most common form is type 1A, caused by autoimmune destruction of β-cell; type 1B is also associated with severe insulin deficiency, but there is no evidence of autoimmunity. Type 1 diabetes is dominated by polyuria, polydipsia, polyphagia and ketoacidosis. Despite an increased appetite, catabolic effects prevail resulting in weight loss and muscle weakness. These effects are the consequence of metabolic derangements, mainly hyperglycemia (Cotran, 2003).

2. Type 2 Diabetes mellitus

Type 2 diabetes mellitus are characterized by two metabolic defects (1) a derangement in β-cell secretion of insulin and (2) a decreased response of peripheral tissues to insulin (insulin resistance) (Cotran, 2003). Unlike type 1 diabetes, there is no evidence that autoimmune mechanisms are involved. Lifestyle clearly plays a role, especially when obesity is considered (Cotran, 2003).

3. Other specific types of diabetes

This category of diabetes includes a wide variety of unrelated disorder that cannot be categorized as either type 1 or type 2 diabetes mellitus as follows;

a. Genetic defect of the β-cell function

b. Genetic defect in insulin action

c. Diseases of the exocrine pancreas

d. Endocrinopathies

e. Drug or chemical-induced diabetes

f. Infections

g. Uncommon forms of immune-mediated diabetes

h. Other genetic syndromes sometimes associated with diabetes

4. Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus refers to diabetes mellitus firstly recognized during pregnancy, caused by insulin resistance and relative insulin deficiency. This type of diabetes occurs in approximately 3 to 5 percent of all pregnancies.

5. Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG)

The term IGT and IFG refer to a metabolic stage intermediate between normal glucose homeostasis and diabetes. This stage includes individuals who have IGT and individuals with fasting levels of more than or equal to 110 mg/dL but less than 126 mg/dL (IFG). IGT and IFG are considered to be a stage in the natural history of disordered glucose metabolism, which can lead to any types of diabetes. There is an increased risk of cardiovascular disease, but little or high risk of microvascular disease. However, some patients may revert to normoglycemia.

DIABETIC COMPLICATIONS

The morbidity associated with long-standing and poor control diabetes of either type results from a number of serious complications. The important morphologic changes related to the many late systemic complications. Diabetes mellitus are likely to be acute complications including ketoacidosis or hyperosmolar coma. Chronic complications involve many organs and tissues including arteries (atherosclerosis),

kidneys (nephropathy), retina (retinopathy) and nerves (neuropathy). These changes are seen in both type 1 and type 2 diabetes (Cotran, 2003).

METABOLIC ABNORMALITIES IN DIABETES

Diabetes mellitus results in the abnormalities of glucose, lipid, proteins and ketone metabolism as follows:

1. Glucose metabolism: Hyperglycemia

Specific abnormalities of glucose metabolism in diabetes may exist and may be related causally to pathogenesis of macro- and microvascular complications (Kashiwagi *et al*., 1996). Numerous investigations have concluded that causal factor primarily responsible for the development of most diabetic complication is probably prolonged exposure to hyperglycemia (Pickup and Gareth, 2003). However, the exact mechanism of hyperglycemia detrimental effect is not clear. It is quite possible that hyperglycemia mediates its adverse effects via multiple mechanisms, since glucose and its metabolites are utilized by numerous pathways (Kahn and Weir, 1994). It appears to involve the polyol pathways which may contribute to increased oxidative stress, a complication in diabetes (Baynes, 1991; Kashiwagi *et al*., 1996, Gingliano *et al*., 1996).

A familiar example of a protein glycated in polyol pathways is glycated hemoglobin (HbA_{1C}). Considerable attention has been given recently to the post-translation glycosylation of proteins in diabetes, particularly with respect to hemoglobin. Chromatography of adult hemoglobin yields a major fraction (more than 90% of the total) of hemoglobin A in front of which are three fast fractions; HbA_{1C} comprise 4% to 6% of HbA , with the other fractions comprising 1-2% each. These glycated hemoglobin are formed non-enzymatically at a rate dependent on the ambient glucose concentration.

HbA_{1C} has been best characterized. Glucose combines with the N-terminal valine of the β-chain of HbA to yield an almidine. This spontaneously undergoes the amadori rearrangement to yield a ketoamine-the terminal product being 1-amino, 1-deoxyfructose (Keen, 1999).

2. Lipid metabolism: Dyslipidemia

Lipid and lipoprotein

Diabetic patients particularly those with type 2 diabetes, commonly have abnormalities of plasma lipids and lipoprotein concentrations, and dyslipidemia outweighs all of the other major cardiovascular risk factors (i.e., hypertension, glucose intolerance, obesity) in this patient population. Individuals with poorly controlled type 1 diabetes also frequently present with dyslipidemia, but the pattern differs from that in type 2 diabetes (DeFronzo, 1998).

Lipid and lipoprotein disturbances occur more frequently in type 2 diabetic patients than those with type 1 diabetes. The characteristic dyslipidemia is already present at the pre-diabetic stage of impaired glucose tolerance. The most common abnormality is hypertriglyceridemia, which is often associated with low levels of high-density lipoprotein (HDL) cholesterol, while total and low density lipoprotein (LDL) cholesterol concentrations are similar to non-diabetic levels (see Table 2-1). The lipid and lipoprotein abnormalities in type 2 diabetes are related closely to insulin resistance and elevated insulin concentrations. Hyperinsulinemia has been shown to precede the development of other metabolic abnormalities (Pickup and Gareth, 2003).

In well-controlled type 1 diabetic patients, serum lipid and lipoprotein concentrations are similar to those in non-diabetic people. Indeed, some studies have reported lower levels of very low density lipoprotein (VLDL) and LDL and higher levels of HDL. However, disordered lipid and lipoprotein metabolism is common in poor glycemic control, with increased concentrations of triglyceride-rich lipoprotein, chylomicrons and VLDL (see Table 2-1). Insulin deficiency is associated with increased hepatic production of apoprotein β-containing lipoproteins and ineffective lipoprotein clearance due to decreased activity of the insulin-dependent lipoprotein lipase. Severely insulin-deficient patients with ketosis may develop severe lipidemia with chylomicronemia. These abnormalities are rapidly improved with insulin therapy, through a decreased hepatic lipoprotein production and an increased lipoprotein lipase activity (Pickup and Gareth, 2003).

Table 2-1 Characteristic of dyslipidemia in diabetes (Pickup and Gareth, 2003)

	Serum lipid						
	Chol	TG	VLDL	LDL	HDL	ApoB	ApoA-1
Type 1DM							
Good control	N / ↓	N / ↓	N / ↓	N / ↓	N / ↑	↑	N / ↑
Poor control	↑	↑	↑	N / ↑	N	↑	N
Nephropathy	↑	↑	↑	↑	↓	↑	↓
Type 2 DM							
Good control	N / ↑	↑	↑	N	↓	N	N / ↓
Poor control	↑	↑	↑	↑	↓	↑	↓

Chol, cholesterol; TG, triglyceride; N, normal; ↓ ↑, lower, higher than normal, respectively.

In 2001, Iszior-Walus *et al*. assessed the determinants and prevalence of hyperlipidemia in 3,159 type 1 diabetic patients. They found that plasma total cholesterol, HDL, and HDL subfractions were higher in women than in men, while plasma triglycerides were higher in men. Total cholesterol, LDL and HDL and HDL subfractions were, as expected, significantly associated with age and HbA_{1c} in both sexes. In a separate study, it was found that after 12 weeks of diabetic induction with a single injection of streptozotocin (STZ; 45 mg/kg BW, i.p.) in rats, there was a significant increase in blood glucose, plasma cholesterol and triglyceride levels (Ozansoy *et al*., 1999).

3. Protein metabolism: Increased protein breakdown

Protein synthesis and breakdown are regulated by substrate availability and the hormonal milieu, the important one of which is insulin. Profound changes in body composition occur after the initiation of therapy in people with type 1 diabetes, especially if insulin deficiency has been severe and prolonged (Geyelin, 1992). Insulin deprivation increases the concentration of circulating amino acids, due to the net increase in protein breakdown, and with an accompanying decline in amino-acid disposal (utilization in protein synthesis or amino-acid oxidation) (Felig *et al*., 1977; Nair *et al*., 1989). Urinary nitrogen excretion, a marker of protein catabolism, increases

during insulin deprivation. If there is a prolonged, insulin deprivation, cachexia and a loss of muscle mass occur (Walsh and Wright, 1995).

Glucagon secretion is enhanced by ingestion of protein and facilitates disposal of glucogenic amino acids such as alanine or glutamine. The elevated glucagon concentrations present in poorly controlled type 1 diabetes stimulate alanine and glutamine uptake, resulting in normal or low concentrations despite increased appearance from protein breakdown due to insulin deprivation (Charlton, 1996). Concentrations of branched-chain amino acids are elevated in these situations, but are rapidly lowered to non-diabetic levels by treatment with insulin (Felig *et al.*, 1977, Nair *et al.*, 1989).

Free fatty acid

Insulin is the main hormonal regulator of lipolysis. Increasing plasma glucose concentrations (e.g. after meal) normally lead to increased insulin secretion, which inhibits lipolysis (Roust and Jensen, 1993). This insulin-induces suppression of FFA concentrations enhances insulin-dependent glucose disposal and insulin-induced suppression of endogenous glucose production (Saloranta *et al.,* 1991). These events do not occur in type 1 diabetes, due to a lack of endogenous insulin secretion, and are completely dependent on appropriate timing and dosing of exogenous insulin.

Conversely, in non-diabetic individuals, falling blood glucose concentrations increase lipolysis due to suppression of insulin secretion (Klein and Wolfe, 1992). The resulting rise in FFAs will stabilize or raise glucose concentrations. However, hypoglycemia caused by exogenous hyper-insulinism will also suppress lipolysis and impair counter-regulation.

Absolute or relative insulin deficiency is responsible for most of the excess FFAs available for oxidation in type 1 diabetes. Elevated FFAs directly impair peripheral glucose uptake (Kelley *et al*., 1993) and, at least acutely, stimulate endogenous glucose production (Ferrannini *et al*., 1983). Another consequence of elevate FFA flux is increased ketogenesis, a precursor to ketoacidosis (Mile *et al*., 1983). Insulin is able to counteract the lipolytic effects of other hormones, so that growth hormone (GH) or

cortisol have little effect on lipolysis unless insulin availability is reduced (Dinneen *et al*., 1995). Similarly, the lipolytic effect of catecholamines is blunted by hyperinsulinemia and accentualed by hypoinsulinemia (Jensen et al., 1987). Although glucagon has no effect on systemic FFA availability, increased concentrations, as seen in uncontrolled diabetes, may drive hepatic metabolism towards ketogenesis (Mile *et al*., 1983).

4. Ketoacidosis

Ketocidosis means dangerously high levels of ketones in the blood. It is a potentially life-threatening complication in patients with diabetes mellitus. It happens predominantly in those with type 1 diabetes, but it can occur in those with type 2 diabetes under certain circumstances. DKA results from an absolute shortage of insulin; in response the body switches to burning fatty acids and producing acidic ketone bodies that cause most of the symptoms and complications (Kitabchi *et al*., 2006).

Ketoacidosis most frequently occurs in those who already have diabetes. It may also be the first presentation of previously undiagnosed diabetes. There is often a particular underlying problem that has led to the DKA episode. This may be intercurrent illness (pneumonia, influenza, gastroenteritis, urinary tract infection), pregnancy, inadequate insulin administration (e.g. defective insulin pen device), myocardial infarction (heart attack), stroke or the use of cocaine. Young patients with recurrent episodes of DKA may have an underlying eating disorder, or may be using insufficient insulin for fear that it will cause weight gain (Powers, 2005). In 5% of cases, no cause for the DKA episode is found (Kitabchi *et al*., 2006).

The ketone bodies have a low pH and turn to the blood acidic (metabolic acidosis). The body initially buffers with the bicarbonate buffering system and other mechanisms to compensate for the acidosis, such as hyperventilation to lower the blood carbon dioxide levels. The hyperventilation attended extreme form such as Kussmaul respiration (Powers, 2005; Kitabchi *et al*., 2006). Ketones bodies participate in osmotic and lead to further electrolyte losses. Vomiting, dehydration, deep gasping breathing, confusion and occasionally coma are typical symptoms (Kitabchi *et al*., 2006).

Ketoacidosis is diagnosed with blood and urine tests; it is distinguished from other, rarer forms of ketoacidosis by the presence of high blood sugar levels. Treatment involves intravenous fluids to correct dehydration, insulin to suppress the production of ketone bodies, treatment for any underlying causes such as infections, and close observation to prevent and identify complications (Dunger *et al.,* 2004; Kitabchi *et al*., 2006).

KETONE METABOLISM IN KETOACIDOSIS

Ketoacidosis arises because of absolute lack of insulin in the body. The lack of insulin leads to increased release of glucose by the liver from glycogen and increased gluconeogenesis. The absence of insulin also leads to the release of free fatty acids (FFA) from adipose tissue. Free fatty acids are then converted in the liver into ketone bodies such as acetone, acetoacetate and β-hydroxybutyrate. β-hydroxybutyrate is transferred into the circulation and then utilized on peripheral tissues. β-hydroxybutyrate can serve as an energy source for the brain in absence of insulin-mediated glucose uptake, which is likely a protective mechanism in case of starvation.

1. Ketone body synthesis pathway

Ketone bodies are synthesized in liver mitochondria by four-step enzymatic pathway (figure 2-1). Two molecules of acetyl-CoA derived from FFA are used to generate into acetoacetyl-CoA by acetyl-CoA thiolase. Acetoacetyl-CoA is then converted by HMG-CoA synthase into HMG-CoA, which is further converted into acetoacetate by HMG-CoA lyase. Finally, acetoacetate is converted into acetone by acetoacetate decarboxylase, and into β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase.

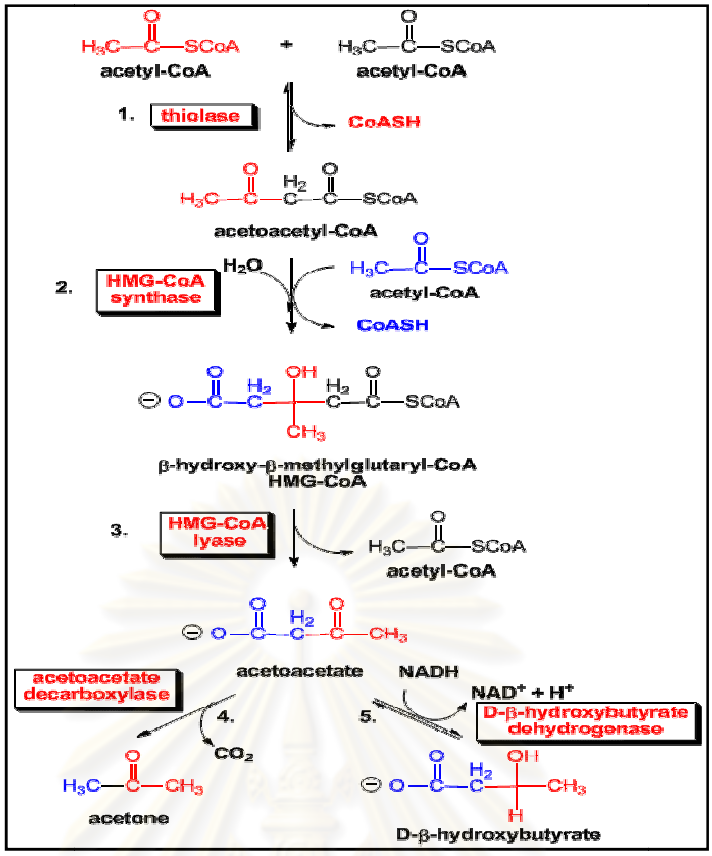

Figure 2-1 Ketone bodies synthesis pathway in liver mitochondria (www.themedicalbiochemistrypage.org)

It has been demonstrated in severe uncontrolled diabetes by using hepatic catheterization that up to 80-90% of the FFA taken up by the liver is converted to ketone bodies (Owen *et al.,* 1977; Wahren *et al.,* 1975). STZ-induced diabetes in the rat was associated with an 80% increase in FFA levels and a 700% increase in β-hydroxybutyrate. This large disproportional rises in FFA and β-hydroxybutyric acid are most likely explained by the more efficient liver in synthesizing ketone bodies from FFA (Midaoui *et al*., 2006).

It was reported that in alloxan-induced diabetes the activity of HMG-CoA synthase from rat liver extracts doubled (Quant *et al.,* 1989). Williamson *et al*., (1968) have also demonstrated that the activity of HMG-CoA synthase, HMG-CoA lyase and acetoacetyl-CoA thiolase was increased by 90%, 35% and 4%, respectively in whole liver homogenate from alloxan-diabetic rats. Furthermore, the overall activity of the hepatic ketone body synthesis pathway, as measured by the activity of HMG-CoA synthase is related to the production of β-hydroxybutyric acid from acetyl-CoA (Midaoui *et al.,* 2006

2. Ketone body utilization pathway

Ketone bodies are utilized in peripheral tissue by three-step enzymatic pathway (figure 2-2). In mitochondria, β-hydroxybutyrate is converted into acetoacetate and then acetoacetyl-coA by β-hydroxybutyrate dehydrogenase and 3-ketoacyl-CoA transferase, respectively. Finally acetoacetyl-CoA is converted into two molecules of acetyl-CoA by acetyl-CoA thiolase.

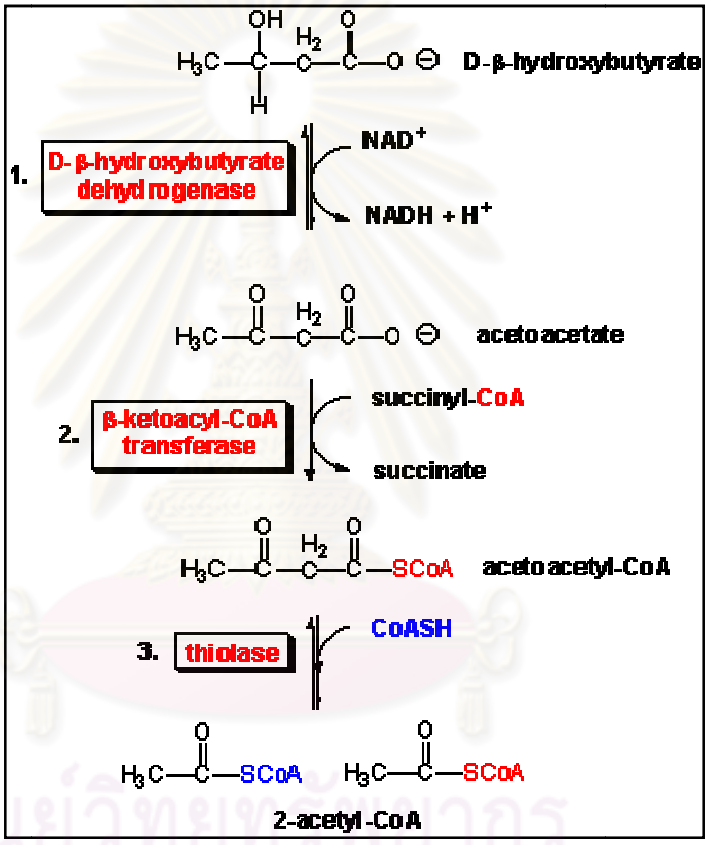

Figure 2-2 Ketone bodies utilization pathway in peripheral tissue (www.themedicalbiochemistrypage.org)

Beatty *et al.* (1959) previously showed that the uptake of acetoacetate by muscle preparations in vitro was lower in diabetic than in normal rats. Bässler *et al.* (1972) observed that the blood levels of ketone bodies rised exponentially with the infusion rate in diabetic rats comparatively with the linear rise observed in normal rats. This suggested that the same rate of ketone bodies production by the liver would lead to much higher blood levels of ketone bodies in diabetic than in normal rats. Furthermore,

Bässler *et al*. (1973) showed that the 3-ketoacyl-CoA transferase activity was decreased by 40% (units/g) in skeletal muscle of diabetic rats. These investigations suggested that the increase in plasma ketone bodies levels observed in diabetic animals was explained, at least in part, by a decrease in their removal, presumably due to a decrease in the activity of 3-ketoacyl-CoA transferase in the muscle.

Effects of exercise on ketone metabolism in normal rats

Winder *et al.* (1974) demonstrated that homogenates of gastrocnemius muscles from endurance-trained rats oxidized 3-β-hydroxybutyrate two to three times as rapidly as homogenates from sedentary rats. Beattie and Winder. (1985) showed that trained rats exhibited lower plasma β-hydroxybutyric acid levels during and after a bout of exercise than their sedentary counterparts. Ohmori *et al*. (1990) showed that trained rats had lower circulating levels of β-hydroxybutyric acid during exercise than sedentary rats. They also demonstrated that ketone body uptake by perfused hindlimb was greater in trained rats than in sedentary rats. Studies conducted in skeletal muscle of nondiabetic animals have demonstrated that 3-β-hydroxybutyrate dehydrogenase, 3-ketoacyl CoA-transferase, and acetoacetyl-CoA thiolase activities were increased by training (Winder *et al.*, 1974).

ANIMAL MODEL OF DIABETES MELLITUS

Several models of diabetes have been used in the previous investigations. There are many models with diabetes as presented in phenotypic forms of type 1 diabetes and type 2 diabetes. None of the species exhibits the full spectrum of functional or structural lesions associated with diabetes in humans, but each offers an opportunity for investigating certain clusters of derangements that are common in diabetic humans, particularly in their formative stages. The major features of common diabetic rat models for type 1 and type 2 diabetes are presented in table 2-2 (Saraceni and Broderick, 2007).

Table 2-2 Characteristic Features of Common Diabetic Rat Models Used in Exercise Studies (Saraceni and Broderick, 2007)

Model	Major Features	Key References
Type 1 diabetes		
Streptozotocin	Acute chemically-induced destruction of pancreatic β-cells: insulinopenia, ketonemia, glucosuria, weight loss, ketoacidosis	(Paulson *et al*., 1987; 1988, Goodyear *et al.,*1988; Nadeau *et al*., 1988 Dompierre *et al*.,1990; Paulson *et al*., 1992; Hall *et al*.,1995; Woodiwiss *et al*., 1996; Nakai *et al*., 2002; Broderick *et al*., 2005)
BB Wor	Spontaneous autoimmune insulinitis and lymphocytic infiltration. Insulinopenia, hyperglycemia, ketonemia, glucosuria, weight loss, ketoacidosis.	(Noble *et al*.,1994; Villanueva *et al*., 2003)
Type 2 diabetes		
JCR:LA	Spontaneous development of type 2 diabetes: obesity, insulin resistance, hyperinsulinemia, hyperglycemia,hypertriglyceridemia, hypercholesterolemia, atherosclerosis	(Russell *et al*., 1989)
OLETF	Spontaneous development of type 2 diabetes: mild obesity, insulin resistance, hyperinsulinemia,hyperglycemia, hypertriglyceridemia.	(Shima *et al*., 1994)
Sand rat & Sucrose-feeding	Nutritionally-induced: mild obesity, insulin resistance, hyperinsulinemia, hyperglycemia, hypertriglyceridemia	(Heled *et al*., 2002, Davidoff *et al*., 2004)
ZDF	Spontaneous development of type 2 diabetes: obesity, insulin resistance, hyperinsulinemia, hyperglycemia,hypertriglyceridemia, hypercholesterolemia.	(Pold *et al*., 2005)

Streptozotocin induced diabetes

In the present study, streptozotocin (STZ) was used to experimentally induce diabetes in rats. STZ [2-deoxy-2-(3-methy-3-nitrosoureido-D-glucopyranoside)] is a nitrosourea derivative isolated from the mold *Streptomyces grisevus* (figure 2-3). The diabetogenic action of STZ is to destroy most islet of beta cells. It is effective in different, species-specific doses, ranging from 25 to 200 mg/kg, in rats, dogs, mice, hamsters, monkeys, miniature pigs, pigs and rabbits (Porte, 1997).

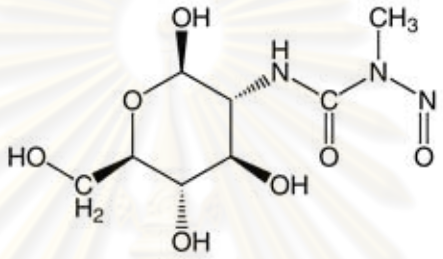

Figure 2-3 Molecular structure of Streptozotocin,
2-deoxy-2-(3-methyl-nitrosoureido)-D-glucopyranose.
(From Wikipedia, 2006)

STZ can induce severe insulin deficient diabetes in rats and other rodents, either when given as a single large dose (50-100 mg/kg in rats) or as multiple smaller dose. In the latter case, diabetes develops more gradually and appears to have an autoimmune, rather than a toxic basis (Pickup and Gareth, 2003). STZ-treated animals, though insulinopenic, retain some insulin-secretion capacity, are not ketotic, and do not usually require insulin support for survival. In fact, a mild diabetic state, resembling an insulin-poor form type 1 diabetes, may be induced in rats by a single low dose of about 35 mg/kg STZ. However, there is a tendency for spontaneous recovery in rats receiving doses below 35 mg/kg (Porte, 1997).

STZ is unstable in solution even at acid pH, and should be injected promptly after dissolving in citrate buffer at pH 5.0. Its *in vivo* life span is less than 15 minutes (Porte, 1997). After the intravenous administration of STZ, an early hyperglycemic phase appears, followed by a hypoglycemic phases and then a permanent diabetic phase occurring at approximately 4, 7 and 24 hours, respectively (Wang, 1996). The precise mechanism of STZ diabetogenicity has been described. STZ may act on both the

membrane and the interior of the β-cell (figure 2-4). It damages β-cell membrane and also induces fragmentation of DNA (Pickup and Gareth, 2003). STZ causes DNA strand breaks in pancreatic islet and stimulates nuclear poly (ADP-ribose) synthetase, and thus depletes the intracellular NAD^+ and $NADP^+$ levels. NAD^+ depletion by STZ inhibits proinsulin synthesis and thus induces diabetes. The pathological and biochemical features of the model may be compatible to those of type 1 diabetes in humans (Ohkuwa, 1995).

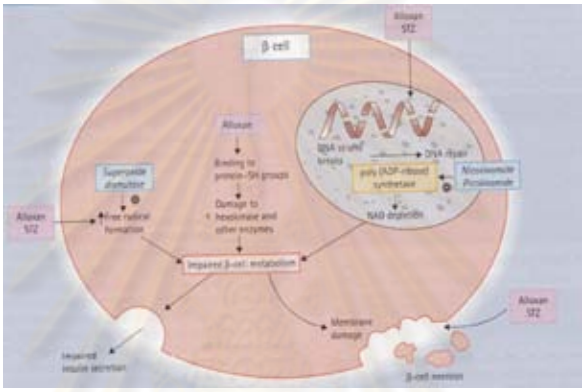

Figure 2-4 Suggested mechanisms of alloxan and streptozotocin toxicity on the β-cell. (modified from Pickup and Gareth, 2003)

THERAPEUTIC PRINCIPLES OF DIABETES

Controlling blood glucose is essential for being healthy and avoiding long-term complications of diabetes. Some patients are able to control blood glucose with diet and exercise. Others may need to use insulin injection or other medications in addition to lifestyle changes. In either case, monitoring blood glucose is a key part of the treatment program. Diagram for modern diabetic management is shown in figure 2-5 (Pickup and Gareth, 2003).

The diabetic treatment can be divided into 7 sub-groups as follows;

1. Dietary control

Based on the study in patients with diabetes mellitus, new diet recommendations were issued by the American Diabetes Association (1994), which suggested that 60% to 70% of calories be derived from the combination of carbohydrate and monounsaturated fat. The intake of carbohydrate and fat was to be individualized on the basis of treatment

goals but might include as much as 20% of calories from monounsaturated fat with a corresponding decrease in the carbohydrate content (to 40% to 50%). Since saturated and polyunsaturated fat intake of 10% each are suggested, the total fat intake could be as high as 40% (American Diabetes Association, 1994). The Diabetes Control and Complications Trial (DCCT) and other studies reinforce the importance of nutrition therapy in the intensive management of type 1 diabetes mellitus (The Diabetes Control and Complications Trial Research Group., 1993). Adherence to an individualized prescribed nutrition plan improves glycated hemoglobin levels in adults (Delahanty, 1993) and has repeatedly been identified as the single behavior most positively correlated with good blood glucose control in children (Burroughs, 1993, Charron-Prochownik *et al.* 1993).

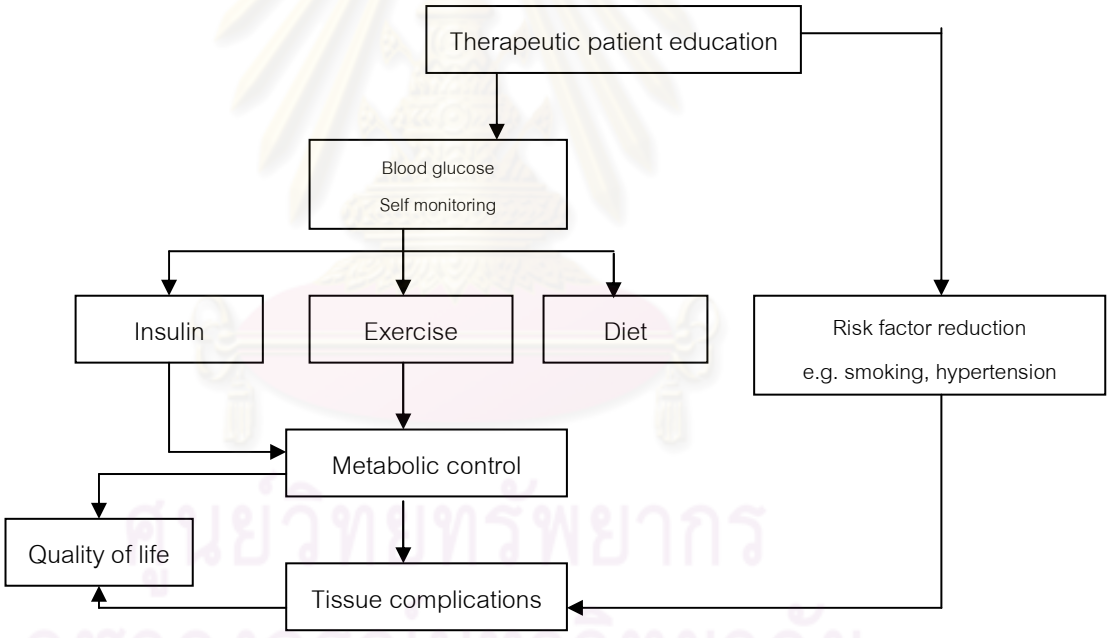

Figure 2-5 Modern diabetes management (Pickup and Gareth, 2003)

2. **Exercise**

Before the discovery of insulin, dietary control and exercise were the principle therapies used in the treatment of diabetes mellitus. In patients with type 1 DM, the ability to exercise is often severely limited because of the associated metabolic abnormalities, including muscle wasting, dehydration, and ketosis. With the advent of

insulin therapy, vigorous exercise became possible for patients with type 1 DM, although difficult to manage. It was soon recognized that exercise potentiates the hypoglycemic effect of injected insulin (Lawrence, 1926) and that the combination of insulin and exercise may lead to acute or delayed symptomatic hypoglycemia and decreased insulin requirements.

3. Insulin therapy and home monitoring blood sugar

Insulin therapy is the non-invasive treatment of type 1 diabetes mellitus but insulin treatment remains difficulties because of the imprecise manner in which subcutaneous insulin is delivered. Treatment is further complicated by how, when, and in what quantities food and snacks are ingested, the glycemic control of foods and liquids, and the effects of activity and day-to-day life stressed (LeRoith, 2004).

4. Immune intervention

Type 1 diabetes mellitus arises as a consequence of immunologically mediated pancreatic islet β-cell destruction. Improvement in their function accounts for the "honeymoon" period often seen during the first year after onset of type 1 diabetes. Moreover, the potential of "rescue" of those residuals β-cell from immune destruction is the basis for immune intervention in human type 1 diabetes. The goals of intervention at disease onset are to halt to a destruction of β-cells, perhaps allowing residual β-cells to recover function, thus modifying the severity of clinical manifestations (LeRoith, 2004).

5. Gene therapy

Gene therapy is eagerly anticipated to play a dominant role in future treatments for numerous diseases. Gene therapy involves modifying the expression of an individual's gene or correction of abnormal genes to treat disease (LeRoith, 2004).

6. Islet transplantations

The treatment of type 1 diabetes by replacing destroyed β-cells with alternative insulin-secreting cells in an attractive concept that has been considered for decades, but only recently has significant clinical success been achieved using cadaveric islet transplantation (LeRoith, 2004).

7. Stem cells

Recent advances in islet transplantation for treatment of patients with type 1 diabetes highlight the need for new sources of islet cells because human cadaveric pancreas will never be a sufficient source for cell replacement therapy for the millions of patients who suffer from this disease (Shapiro *et al*., 2000). Pluripotent stem cells are capable of self-renewal and differentiation into all cell types found in the embryo and adults. Embryonic stem cells derived from the inner cell mass of blastocytes are pluripotent. Pluripotent stem cells could theoretically provide an unlimited supply of cells of any adult type, including islets, if conditions for continuous proliferation and differentiation could be established in culture (LeRoith, 2004).

GENERAL CONSIDERATION OF EXERCISE

Physical activity may be defined as the muscular activity. Therefore physical activity results in the expenditure of energy proportional to muscular work. Exercise represents a subset of physical activity that is planned, with a goal of improving or maintaining fitness (Powers and Howley, 2001).

Exercise or work of a fairly vigorous nature, leads to a number of important bodily changes. The changes in function of the body are brought about by both single and replaced bouts of exercise. A single bout of exercise is called “acute exercise” whereas repeated bouts of exercise over several days or month is called “chronic exercise” or “exercise training” (Lamb, 1984).

The functional changes that occur when one exercises a single time are called responses to exercise. Theses functional changes are sudden, temporary and

disappeared shortly after the exercise period is finished. They are the increase in heart rate, the rise in blood pressure, the increase in breathing, the increase in blood flow to the working muscles and the decrease in blood flow to the stomach and the kidney. Each of these responses will persist no longer than a few minutes after the exercise is over (Lamb, 1984; Fox *et al.*, 1993).

Exercise training has the major objective of facilitating biologic adaptations that improve performance in specific tasks. An “adaptation” is a more or less persistent change in structure or function following training that apparently enables the body to respond more easily to subsequent exercise bouts. Ordinarily, adaptations are not seen until several weeks of training have passed, but some occur after only four or five days of training. The training is influenced by many factors such as intensity, duration, frequency and mode of exercise (Lamb, 1984; McArdle *et al.*, 2006). Each of these is important for physical adaptations. To achieve the training improvement, the following major factors must be considered (Roberg and Roberts, 1996)

Intensity

Intensity refers to the level of stress achieved during the exercise period. Intensity is determined best from measurements of oxygen consumption, but indirect method are heart rate, respiration rate, or from the rating of perceived exertion (PPE). Exercise sessions can be low intensity or high intensity. Low intensity exercise would be equal or 50% to 60% of an individual’s maximal heart rate whereas high intensity exercise would be 85% to 90% of an individual’s maximal heart rate. It is best to begin an exercise program at a low intensity and gradually increase the intensity over time.

Duration

Duration refers to the length of the training session. Duration and intensity are inversely related; that is, if the intensity of the exercise is high, the duration is generally low, and vice versa. The duration of the exercise session can be affected by environmental factors (e.g., heat, humidity, altitude). It can also be affected by the present fitness level or energy supply of an individual.

Frequency

Frequency refers to the number of training sessions per week. It is recommended that individuals try to exercise 4 or 5 days per week. The frequency of exercise depends on the type of exercise performed and the fitness status and goals of the individual.

Mode

Mode refers to the type of activity performed during the exercise session. Various modes of exercise can affect the components of fitness differently. Choosing the correct mode of exercise is important because it has a direct effect on the outcome.

BENEFICIAL EFFECTS OF EXERCISE IN DIABETES MELLITUS

Exercise training is known to have beneficial effects on diabetes. The benefits of exercise for the diabetic population are illustrated in figure 2-6 (modified from Saraceni and Broderick, 2007). The effects on glucose, lipid and cardiovascular system are described below.

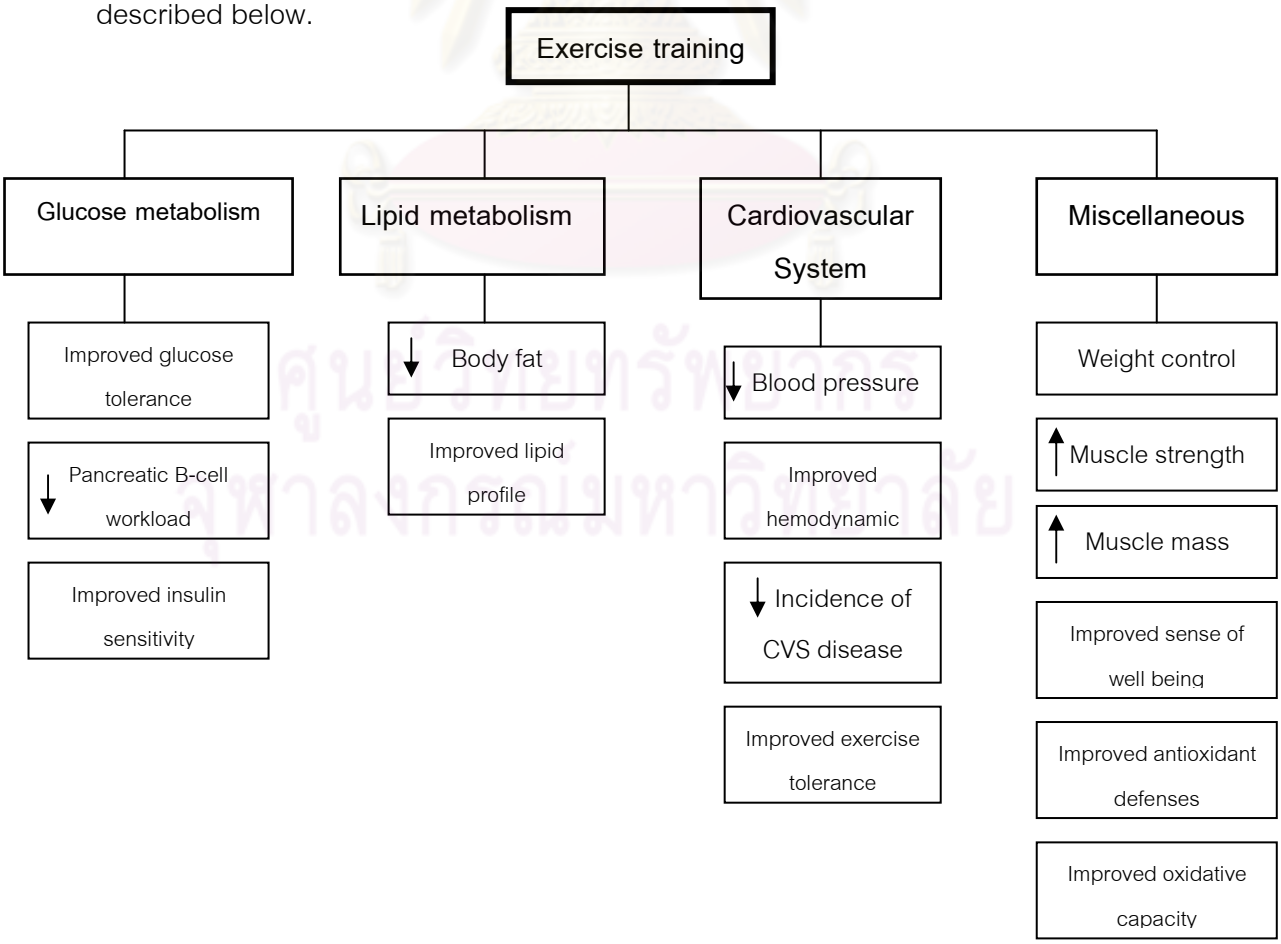

Figure 2-6 Beneficial effects of exercise for the diabetic patients (modified from Saraceni and Broderick, 2007).

4. Effects on glycemic control

One of the beneficial effects of exercise on diabetes is an increase in glucose uptake and metabolism (Enevoldsen *et al*., 2000). Glucose transport has been shown to be defective in diabetic humans and STZ-induced diabetic animals (Garvey *et al*., 1993). STZ-induced diabetes results in decrease in myocardial sacrolemmal GLUT-4 protein, responsible for insulin-stimulated glucose uptake, total membrane GLUT-4 protein extract, and GLUT-4 mRNA (Garvey *et al*., 1993). It was demonstrated that exercise training enhanced myocardial glucose metabolism in STZ-induced diabetic rats and young control rats. In addition; moderate treadmill exercise training increased myocardial GLUT-4 level by 40% in STZ-induced diabetic rats (Hall *et al*., 1995).

2. Effects on lipid profile

Moreover, as an exercise continues, fat rather than carbohydrate becomes the predominant fuel that is burned. In both lean and obese diabetic subject exercise training causes significant reductions in the VLDL, triglyceride concentration and, to a lesser extent, in LDL cholesterol. HDL cholesterol also has been shown to increase. These beneficial effects on lipids are observed in both type 2 and type 1 individuals (DeFronzo, 1998). Dose-response relationships between exercise training volume and blood lipid changes suggest that exercise can favorably alter blood lipids at low training volumes, although the effects may not be observable until certain exercise thoroughly are met (Durstine *et al*., 2001). Moreover, exercise down-regulated hepatic fatty acid synthase in STZ-treated rats (Fiebig *et al*., 2001) and reduced triglyceride secretion rates (TGSR) in diabetic rats (Tan *et al*., 1982).

3. Effects on ketone metabolism

In STZ-induced insulinopenia, the inability to metabolize carbohydrate fuel source compels tissues to utilize ketone bodies and lipids thereby depleting fat stores and inducing weight loss (Howarth *et al*., 2005). The development of moderate hyperketonemia, particularly the accumulation of acetoacetate, has been described in

diabetic rats proceeding prolonged exercise. A study has strongly correlated habitual exercise with the marked reduction of plasma ketone bodies and glucagon in mildly diabetic rats and plasma ketone bodies, particularly acetoacetate, in severely diabetic rats (Ohmori *et al*., 1990).

A study has shown that STZ-induced diabetes is associated with a significant increase in plasma free fatty acids (FFA) and β-hydroxybutyric acid levels in sedentary rats. It is also shown that exercise training can normalize plasma FFA levels and markedly reduce β-hydroxybutyric acid levels in these diabetic rats. Furthermore, exercise training also decreases hepatic ketone body synthesis measured as the activity of the hydroxymethyl glutaryl-CoA (HMG-CoA), a rate-limiting enzyme in hepatic ketogenesis (Lang *et al*., 2002). In a series of studies by Midaoui *et al.* in 2005 and 2006, plasma concentrations of β-hydroxybutyric acid in diabetic rats may be reduced with physical training despite any notable changes in plasma glucose or insulin levels. This exercise-induced reduction in circulating concentrations of ketone bodies has been attributed to decreased production in the liver or increased uptake by peripheral tissues. This enhanced capacity to utilize ketone bodies may be partly mediated by the action of 3-ketoacyl CoA-transferase in skeletal muscle and the subsequent decrease of β-hydroxybutyric acid levels, a mechanism seemingly defective in sedentary diabetic rats (Midaoui *et al*., 2005). Alternatively, physical training in diabetic rats depresses HMG-CoA synthase, a rate-limiting enzyme in hepatic ketogenesis (Midaoui *et al*., 2006). Exercise training has also been demonstrated to result in a significant reduction in plasma free fatty acid, triglyceride concentrations, total cholesterol, and low-density lipoprotein with resting blood glucose concentrations remaining virtually unaltered (Goodyear *et al*., 1988; Paulson, 1992; Hawley, 2004; Diabetes prevention program research group, 2005).

MONOUNSATURATED FATTY ACID (MUFA)

Monounsaturated fats or MUFA are fatty acids that have 18 carbon atoms, a double bond in the fatty acid chain and all of the remainder of the carbon atoms in the chain are single-bonded. By contrast, polyunsaturated fatty acids have more than one double bond.

Fatty acids are long-chained molecules having a methyl group at one end and a carboxylic acid group at the other end. Fatty acid fluidity increases with increasing number of double bonds. Therefore, monounsaturated fatty acids have a higher melting temperature than polyunsaturated fatty acids but lower than saturated fatty acids. Monounsaturated fatty acids are liquids at room temperature and semisolid or solid when refrigerated.

Natural sources

Monounsaturated fats are found in natural foods such as nuts and avocados and are the main component of tea seed oil and olive oil (oleic acid). Canola oil is 57%–60% monounsaturated fat, olive oil is about 75% monounsaturated fat while tea seed oil is commonly over 80% monounsaturated fat. Other sources include macadamia nut oil, grape seed oil, groundnut oil (peanut oil), sesame oil, corn oil, whole grain wheat, cereal, oatmeal, safflower oil, sunflower oil, tea-oil camellia, avocado oil. The dietary substitution of groundnut oil containing 46% of MUFA has slightly but significantly decreased the blood glucose, HbA_{1c}, lipid peroxidation, and lipid profile and increased antioxidant levels in diabetic rats (Ramesh *et al*., 2006).

Molecular description

Common monounsaturated fatty acids are palmitoleic acid (16:1 n-7), cis-vaccenic acid (18:1 n-7) and oleic acid (18:1 n-9). Palmitoleic acid has 16 carbon atoms with the first double bond occurring 7 carbon atoms away from the methyl group (and 9 carbons from the carboxyl end). It can be lengthened to the 18-carbon cis-vaccenic acid. Oleic acid has 18 carbon atoms with the first double bond occurring 9 carbon atoms away from the methyl group. The illustration below shows a molecule of oleic acid in Lewis formula (figure 2-7).

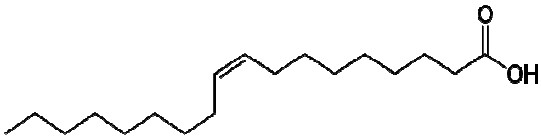

Figure 2-7 Oleic-acid-skeletal

Effect of high-MUFA diets on diabetes mellitus

1. Lipid profile

A meta-analysis of various studies comparing high-MUFA diet and low-carbohydrate diet to diet therapy in patients with type 2 diabetes revealed that high-MUFA diets improved lipoprotein profiles as well as glycemic control whereas reduced fasting plasma triglyceride and VLDL-cholesterol concentrations by 19% and 22%, respectively, and cause a modest increase in HDL-cholesterol concentrations (Garg *et al.,* 1988; Garg, 1994; Parillo *et al.,* 1992; Rusmussen *et al.,* 1993; Campbell *et al.*, 1994; Garg, 1994). Kotake *et al.* (2004) demonstrated that a high-MUFA diet can lower serum triglyceride levels as mechanism by reduced hepatic triglyceride production in normal mice and improved disorder of glucose metabolism in diabetic mice. Partial replacement of complex digestible carbohydrates with MUFA (avocado as one of its main sources) in the diet of type 2 diabetic patients improved the lipid profile favorably.

Foods containing monounsaturated fats lower low-density lipoprotein cholesterol (LDL-C), while possibly raising high-density lipoprotein cholesterol (HDL-C) (Merck & Co. Inc, 2009). However, their true ability to raise HDL-C is still in debate.

In children, consumption of monounsaturated oils is associated with healthier serum lipid profile (Sanchez-Bayle *et al.*, 2008).

2. Glycemic control

A number of studies in both normal and diabetic subjects have shown that substituting MUFA for carbohydrate (CHO) can improve glycemic control, and possibly insulin sensitivity, two effects that can have critical short-term benefits for tube-fed patients (Schrezenmeir, 1998). However, in many of the studies, there were significant improvements in glycemic control with the high-MUFA diets as compared with the high-CHO diets (Coulston *et al.,* 1989; Rusmussen *et al.*, 1993; Campbell *et al.,* 1994, Garg *et al.,* 1994, Chen *et al.,* 1995). In particular, several studies showed significant declines in glycosuria with high-MUFA diets (Garg *et al.,* 1988; Garg, 1994; Campbell *et al.,* 1994).

However, in some studies glycemic control remained stable and showed no evidence of deterioration in subjects receiving high-MUFA diets (Nielson *et al.,* 1995). In those studies in which glucose levels were lower on high-MUFA diets, there was a corresponding reduction in plasma insulin levels (Garg, 1994; Coulston *et al*., 1989). In a crossover study, eight males with type 2 diabetes were given a high-CHO diet and a high-MUFA diet, each for a 3-week period (Garg, 1994). The results showed that despite a marked improvement in plasma triglyceride and VLDL-cholesterol and higher HDL on the high-MUFA diet, glucose and insulin responses to a test meal were identical on the two diets. Both postprandial glucose and plasma insulin levels were significantly lower, respectively, and fasting insulin-mediated glucose disposal was significantly greater in the subjects receiving a MUFA-rich diet compared with that of a high-CHO diet, each given for 15 days to 10 subjects with type 2 diabetes (Parillo *et al*., 1992).

A diet rich in MUFA fat has beneficial effects on blood pressure and glucose metabolism by a reduced peak plasma glucose concentrations, without adverse effects on lipid composition in type 2 diabetic patients (Rusmussen *et al*., 1993). Partial replacement of digestible complex maintains an adequate glycemic control, and offers a good management alternative (Isreal *et al*., 1994). A high-MUFA enteral formula suppresses post-prandial hyperglycemia without exaggerated insulin secretion compared with a high-carbohydrate enteral diet in patients with type 2 diabetes and healthy subjects (Yokoyama *et al.,* 2008).

From the above literature review, it is hypothesized that high-MUFA diet in combination with exercise training may synergistically play a role in glycemic control and improvement of ketone metabolism in diabetic rats. Therefore, the aim of the present study was to evaluate whether high-monounsaturated fat diet in combination with exercise training could improve glycemic control and ketone metabolism or not and whether the effect on ketone metabolism was related to a decreased hepatic ketone bodies synthesis and an increased ketone bodies utilization in gastrocnemius muscle in diabetic rats.

CHAPTER III

MATERIALS AND METHODS

This study was an animal experimental research design. It was aimed to examine whether high-monounsaturated fat diet with/without exercise training can improve the serum concentration of ketone bodies and glycemic control in streptozotocin-induced diabetic rats. In addition, the mechanism of action on ketone metabolism is investigated whether it is mediated through changes in the enzymatic activity of the hepatic ketone body synthesis pathway and/or peripheral ketone utilization pathway. The experimental protocol of this study consisted of two parts. The first part was a determination of biochemical characteristics, including plasma glucose, plasma glycated hemoglobin (HbA_{1C}), plasma insulin, serum free fatty acid and serum β-hydroxybutyrate. The second part was an assessment of the activity of β-hydroxybutyrate dehydrogenase and 3-ketoacyl-CoA transferase, enzymes used in hepatic ketone body synthesis pathway and ketone body utilization in liver and gastrocnemius muscle, respectively, using spectrophotometer technique. All protocols and procedures employed in this study were reviewed and approved by the committee of Animal Care, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (Approval No. 08/52).

Reagents

Acetyl-CoA (Sigma Chemical, USA)

Acetyl phosphate (Sigma Chemical, USA)

ACCU-CHEK® (ROCH, Germany)

β-hydroxybutyrate assay kit (Bio vision, USA)

β-NADH (Sigma Chemical, USA)

Citrate buffer (Sigma Chemical, USA)

Distilled wate

Dithiothreitol (Sigma Chemical, USA)

EDTA (Merck, Germany)

Iodoacetamide (Sigma Chemical, USA)

KOH (Merck, Germany)

Liquid N_2

$MgCl_2$ (Merck, Germany)

MOPS (3-(N-morpholino) propanesulfonic acid) (Sigma Chemical, USA)

NEFA (non-esterified fatty acid) (Wako, Germany)

NEMBUTAL® (CEVA, France)

Normal Saline

Olive oil® (SABROSO, Spain)

Palm oil® (TESCO, Thailand)

Phosphotransacetylase (pTA) (Sigma Chemical, USA)

SPI Bio, insulin (Cayman Chemical Company, USA)

Streptozotocin (STZ) (Sigma Chemical, USA)

Succinyl-CoA (Sigma, USA)

Sucrose (UNIVAR, Australia)

Trisma® hydrochloride (Sigma Chemical, USA)

Tris (hydroxymethyl) amino-methane (Sigma Chemical, USA)

Triton X-100 (Sigma Chemical, USA)

Animal Preparation

Male Wistar rats with an average initial body weight of 180 to 220 g were purchased from National Laboratory Animal Center, Mahidol University, Salaya, Thailand. They were allowed to rest for five to seven days after arrival at Animal Center, 3rd floor Pathology building, Faculty of Medicine, Chulalongkorn University. The rats were kept in a room where temperature was 25±1°C in condition (12 hour day: 12 hour night). Two rats were housed per stainless steel cage and fed *ad libitum* with regular dry rat chow and water. The rats were weighed once a week.

Experimental Design

A total of 38 Wistar rats was used in this study and induced to be diabetic with streptozotocin. The animals were then randomly divided into five groups of eight animals each as follows:

Group I: (DM+regular diet, n=7). Diabetic rats were fed regular diet *ad libitum* (10.95% fat, 370 kcal/100 g diet) as a control group.

Group II: (DM+SFA, n=8). Diabetic rats were fed high-saturated fat diet *ad libitum* (35% fat, 456 kcal/100 g diet). Palm oil was used as a source of saturated fatty acid (SFA).

Group III: (DM+MUFA, n=8). Diabetic rats were fed high-monounsaturated fat diet *ad libitum* (35% fat, 456 kcal/100 g diet). Olive oil was used as a source of monounsaturated fatty acid (MUFA)

Group IV: (DM+Ex, n=7). Diabetic rats were fed regular diet *ad libitum* and trained following an exercise training protocol as described below.

Group V: (DM+MUFA+Ex, n=8). Diabetic rats were fed high-monounsaturated fat diet *ad libitum* and trained following an exercise training protocol as described below.

The experimental period lasted for twelve weeks. At the end of the experiment, in the morning, 24 hours after the last exercise bout, the animals were transferred into a quiet cage for assessment of the final body weight. After a 4 hour fasting, the rats were sacrified using an intra-peritoneal injection of overdose sodium pentobarbital (100 mg/kg BW). Cardiac blood (10 mL) was taken and immediately transferred into a tube containing 1.25 mg/mL sodium fluoride (NaF) for assessment of plasma glucose, a tube containing 1.25 mg/mL EDTA for assessment of plasma HbA_{1C} and plasma insulin, and whole blood for assessment of serum free fatty acid and β-hydroxybutyrate. The plasma and serum were rapidly separated from blood cells by centrifugation and kept frozen at -80°C. Thoracic abdominal wall were opened by a midline incision. The liver and the right gastrocnemius muscle were removed, weighed, cut into pieces and submersed in liquid N_2 for 2 minutes and then kept frozen at -80°C for further analysis of β-

hydroxybutyrate dehydrogenase and 3-ketoacyl CoA-transferase activity, respectively. Diagram of experimental design is shown in figure 3-1

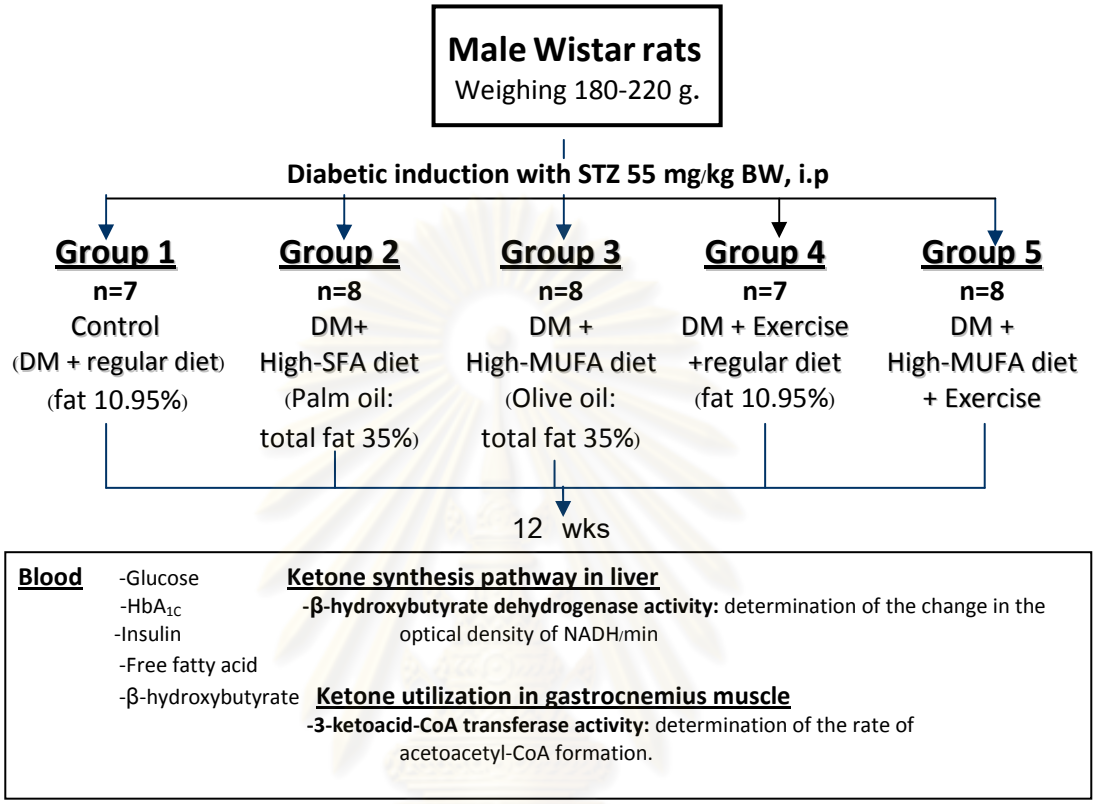

Figure 3-1 Diagram of experimental design

Diabetic Induction

To induce diabetes mellitus, streptozotocin (STZ) (Sigma Chemical, USA) was prepared by dissolving in an acidified citrate buffer (0.1 M, pH 4.5) (Sigma Chemical Co.) and injected immediately into the peritoneum of 4-hour-fast rats, at a single-dose of 55 mg/kg (Rakeiten *et al.*, 1963). Two days later, after a 4-hour-fast, glucose concentration in the tail blood of the STZ-injected animals was assessed with a glucometer (Advantage Glucometer, Boehringer Mannheim, Germany) (figure 3-2). Samples were analyzed by applying a drop of blood to a control strip inserted into the monitor and only the animals with a value of glucose between 250-300 mg/dL were recruited in the protocol.

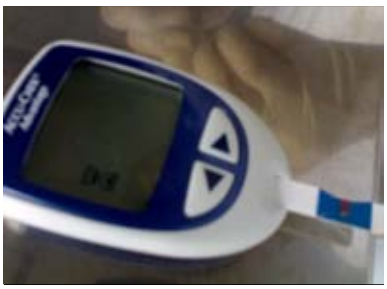

Figure 3-2 Glucometer are used to determine glucose concentration in rat tail vein

High-monounsaturated fat (high-MUFA) and high-saturated fat (high-SFA) diet preparations.

High-MUFA and high-SFA diet were prepared every two days by throughly mixing 80 g of regular dry diet (CP mice fed®) with 20 mL of olive oil (SABROSO®) or palm oil (TESCO®), respectively (see page 88-89). Olive oil and palm oil were purchased from local market and stored at 25°C. The diets then contained 35 percent of fat and yielded 456 kcal of energy per 100 g diet.

Exercise training Protocol

The exercise training protocol used in the present study was modified from the studies of Midaoui *et al.* (2005, 2006), which was shown to improve ketone metabolism but not affect glycemic control in STZ induced diabetic rats. The exercise training program of moderate intensity was carried out progressively on a motorized treadmill (SPORTART 1190) with 8% slope (figure 3-3) twice daily in the morning (between 10 to 12 a.m.) and in the afternoon (between 5 to 7 p.m.), 5 days per week for twelve weeks. The rats began running at a speed of 22 m/min for 10 minutes and the speed was gradually increased up to 26 m/min for 50 minutes (Table 3-1).

Table 3-1 Exercise traininig program on motorized treadmill

(modified from Midaoui *et al.*, 2006)

Week	Duration (minutes)	% incline	Speed (m/min)	Speed (km/hr)
1	10	8	22	1.2
2	15	8	23	1.3
3	20	8	23	1.3
4	25	8	23	1.3
5	25	8	25	1.4
6	30	8	25	1.4
7	35	8	25	1.4
8	40	8	25	1.4
9	40	8	26	1.5
10	45	8	26	1.5
11	50	8	26	1.5
12	50	8	26	1.5

Figure 3-3 The rats employed exercise training by running on a motorized treadmill

Data Collection and Determination

1. Physical and physiological characteristics

The physical and physiologic characteristics of the animal were recorded as follows:

-Final body weight

-Liver weight

-Gastrocnemius weight

-Muscle weight per body weight

-Daily caloric intake

The weight of the rats was recorded once a week. Daily Caloric intake was monitored in the morning (10.00 a.m. to 11.00 a.m.). At the end of the experiment, in the morning, 24 hours after the last exercise bout, the animals were transferred into a quiet cage for assessment of the final body weight. Thoracic abdominal wall were opened by a midline incision. The liver and the right gastrocnemius muscle were removed, weighed, cut into pieces and submersed in liquid N_2 for 2 minutes and then kept frozen at -80^{o}C for further analysis.

2. Biochemical characteristics

The biochemical characteristics of the animal were determined as follows:

-Plasma glucose

-Glycated hemoglobin (HbA_{1C})

-Plasma insulin levels

-Serum free fatty acid (FFA)

-Serum β-hydroxybutyrate

2.1 Plasma glucose

The cassette COBAS INTEGRA glucose HK (GLUC2) contains an *in vitro* diagnostic reagent system intended for use on COBAS INTEGRA systems for the quantitative determination of the glucose concentration in serum, plasma, urine, and cerebrospinal fluid (CSF). Glucose measurements are used in the diagnosis and treatment of carbohydrate metabolism disorders including diabetes mellitus and idiopathic hypoglycemia.

Procedure

Plasma glucose was analyzed using enzymatic method with hexokinase. Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6-phosphate by $NADP^+$ to form NADPH

$$\text{D-glucose + ATP} \xrightarrow{HK} \text{D-glucose-6-phosphate + ATP}$$

$$\text{D-glucose-6-phosphate + } NADP^+ \xrightarrow{G6PDH} \text{D-6-phosphogluconate + NADPH + } H^+$$

The concentration of the NADPH formed is directly proportional to the glucose concentration. It is determined by measuring the increase in absorbance at 340 nm using COBAS INTEGRA HK (GLUC2) analyzer by Bangkok RIA lab CO, LTD.

2.2 Glycated hemoglobin (HbA_{1C})

COBAS INTEGRA Tina-quant Hemoglobin A1c Gen, 2 (A1C-2) is an *in vitro* diagnostic reagent system intended for use on Roche clinical chemistry analyzers for the quantitative determination of percent hemoglobin A1c (%) in whole blood. Hemoglobin A1c results are useful for the monitoring of long term blood glucose control in individuals with diabetes mellitus.

Procedure

HbA_{1C} was analyzed by chromatographic methods using COBAS INTEGRA 800 analyzer, the detection of tetradecyl trimethylammonium bromide (TTAB) reaction with HbA in blood by Bangkok RIA lab CO, LTD.

The anticoagulated whole blood specimen is hemolyzed automatically on the COBAS IVTEGRA 800 analyzer with COBAS INTEGRA Hemolyzing Reagent Gen, 2. This method uses TTAB as the detergent in the hemolyzing reagent to eliminate interference from leucocytes (TTAB does not lyse leucocytes). Sample pretreatment to remove labile HbA_{1C} is not necessary. All hemoglobin varients which are glycated at the

B-chain, N-terminal and which have antibody recognizable regions identical to that of HbA_{1C} are determined by this assay.

2.3 Plasma insulin levels

Plasma insulin levels were analyzed by enzyme immunoassay (EIA), which is based on the competition between unlabelled rat insulin and acetylcholinesterase (AchE) linked to rat insulin (tracer) for limited specific Guinea-Pig anti-rat insulin antiserum sites. The complex Guinea-Pig antiserum-rat insulin (free insulin or tracer) binds to the Goat anti-Guinea-Pig antibody that is attached to the wall. The plate is then washed and Ellman's Reagent (enzymatic substrate for AchE and chromogen) is added to the wells. The AchE tracer acts on the Ellman's Reagent to form a yellow compound. The intensity of the colour, which is determined by spectrophotometry, is proportional to the amount of tracer bound to the wall and is inversely proportional to the amount of free rat insulin present in the well during the immunological incubation.

Procedure

1. EIA buffer: dispense 100 µL to non-specific binding (NSB) wells and 50 µL to maximum binding (Bo) wells.

2. Rat insulin standard: dispense 50 µL of each of the six standards (S1 to S6) in duplicate to appropriate wells. Start with the lowest concentration standard (S6) and equilibrate the tip in the next higher standard before pipetting.

3. Quality control and samples: dispense 50 µL in duplicate to appropriate wells. Highly concentrated samples may be diluted in EIA buffer.

4. Rat insulin AchE tracer: dispense 50 µL to each well.

5. Rat insulin antiserum: dispense 50 µL to each well except the non-specific binding (NSB) wells.

6. Cover the plate with a plastic film and incubate for 16-20 hours at $4^{\circ}C$.

7. Empty the plate by turning over and shaking. Then, wash each well five times with the wash buffer (300 µL/well).

8. Dispense 200 µL of Ellman's reagent to the 96 wells

9. Incubate in the dark (plate covered with an aluminium sheet) at room temperature. Optimal development is obtained using an orbital shaker for 1.5 hour.

10. The plate should be read between 405 and 414 nm (yellow colour) when the maximum binding (Bo) wells reach an absorbance of 0.2-0.8 unit. The enzyme immunoassay protocol was set as described below.

Enzyme immunoassay protocol (volume are in µL)					
	Blank	Non-specific binding (NSB)	Maximum binding (Bo)	Standard	Sample
Buffer	-	100	50	-	-
Standard	-	-	-	50	-
Sample	-	-	-	-	50
Tracer	-	50	50	50	50
Antiserum	-	-	50	50	50
Cover the plate, incubate at 4°C for 16-20 hours					
Wash the plate 5 time					
Ellman's reagent	200	200	200	200	200
Incubate the plate with an orbital shaker in the dark at room temperature					
Read the plate between 405 and 414 nm					

Calculation

1. Calculate the average absorbance for each NSB, Bo, standards and samples.

2. Calculate the B/Bo (%) for each standard and sample: (average absorbance of standards or sample – average absorbance of NSB) divided by (average absorbance of Bo – average absorbance of NSB) & multiplied by 100.

3. Using a semi-log graph paper, plot the B/Bo (%) for each standard point (y axis) vs. the concentration (x-axis). Draw a best-fit line through the points.

4. To determine the concentration of the samples, find the B/Bo (%) value on the y axis. Read the corresponding value on the x axis which is the concentration of the unknown sample. Samples with a concentration greater than 4 ng/mL should be re-assayed after dilution in EIA buffer.

2.4 Serum free fatty acid (FFA)

NEFA C is an *in vitro* assay for the quantitative determination of non-esterified fatty acid (NEFA) in serum. NEFA in serum binding albumin, is used as an important energy source of peripheral tissues. The amount of NEFA in serum depends on a balance between intake in liver and peripheral tissues, and the release or decrease of NEFA is observed in diabetes, hepatic diseases or endocrine diseases.

NEFA had been assayed by organic solvent extraction method, which was complicated to operate. Enzymatic method using Acyl-CoA Oxidase (ACOD) has become widespread due to excellent specificity and concise procedure. NEFA C is the reagent kit for NEFA assay based on enzymatic method using 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) as a violet color agent.

Procedure

1. Preparation of reagents

R_1: Prepare Reagent 1 by mixing one bottle of each Color A (for 10 mL) and Solvent A (10 mL). (**Color A**= 0.27 units/ml Acyl-CoA synthetase (ACS, *Pseudomonas sp.*), 0.73 mmol/L coenzyme A (CoA), 4.5 mmol/L adenosine 5'triphosphate disodium salt (ATP, *Bacterium sp.),* **Solvent A**= 50 mmol/L Phosphate buffer, pH 7.0)

R_2: Prepare Reagent 2 by mixing one bottle of each Color B (for 20 mL) and Solvent B (20 mL). (**Color B**= 5.5 units/mL Acyl-CoA oxidase (ACOD, *Arthrobacter sp.*), 6.8 units/mL peroxidase (POD, *Horseradish*), **Solvent B=** 1.2 mmol/L 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA))

After preparing the reagent 2, store below 25°C and use within the day, or store at 2-10°C and use within 5 days.

2. Material required but not supplied

Test tube, pipette (for specimen, for reagent), water bath or heating block capable of maintaining 37°C, Spectrophotometer or Colorimeter that have 550 nm filter.

3. **Standard procedure**

	Sample (S)	Standard (Std)	Reagent Blank (BI)	Sample Blank* (SBI)
Specimen	Serum 0.05 mL	Standard 0.05 mL	Distilled or deionized water 0.05 mL	---
Reagent 1	1.0 mL	1.0 mL	1.0 mL	1.0 mL
Mix well and incubate for 10 minutes at 37°C				
Reagent 2	2.0 mL	2.0 mL	2.0 mL	2.0 mL
Specimen	---	---	---	Serum 0.05 mL
Mix well and incubate for 10 minutes at 37°C				
After cooling the solution to room temperature, measure the absorbance (Es) of Sample (S) and the absorbance (Estd) of Standard (Std) against reagent blank (BI) within 30 minutes. Spectrophotometer 550 nm				

*1 Sample Blank is not required for common samples, but required for lipidemia or hemolysis sample.

Calculation

1. Calculation method from calibration curve

   Calculate NEFA concentration from the calibration curve which was made previously.

2. Calculation method from expression

$$\text{NEFA (mEq/L)} = \frac{Es}{Estd} \times 10$$

Remarks, when sample blank is measured, calculate Es by subtracting sample blank from sample O.D. to get NEFA concentration.

2.5 Serum **β**-hydroxybutyrate

Diabetic ketoacidosis occurs when circulating insulin levels drop to very low levels, shutting off the supply of glucose to the body. The physiological response is for the liver to produce ketone bodies (acetoacetate, acetone, and primarily β-hydroxybutyrate) from the acetyl-CoA produced from fatty acid oxidation. The very high rate of ketone body production outstrips the body's ability to utilize them as an energy source and blood concentration builds up. As rather strong acids, they result in a product which reacts with colorimetric probe with an absorbance band at 450 nm. The kit is an easy and convenient assay to measure β-hydroxybutyrate levels in biological samples. The assay is linear for 1-20 nmol β-hydroxybutyrate in up to 100 µL samples or 0.01-0.2 mM of β-hydroxybutyrate samples.

Procedure

1. Standard Curve Preparations: Dilute the β-hydroxybutyrate Standard to 1.0 mM by adding 10 µL of the Standard to 90 µL of distilled water, mix well. Add 0, 4, 8, 12, 16, 20 µL to a series of wells. Adjust volume to 50 µL/well with Assay Buffer to generate 0, 4, 8, 12, 16 and 20 nmol per well of the β-hydroxybutyrate Standard.

2. Sample Preparation: β-hydroxybutyrate concentrations can vary over a wide range from normal range: 20 µM-1 mM to diabetic range: 3-5 mM in serum and 10 times that in urine during about 5 µL equivalent of such samples can be tested directly. Add samples to test wells. Adjust the volume to 50 µL with β-hydroxybutyrate assay Buffer.

3. Development:

Mix enough reagents for the number of samples and standard to be performed: For each well, prepare a total 50 µL reaction mix.

β-hydroxybutyrate Assay Buffer	46 µL
β-hydroxybutyrate Enzyme Mix	2 µL
β-hydroxybutyrate Substrate Mix	2 µL

Mix and add 50 µL of the reaction mix to each well containing β-hydroxybutyrate Standard or Samples.

4. Incubate at room temperature for 30 minutes, protect from light.

5. Measure O.D. at 450 nm

Calculation

Correct background by subtracting the 0 β-hydroxybutyrate control from all standard and sample reading. Plot standard curve nmol/well vs. standard reading. Apply sample readings to the standard curve to get the amount of β-hydroxybutyrate in the sample wells.

The β-hydroxybutyrate concentration in the test samples:

$$C = Ay/Sv \quad (nmol/\mu L;\ \mu mol/mL;\ or\ mM)$$

Where: **Ay** is the amount of β-hydroxybutyrate (nmol) in sample from the standard curve

Sy is the sample volume (μL) added to the sample well.

β-hydroxybutyric acid molecular weight: **104.1**

3. Enzymatic activity in ketone metabolism

3.1 Hepatic ketone body synthesis pathway: Assessment of β-hydroxybutyrate dehydrogenase activity

Ketone bodies are synthesized in liver mitochondria by the four-step enzymatic pathway shown in Figure 3-4. From the enzymatic reaction in this pathway, when each molecule of β-hydroxybutyrate is generated from acetoacetate, one molecule of NADH (nicotinamide adenine dinucleotide) is used. The activity of β-hydroxybutyrate dehydrogenase in the hepatic ketone body synthesis pathway was assessed by measuring the change in the optical density of NADH after the addition of acetyl-CoA to a medium containing the liver extracts. Because the liver extract also contains acetyl-CoA hydrolase, which produced free CoA, which tends to reverse the first reaction, the acetyl-CoA-regenerating system was used in the assay as described by Lynen *et al.* (1958).

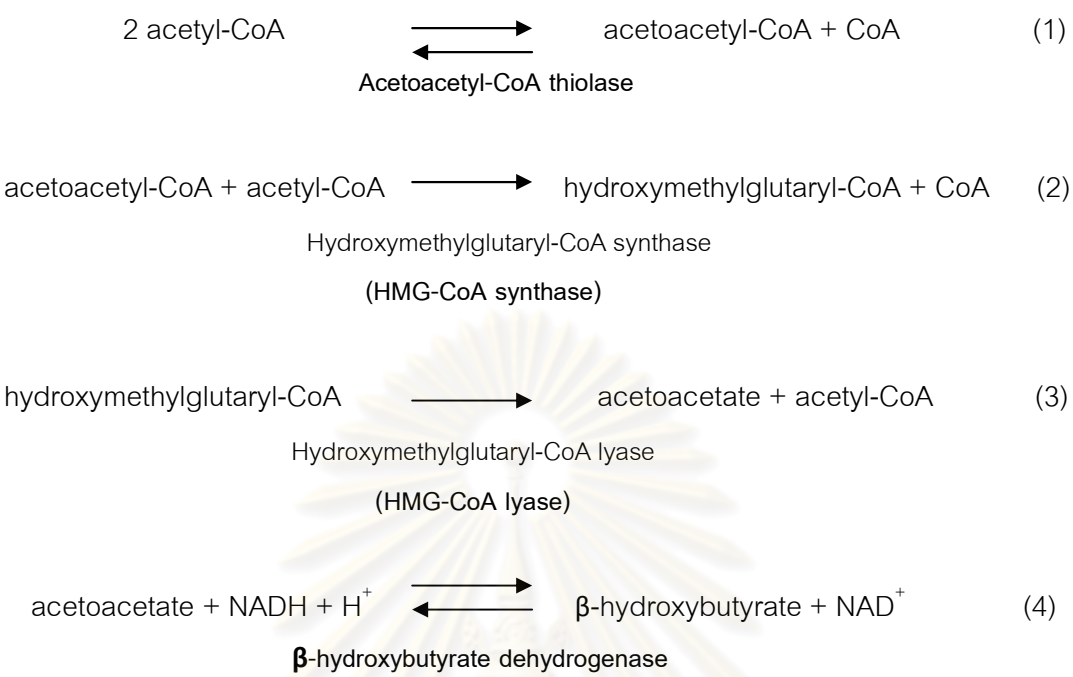

Figure 3-4 Hepatic ketone body synthesis pathway.

In brief, this consists of adding acetyl phosphate and phosphotransacetylase (pTA, from *Bacillus stearother-mophilus;* Sigma Chemical, USA) to the incubation medium to convert free CoA to acetyl-CoA, thus driving the enzymatic cascade in the appropriate direction.

At the time of assay, one piece of liver was pulverized in a percussion mortar kept in liquid N_2. Subsequent operations were carried out at 4°C. Each gram of liver powder was homogenized in 3 mL of extraction buffer (0.3 M sucrose, 0.5 mM EDTA, 10 mM MOPS-KOH, pH 7.2) with a motor homogenizer, ULTRA TURRAX® at level 2 for 30 seconds and then centrifuged at 600 *g* for 5 minutes. Sample (100 µL) of the resulting supernatant was treated with 7.5 µL of 20% (wt/vol) Triton X-100 to expose enzymes of the ketone body synthesis pathway. The incubation buffer contained 50 mM Tris-HCl and 0.2 mM dithiothreitol, pH 8, at 30°C. The incubation mixture (final volume 1,300 µL) consisted of incubation buffer 130 µL, distilled water 140 µL, acetyl phosphate (5 mM) 900 µL, 10 units of pTA 10 µL, 100 µL of 10 µM NADH (Sigma Chemical, USA) and 10 µL of liver extract. The optical density was read until stabilization to avoid modification in

NADH concentration resulting from other enzymatic reactions using endogenous substrates, and then 10 µL of 100 µM acetyl-CoA was added to start the reaction.

The hepatic ketone body synthesis pathway activity was measured at 340 nm, at 30°C, in a 1-cm light path (SHIMADZU® Spectrophotometer UV-1601PC, UV-visible). By definition, 1 unit of enzyme activity causes 1 µmol/min of NADH to be transformed into NAD.

3.2 Peripheral ketone body utilization pathway: Assessment of 3-ketoacyl CoA-transferase activity.

Ketone bodies are utilized by peripheral tissue such as muscle by the three-step enzymatic pathway as shown in Figure 3-5.

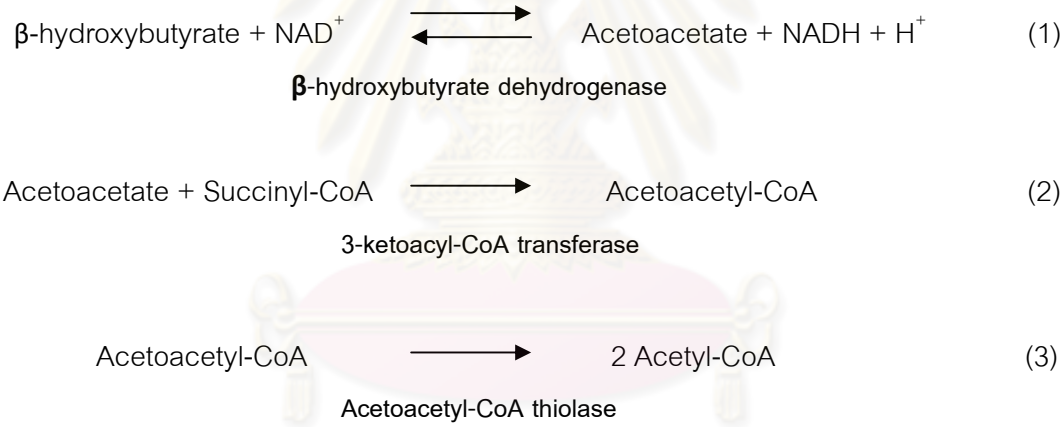

Figure 3-5 Peripheral ketone body utilization pathway.

The gastrocnemius muscle 3-ketoacyl CoA-transferase activity was determined spectrophotometrically according to the method of Williamson *et al.* (1971), as modified by Rebrin *et al.* (2007). All further procedures were carried out at 2-4°C.

The thawed gastrocnemius muscle was placed in cold extraction buffer, each gram of gastrocnemius muscle was homogenized in 4 mL of extraction buffer [Tris-(hydroxymethyl)-aminomethane-sucrose] and finely minced with scissors. The gastrocnemius muscle was homogenized with a motor homogenizer, ULTRA TURRAX® at level 3 for 30 seconds. The homogenate, cooled in ice water, was immediately exposed to ultrasonic vibration for exactly 45 seconds (3 x 15 seconds interspaced by

15 seconds) 40% amplitude (SONICS Vibra-cell™ AMPLITUDE). The ultrasonically treated homogenate was then centrifuged for 20 minutes at 30,000 *g* by high-speed centrifuge (BECKMAN Avanti™J-25I) and the supernatant was decanted and saved.

The activity of 3-ketoacyl CoA-transferase was measured with a SHIMADZU® Spectrophotrometer UV-1601PC, UV-visible. The assay is based on the determination of the rate of acetoacetyl-CoA formation from succinyl-CoA and acetoacetate.

For the determination of the rate of acetoacetyl-CoA formation from succinyl-CoA and acetoacetate the silica cuvettes (1 cm) contained in a final volume of 1000 µL: distilled water 300 µL, tris-HCl buffer, pH 8.5, 50 µmol 500 µL, $MgCl_2$ 5 µmol 50 µL, iodoacetamine 5 µmol 50 µL (to inhibit acetoacetyl-CoA thiolase and enzymes of the hydroxymethylglutaryl-CoA pathway), succinyl-CoA 0.1 µmol 50 µL and acetoacetate 50 µmol 50 µL. The homogenate sample (0.1 mL) was added, and the rate of increase in extinction at 313 nm, temperature 25°C, light path = 1 cm, was measured for 2 minutes.

Data analysis

All data were presented as means and standard deviations (SD). For comparisons among groups of animals, one way analysis of variance (ANOVA) were used and the differences in pairs of means among groups were made by Post Hoc test. If the statistical probability (p-value) was less than or equal to 0.05, the differences were considered to be statistically significant.

CHAPTER IV

RESULTS

The results of this study are arranged into 3 parts. The first part includes the outcomes relating to physical and physiological characteristics of the animals. The second part is composed of the outcomes on biochemical characteristics. The last part shows the outcomes on enzymatic activities involving in ketone metabolism. The list of grouped outcome variables is as follows.

Part 1 Physical and physiological characteristics

: Final body weight

: Liver weight

: Gastrocnemius weight

: Muscle weight per body weight

: Daily caloric intake

Part 2 Biochemical characteristics

: Plasma glucose

: Glycated hemoglobin (HbA_{1C})

: Plasma insulin levels

: Serum free fatty acid (FFA)

: Serum β-hydroxybutyrate

Part 3 Enzymatic activities in ketone metabolism

: β-hydroxybutyrate dehydrogenase activity (Marker of hepatic ketone body synthesis)

: 3-ketoacyl CoA-transferase activity (Marker of muscle ketone body utilization)

1. Physical and physiological characteristics

1.1 Final body weight

Figure 4-1 shows final body weight of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

The final body weights of the 5 groups at wk 12 were 311.90 $\pm$ 45.77, 389.30 $\pm$ 21.18, 360.30 $\pm$ 76.11, 372.60 $\pm$ 50.37 and 349.25 $\pm$ 48.09 grams, respectively. In DM+SFA group the final body weight was significantly increased when compared with control (p=0.048). There were no significant differences among DM+MUFA, DM+Ex+regular diet, DM+MUFA+Ex and Control.

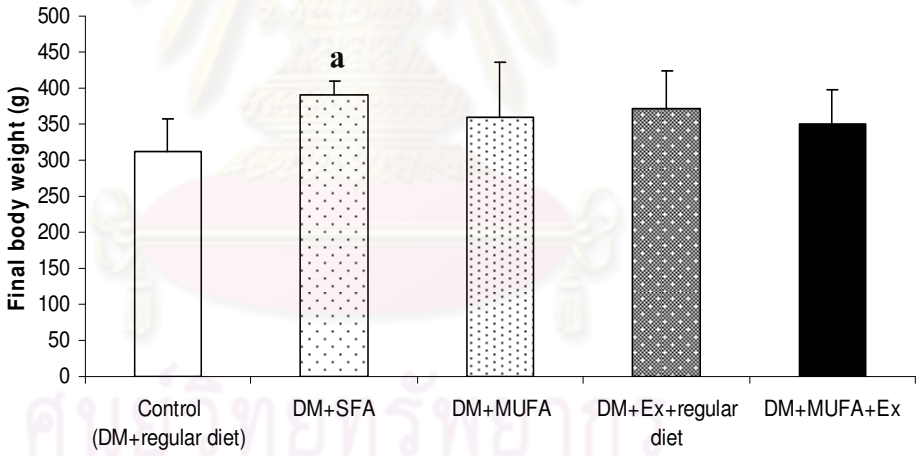

Figure 4-1 Final body weight (g) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups.

Data were expressed as mean $\pm$ SD.

[a] p<0.05 vs. Control

1.2 Liver weight

Figure 4-2 shows liver weight of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, the liver of those rats weighed 14.76 $\pm$ 2.31, 16.49 $\pm$ 1.65, 14.63 $\pm$ 2.01, 15.12 $\pm$ 1.98 and 14.52 $\pm$ 1.09 grams, respectively. There were no significant differences of liver weight among each group.

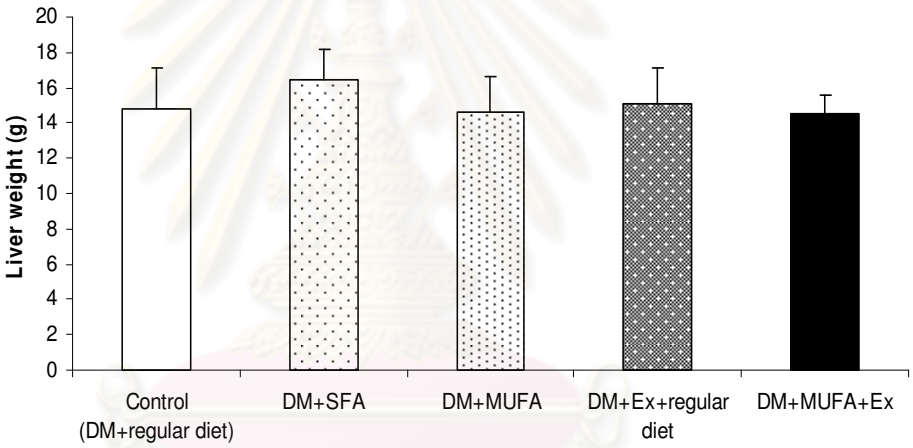

Figure 4-2 Liver weight (g) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups.
Data were expressed as mean $\pm$ SD.

1.3 Gastrocnemius weight

Figure 4-3 shows gastrocnemius weight of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, gastrocnemius muscle of those rats weighed 1.37 $\pm$ 0.25, 1.82 $\pm$ 0.27, 1.59 $\pm$ 0.31, 1.72 $\pm$ 0.31, and 1.73 $\pm$ 0.26 grams, respectively. In DM+SFA group, there was a significant increase in gastrocnemius weight when compared with control group (p=0.035). Gastrocnemius weight did not differ significantly among Control, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex.

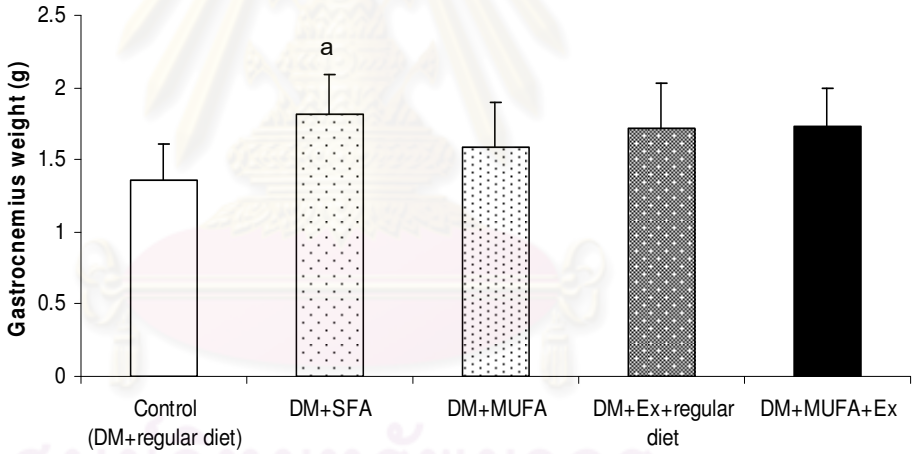

Figure 4-3 Gastrocnemius weight (g) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups.
Data were expressed as mean $\pm$ SD.
[a] p<0.05 vs. Control

1.4 Muscle weight per body weight

Figure 4-4 shows muscle weight per body weight of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, those rats had muscle weight per body weight of 4.39 $\pm$ 0.27, 4.66 $\pm$ 0.57, 4.43 $\pm$ 0.19, 4.59 $\pm$ 0.38 and 4.96 $\pm$ 0.38 mg/g, respectively. Muscle weight per body weight among groups did not differ significantly.

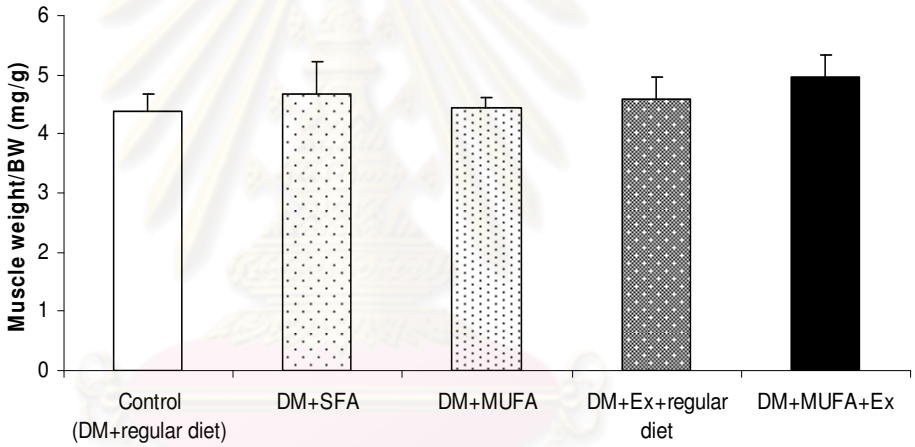

Figure 4-4 Muscle weight per body weight (mg/g) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups. Data were expressed as mean $\pm$ SD.

1.5 Daily caloric intake

Figure 4-5 shows daily caloric intake of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

The average daily caloric intake of those rats over 12 wk were 130.58 $\pm$ 21.39, 106.38 $\pm$ 10.53, 102.02 $\pm$ 10.32, 138.78 $\pm$ 15.99, 101.14 $\pm$ 16.75 kilocalories, respectively. Daily caloric intake of high fat diet groups (DM+SFA, DM+MUFA, DM+MUFA+Ex) were significantly decreased when compared with the two regular diet groups. (DM+SFA vs. Control, p=0.001; DM+MUFA vs. Control, p=0.001; DM+MUFA+Ex vs. Control, p=0.001). (DM+SFA vs. DM+Ex+regular diet, p=0.001; DM+MUFA vs. DM+Ex+regular diet, p=0.001; DM+MUFA+Ex vs. DM+Ex+regular diet, p=0.001).

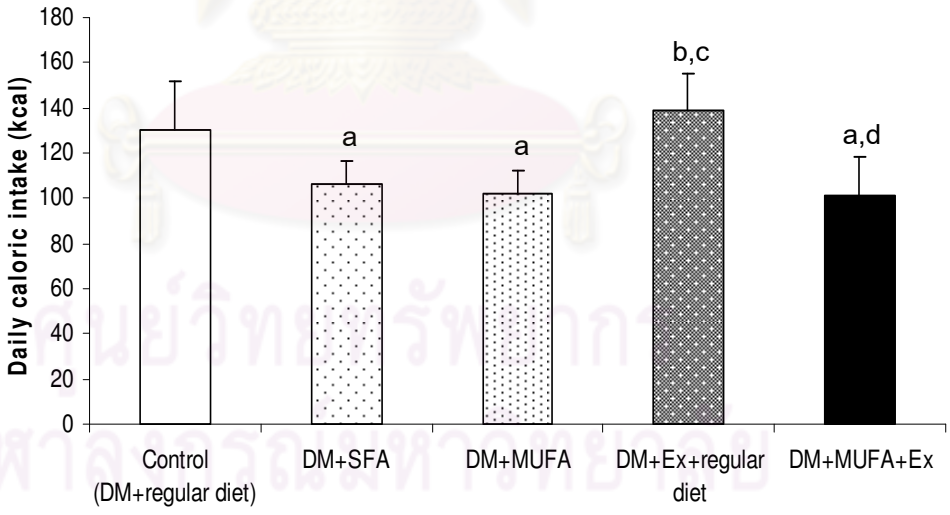

Figure 4-5 Daily caloric intake (kcal) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups.

Data were expressed as mean $\pm$ SD.

[a] p<0.05 vs. Control

[b] p<0.05 *vs*. DM+SFA

[c] p<0.05 *vs*. DM+MUFA

[d] p<0.05 *vs*. DM+Ex+regular diet

Table 4-1 The summary of the results of final body weight (g), liver weight (g), gastrocnemius weight (g), muscle weight per body weight (mg/g) and daily caloric intake (kcal) of rats in Control (DM+regular diet), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet), Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

Groups	Final body weight (g)	Liver weight (g)	Gastrocnemius weight (g)	Muscle weight/BW. (mg/g)	Daily caloric intake (kcal)
Control (DM+regular diet)	311.90 ± 45.77 (n=7)	14.76 ± 2.31 (n=7)	1.37 ± 0.25 (n=7)	4.39 ± 0.27 (n=7)	130.58 ± 21.39 (n=7)
DM+SFA	389.30 ± 21.18[a] (n=8)	16.49 ± 1.65 (n=8)	1.82 ± 0.27[a] (n=8)	4.66 ± 0.57 (n=8)	106.38 ± 10.53[a] (n=8)
DM+MUFA	360.30 ± 76.11 (n=8)	14.63 ± 2.01 (n=8)	1.59 ± 0.31 (n=8)	4.43 ± 0.19 (n=8)	102.02 ± 10.32[a] (n=8)
DM+Ex+regular diet	372.60 ± 50.37 (n=7)	15.12 ± 1.98 (n=7)	1.72 ± 0.31 (n=7)	4.59 ± 0.38 (n=7)	138.78 ± 15.99[b,c] (n=7)
DM+MUFA+Ex	349.25 ± 48.09 (n=8)	14.52 ± 1.09 (n=8)	1.73 ± 0.26 (n=8)	4.96 ± 0.38 (n=8)	101.14 ± 16.75[a,d] (n=8)

Data were expressed as mean ± SD.

[a] $p<0.05$ vs. Control

[b] $p<0.05$ *vs*. DM+SFA

[c] $p<0.05$ *vs*. DM+MUFA

[d] $p<0.05$ *vs*. DM+Ex+regular diet

2. Biochemical characteristics

2.1 Plasma glucose

Figure 4-6 shows plasma glucose of DM+regular diet (Control). Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, plasma glucose levels were 428.40 ± 25.98, 390.50 ± 63.23, 309.42 ± 104.97, 366.86 ± 67.95 and 317.50 ± 81.76 mg/dL, respectively. Plasma glucose was significantly lower in DM+MUFA (p=0.036) and DM+MUFA+Ex (p=0.047) groups when compared with the control group. There were no significant differences between Control, DM+SFA and DM+Ex+regular diet.

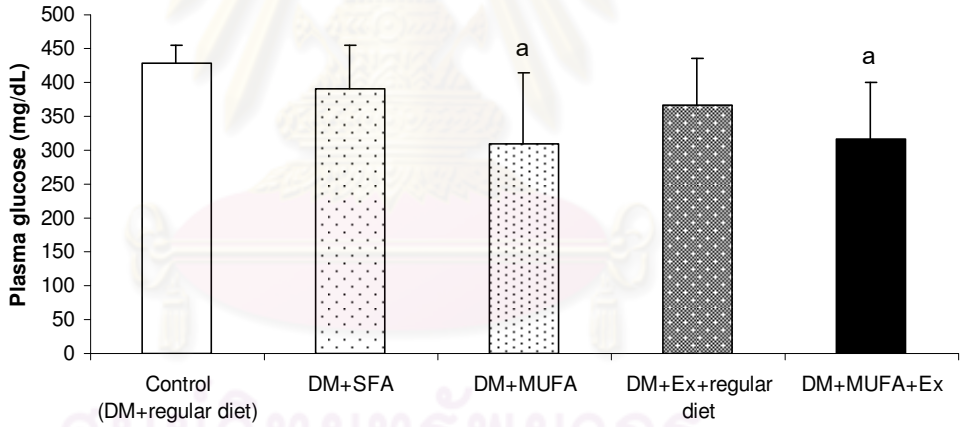

Figure 4-6 Plasma glucose (mg/dL) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups.

Data were expressed as mean ± SD.

[a] $p<0.05$ vs. Control

2.2 Glycated hemoglobin (HbA_{1C})

Figure 4-7 shows glycated hemoglobin (HbA_{1C}) of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, the levels of glycated hemoglobin (HbA_{1C}) of those rats were 8.20 $\pm$ 0.85, 7.20 $\pm$ 0.79, 6.83 $\pm$ 0.88, 7.60 $\pm$ 1.08 and 6.20 $\pm$ 1.06 %, respectively. In DM+MUFA+Ex, there was a significant decrease in glycated hemoglobin (HbA_{1C}) when compared with the control group (p=0.002). Glycated hemoglobin levels did not show a significant difference among Control, DM+SFA, DM+MUFA and DM+Ex+regular diet.

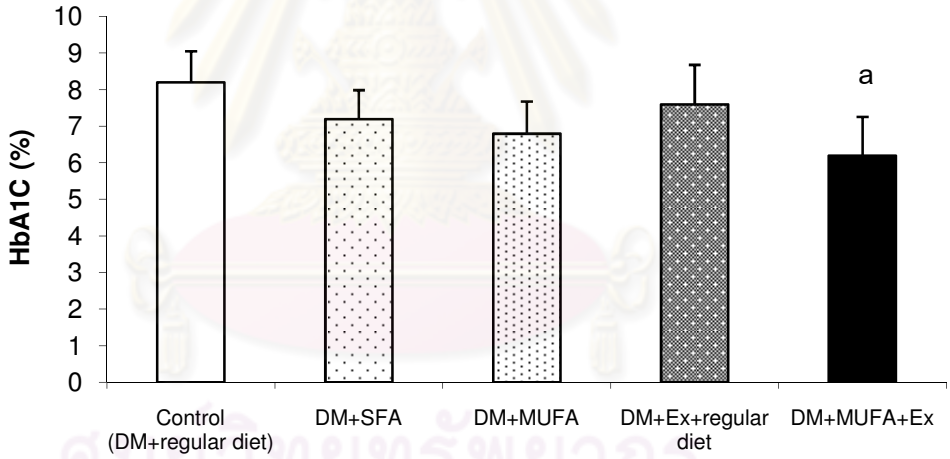

Figure 4-7 Glycated hemoglobin (HbA_{1C}) (%) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups. Data were expressed as mean $\pm$ SD.

[a] p<0.05 vs. Control

2.3 Plasma insulin levels

Figure 4-8 shows plasma insulin levels of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, insulin levels of those rats were 1.87 $\pm$ 0.15, 1.93 $\pm$ 0.44, 1.86 $\pm$ 0.16, 1.66 $\pm$ 0.16 and 1.80 $\pm$ 0.25 ng/mL, respectively. Insulin levels among all groups did not differ significantly.

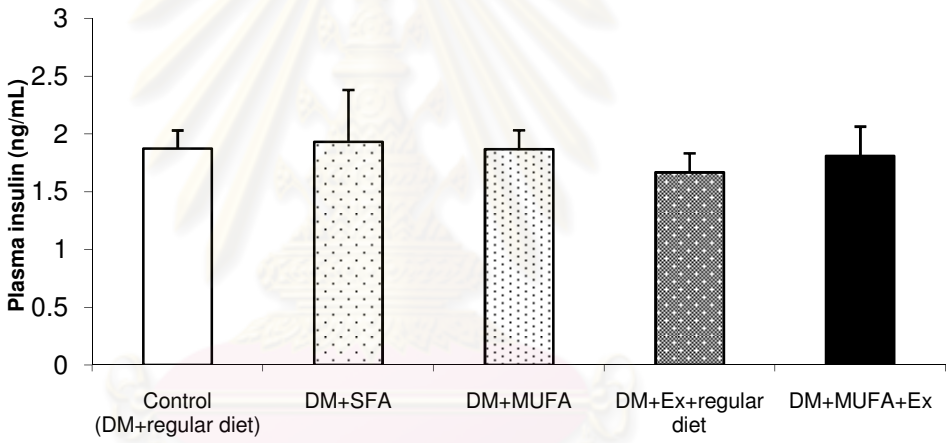

Figure 4-8 Plasma insulin levels (ng/mL) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups.

Data were expressed as mean $\pm$ SD.

2.4 Serum free fatty acid (FFA)

Figure 4-9 shows serum free fatty acid (FFA) of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, serum free fatty acid (FFA) levels of those rats were 0.82 $\pm$ 0.16, 0.83 $\pm$ 0.10, 0.69 $\pm$ 0.15, 0.53 $\pm$ 0.07 and 0.52 $\pm$ 0.21 mmol/L, respectively. Free fatty acid showed a significant decrease in DM+Ex+regular diet (p=0.007) and DM+MUFA+Ex (p=0.008) groups when compared with the control group. Free fatty acid levels in DM+Ex+regular diet (p=0.004) and DM+MUFA+Ex (p=0.004) groups were decreased significantly when compared with DM+SFA.

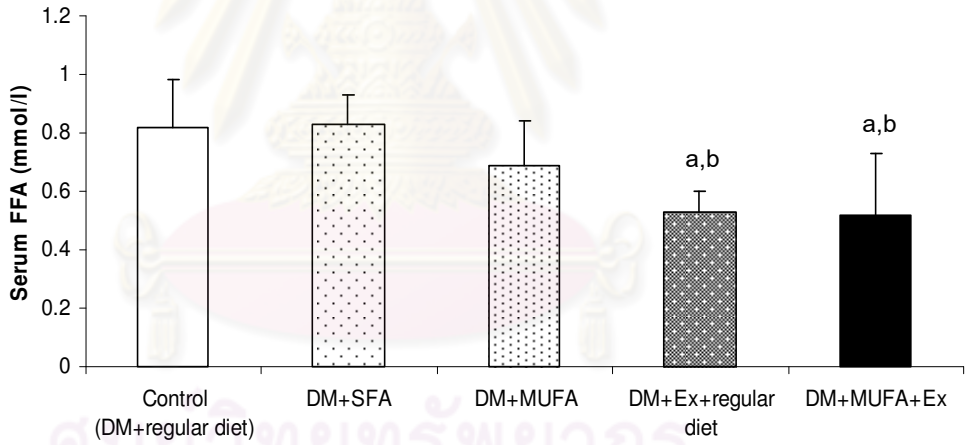

Figure 4-9 Serum free fatty acid (FFA) (nmol/L) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups. Data were expressed as mean $\pm$ SD.

[a] p<0.05 vs. Control [b] p<0.05 *vs*. DM+SFA

2.5 Serum β-hydroxybutyrate

Figure 4-10 shows serum β-hydroxybutyrate of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, serum β-hydroxybutyrate levels of those rats were 5.53 ± 3.07, 3.45 ± 0.85, 4.76 ± 4.39, 2.91 ± 0.68 and 2.43 ± 0.82 mmol/L, respectively. The levels of β-hydroxybutyrate showed a significant decrease in DM+MUFA+Ex when compared with Control (p=0.048). The levels of β-hydroxybutyrate in DM+Ex+regular diet did not differ significantly (p=0.152) when compared with Control.

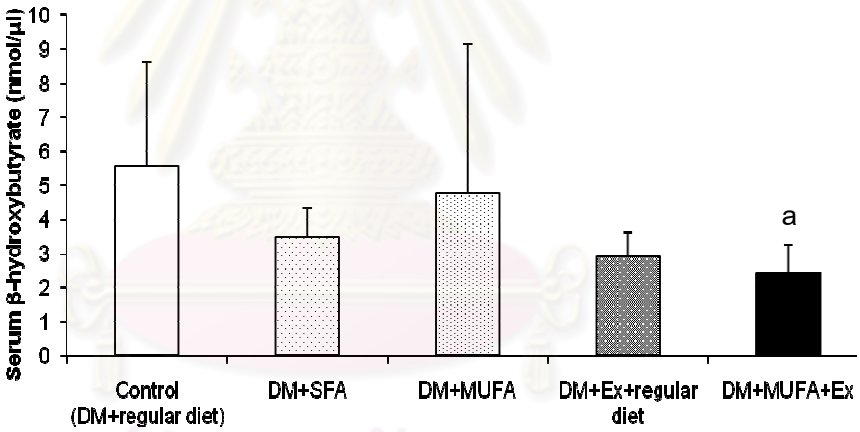

Figure 4-10 Serum β-hydroxybutyrate (nmol/µL) of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups. Data were expressed as mean ± SD.

[a] p<0.05 vs. Control

Table 4-2 The summary of the results of plasma glucose (mg/dL), glycosylated hemoglobin (HbA_{1C}) (%), plasma insulin levels (ng/mL), serum free fatty acid (FFA) (mmol/L) and serum β-hydroxybutyrate (nmol/μL) of rats in Control (DM+regular diet), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet), Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

Groups	Plasma glucose (mg/dL)	HbA_{1C} (%)	Plasma insulin levels (ng/mL)	Serum FFA (mmol/L)	Serum β-hydroxybutyrate (nmol/ μL)
Control (DM+regular diet)	428.40 ± 25.98 (n=7)	8.20 ± 0.85 (n=7)	1.87 ± 0.15 (n=7)	0.82 ± 0.16 (n=6)	5.53 ± 3.07 (n=7)
DM+SFA	390.50 ± 63.23 (n=8)	7.20 ± 0.79 (n=8)	1.93 ± 0.44 (n=7)	0.83 ± 0.10 (n=7)	3.45 ± 0.85 (n=6)
DM+MUFA	309.40 ± 104.97[a] (n=8)	6.80 ± 0.88 (n=8)	1.86 + 0.16 (n=7)	0.69 ± 0.15 (n=7)	4.76 ± 4.39 (n=7)
DM+Ex+regular diet	366.80 ± 67.95 (n=7)	7.60 ± 1.08 (n=7)	1.66 ± 0.16 (n=7)	0.53 ± 0.07[a,b] (n=7)	2.91 ± 0.68 (n=6)
DM+MUFA+Ex	317.50 ± 81.76[a] (n=8)	6.20 ± 1.06[a] (n=8)	1.80 ± 0.25 (n=8)	0.52 ± 0.21[a,b] (n=6)	2.43 ± 0.82[a] (n=8)

Data were expressed as mean ± SD.

[a] $p<0.05$ vs. Control

[b] $p<0.05$ *vs*. DM+SFA

[c] $p<0.05$ *vs*. DM+MUFA

[d] $p<0.05$ *vs*. DM+Ex+regular diet

3. Enzymatic activities in ketone metabolism

3.1 **β**-hydroxybutyrate dehydrogenase activity (Marker of hepatic ketone body synthesis)

Figure 4-11 β-hydroxybutyrate dehydrogenase activities of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, β-hydroxybutyrate dehydrogenase activities of those rats were 0.80 ± 0.27, 0.98 ± 0.32, 0.47 ± 0.30, 0.40 ± 0.21 and 0.32 ± 0.13 μmoles of NADH/min/g of liver, respectively. In DM+Ex and DM+MUFA+Ex, there was a significant decrease in β-hydroxybutyrate dehydrogenase activities when compared with Control (p=0.020); (p=0.009) and when compared with DM+SFA (p=0.001); (p=0.001) for DM+Ex and DM+MUFA+Ex, respectively. DM+MUFA showed a significant decrease in β-hydroxybutyrate dehydrogenase activities when compared with DM+SFA group (p=0.003).

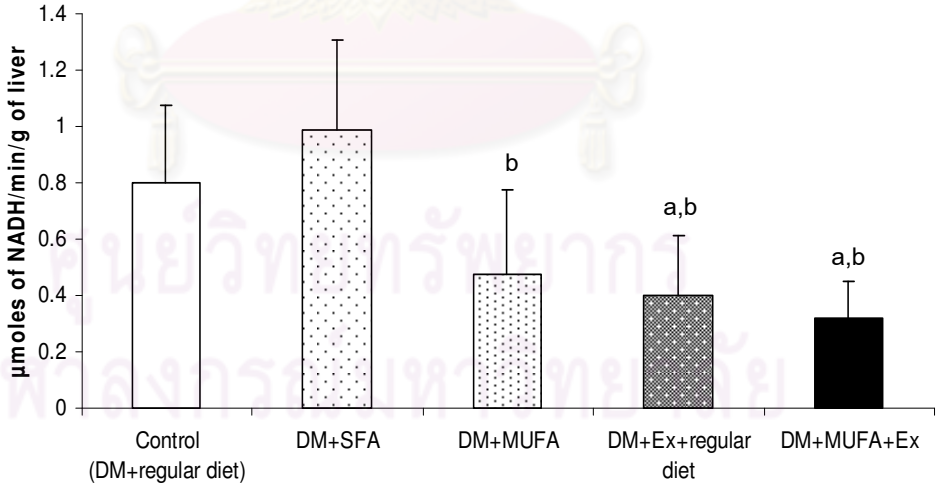

Figure 4-11 β-hydroxybutyrate dehydrogenase activity (Marker of hepatic ketone body synthesis) expressed per gram of liver of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups.

Data were expressed as mean ± SD.

[a] P<0.05 vs. Control [b] P<0.05 *vs*. DM+SFA

3.2 3-Ketoacyl CoA-transferase activity (Marker of muscle ketone body utilization)

Figure 4-12 shows 3-ketoacyl CoA-transferase activities of DM+regular diet (Control), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet) and Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

At week 12, 3-ketoacyl CoA-transferase activities of those rats were 0.07 $\pm$ 0.01, 0.08 $\pm$ 0.01, 0.09 $\pm$ 0.02, 0.10 $\pm$ 0.02 and 0.11 $\pm$ 0.01 µmoles of acetoacetyl-CoA/min/g of muscle, respectively. In DM+Ex+regular diet, there was a significant increase in the enzymatic activity of muscle ketone body utilization when compared with the control group (p=0.003). In DM+MUFA+Ex, there was a significant increase in 3-ketoacyl CoA-transferase activities when compared with the Control (p=0.001), DM+SFA (p=0.01) and DM+MUFA (p=0.012).

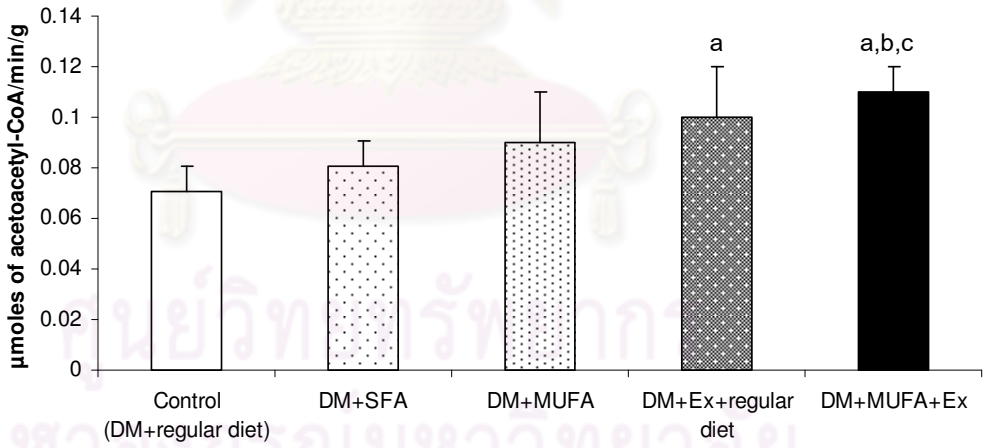

Figure 4-12 3-ketoacyl CoA-transferase activity (Marker of muscle ketone body utilization) expressed per gram of muscle of rats in Control (DM+regular diet), DM+SFA, DM+MUFA, DM+Ex+regular diet and DM+MUFA+Ex groups.

Data were expressed as mean $\pm$ SD.

[a] p<0.05 vs. Control [b] p<0.05 *vs*. DM+SFA

[c] p<0.05 *vs*. DM+MUFA

Table 4-3 The summary of the results of β-hydroxybutyrate dehydrogenase activity (marker of hepatic ketone body synthesis) expressed per gram of liver and 3-ketoacid CoA-transferase activity (marker of muscle ketone body utilization) expressed per gram of muscle of rats in Control (DM+regular diet), Diabetes + high-saturated fat diet (DM+SFA), Diabetes + high-monounsaturated fat diet (DM+MUFA), Diabetes + Exercise training + regular diet (DM+Ex+regular diet), Diabetes + high-monounsaturated fat diet + Exercise training (DM+MUFA+Ex) groups.

Groups	β-hydroxybutyrate dehydrogenase activity (μmoles of NADH/min/g of liver)	β-ketoacyl-CoA transferase activity (μmoles of acetoacetyl-CoA/min/g of muscle)
Control (DM+regular diet)	0.80 ± 0.27 (n=7)	0.07 ± 0.01 (n=7)
DM+SFA	0.98 ± 0.32 (n=8)	0.08 ± 0.01 (n=8)
DM+MUFA	0.47 ± 0.30[b] (n=8)	0.09 ± 0.02 (n=8)
DM+Ex+regular diet	0.40 ± 0.21[a,b] (n=7)	0.10 ± 0.02[a] (n=7)
DM+MUFA+Ex	0.32 ± 0.13[a,b] (n=8)	0.11 ± 0.01[a,b,c] (n=8)

Data were expressed as mean ± SD.

[a] $p<0.05$ vs. Control

[b] $p<0.05$ *vs*. DM+SFA

[c] $p<0.05$ *vs*. DM+MUFA

CHAPTER V

DISCUSSION AND CONCLUSION

In diabetes mellitus, there is a deficiency of insulin and/or insulin resistance which leads to metabolic abnormalities including hyperglycemia, dyslipidemia and ketosis. Under condition of cellular carbohydrate deprivation, there is an increase in plasma FFA and ketone body concentrations resulting from increased lipolysis to compensate the fall in available carbohydrate (Balasse and Fery, 1989). The concentration of ketone bodies in the blood represents the balance between their production and utilization rates by tissues. Increased ketone body levels thus result from either their increased hepatic ketone body synthesis or their decreased muscle ketone body utilization.

In the present study, the experiments were aimed to investigate whether high-monounsaturated fat diet in combination with exercise training might play roles in improving glycemic control and ketone metabolism in diabetic rat model induced by streptozotocin (STZ).

STZ had highly specific cytotoxic action on the β-cell of the Islets of Langerhans. This drug has been widely used, especially in rats, to induce the experimental model of type 1 diabetes mellitus. Numerous studies are performed with this model, providing evidence of its usefulness in studying diabetic complications (Crijns, 1999). In the present study, hyperglycemia was confirmed by 48 hours after intraperitoneal administration of 55 mg/kg STZ.

The optimal diet for diabetic patients has long been a subject of controversy. Dietary therapy was the only treatment available in the era before insulin therapy (Osler, 1892). As elucidated by Joslin (Joslin, 1928), dietary CHO had to be restricted in patients with type 1 diabetes because of impaired CHO metabolism. There are the good foods of life such as meat, cream, butter, cheese, and eggs. Such diets were ketogenic was and consisted mainly of fat and protein. Joslin also commented that this type of diet

atherogenic and made the point that, if patients did not die of diabetic ketogenesis, they would probably die of coronary artery disease.

With the discovery of insulin in 1921 (Banting and Best, 1922), it became possible to introduce CHO-containing foods into the diabetic diet, but this approach was poorly accepted. However, research in the 1950s, suggested that a high-CHO, low-fat diet could be used in diabetic patients to lower cholesterol without increasing the plasma triglycerol concentration (Stone and Conor, 1963). More recently, a diet low in saturated fat has become accepted (American Diabetes Association Position Statement, 2002), but controversy has focused on whether the diabetic diet should be higher in MUFA or higher in CHO. It was demonstrated that a high-MUFA diet would avoid the possible plasma triacylglycerol and glucose-elevating effects of a high-CHO diet in diabetic patients (Garg, 1998).

In the past, Mediterranean region have reported low mortality rates from cardiovascular disease (CVS) despite the consumption of diets high in total fat (Key *et al.*, 1986). A common feature of Mediterranean diets is the use of olive oil containing MUFA as the principle fat, suggesting that the consumption of MUFA is associated with reducing the CVS risk in this region. Many studies have shown that effects of high-MUFA diets lowered blood triglyceride (Garg, 1988, 1994; Campbell *et al.,* 1994; Parillo *et al.*, 1992) and VLDL-cholesterol (Garg, 1988, 1994) levels and elevated HDL-cholesterol levels (Garg, 1988, 1992) in diabetic patients compared with low-fat diets. In addition, high-MUFA diets were seen to be more effective than low-fat diets for efficient glycemic control (Garg, 1988, 1994; Campbell *et al.*, 1994, Parillo *et al.*, 1992) and improving insulin sensitivity (Garg *et al.*, 1988, Parillo *et al.*, 1992) in diabetic patients.

Based on the study in patients with diabetes mellitus, new diet recommendations were issued by the American Diabetes Association (1994), which suggested that 60% to 70% of calories be derived from the combination of carbohydrate and monounsaturated fat. The intake of carbohydrate and fat was to be individualized on the basis of treatment goals but might include as much as 20% of calories from monounsaturated fat with a corresponding decrease in the carbohydrate content (to 40% to 50%). Since saturated

and polyunsaturated fat intake of 10% each are suggested, the total fat intake could be as high as 40% (American Diabetes Association, 1994). High-MUFA supplied 38% of the total calories from fat was lowered serum glucose levels in STZ-induced diabetic rats (Kotake *et al.,* 2004). Consequently, during 12 weeks of experimental period in this study, diabetic rats in (DM + SFA, DM + MUFA and DM + MUFA + Ex) groups were fed diets containing 35 percent of fat.

Although high-MUFA diet has been associated with improvement in glycemic control and dyslipidemia in controlled metabolic studies (Garg, 1998), the results are not necessarily applicable to free-living subjects. In the present study all the rats were fed *ad libitum* and thus allowed to regulate their own energy intake. In a study of Gerhard *et al.* (2004), an *ad libitum* high-MUFA diet did not cause body weight loss in patients with type 2 diabetes (Gerhard *et al*., 2004).

EFFECTS ON FINAL BODY WEIGHT, LIVER WEIGHT, GASTROCNEMIUS WEIGHT, MUSCLE WEIGHT PER BODY WEIGHT AND DAILY CALORIC INTAKE.

In this study, it was found that final body weight was significantly increased only in DM+SFA group, whereas daily caloric intake was lower in DM+SFA, DM+MUFA and DM+MUFA+Ex groups when compared with control (DM+regular diet) group.

A study showed that energy-controlled high-MUFA diets do not promote weight gain and are more acceptable than low-fat diet for weight loss in obese subjects (Ros, 2003). In the present study, DM+Ex group did not differ in body weight when compared with control group. This result is consistent with those of Midaoui *et al.* (2005, 2006) that the final body weight in trained diabetic rats did not differ when compared with sedentary diabetic rats. Furthermore, it was demonstrated that isocaloric replacement of SFA by supplements of virgin olive oil did not cause changes in the body weight and could improve homeostasis model analysis-insulin resistance (HOMA-ir) and fasting pro-insulin levels in insulin-resistant subjects (Paniagua *et al*., 2007).

Noticeably, the results showed that when the rats were allowed to eat *ad libitum*, the rats fed with high-fat diet consumed less calories than those fed with regular diet. Some previous studies have found that high-fat diet often decreases feed intake in dairy

cows (Schauff and Clark, 1992; Christensen *et al*., 1994). Mechanisms regulating fat-induced depression of food intake have been established. Reducing equivalents generated by fatty acid oxidation are reportedly involved in fat-induced depression of feed intake in rats (Sharrer and Langhans, 1986). In addition, feedback satiety signal may be generated to prevent further influx of fuels (Palmquist, 1994). Another mechanism is that the high-fat diet decreased feed intake by increasing concentrations of lipid metabolites, thus increasing post-feeding plasma cholecystokinin and pancreatic polypeptide concentrations, both of which are satiety hormones (Choi and Palmquist, 1996).

However, it is revealed that liver weight and muscle weight per body weight in all groups did not significantly differ when compared with control group. Midaoui *et al.* also found that the liver weight and gastrocnemius weight in trained diabetic rats group did not differ when compared with sedentary diabetic rats group (Midaoui *et al*., 2005, 2006).

EFFECTS ON GLYCEMIC CONTROL: PLASMA GLUCOSE, HbA_{1c}, AND PLASMA INSULIN LEVELS.

The present study demonstrated no change in plasma glucose and HbA_{1c} in DM+Ex group when compared with control group, although there is a tendency to decrease. The exercise training protocol used in this study was modified from Midaoui *et al.* (2005, 2006) and this finding in glycemic control was also consistent with the previous study (Midaoui *et al*., 2005, 2006). They found that the exercise trained diabetic rats fed *ad libitum* regular diet did not show a decrease in plasma glucose.

Exercise training has been broadly known to have beneficial effects on diabetes, including reduction of blood glucose. These include decreased blood glucose level (Dall'Aglio *et al*., 1983; Goodyear *et al*., 1988; Chakraphan *et al*., 2005; Nakai *et al*., 2002; Boule *et al.,* 2005; Ozakaya *et al*., 2007), increased insulin sensitivity, improved glucose homeostasis, lowered FFA and decreased triglyceride synthesis from liver (Beattie and Winder, 1985; Winder *et al*., 1974; Midaoui *et al*., 1996, Goodyear *et al.,* 1988; Chakraphan *et al*., 2005; Nakai *et al*., 2002; Ozakaya *et al*., 2007). However, there

are some controversial reports that exercise training does not affect blood glucose level (Midaoui *et al*., 1996, 2005, 2006).

In general, regular exercise training has an impact on glucose homeostasis in skeletal muscle of STZ-diabetic rats as the following mechanisms: 1) increase in GLUT-4 content, a protein found in intracellular vesicles and then translocated to the plasma membrane in response to insulin and exercise, 2) increase in insulin-mediated glucose uptake, 3) and a two-fold enhanced capacity of the pyruvate dehydrogenase complex (PDH) activity, the rate limiting step enzyme of aerobic glucose oxidation in tissues (Nakai *et al.,* 2002). Pold *et al*., 2005 have described an increased GLUT-4 protein expression in exercised skeletal muscle and subsequent increase in whole body insulin sensitivity in the Zucker diabetic fatty (ZDF) rat, a model that closely resembles human type 2 diabetes (Pold *et al*., 2005). Physical training represented the effects of PDH activity and glucose metabolism. Physical training in chronic insulin deficiency may increase glucose uptake in contracting skeletal muscle and independently activated PDH complex activity (Nakai *et al*., 2002).

In comparison with control group, it was demonstrated that MUFA diet and MUFA plus exercise could significantly decrease plasma glucose levels while exercise alone did not. Thus, it is suggested that the effect on reduced plasma glucose in diabetic rats in this study is mainly due to MUFA. Plasma insulin levels in all groups remained in the low levels and did not differ significantly among groups, suggesting that MUFA may improve glucose control with little or no effect of insulin.

Long term glycemic control has been shown only in DM+MUFA+Ex group, as indicated by a significant decrease in glycated hemoglobin, which tended to decrease to a normal level after twelve-week period. However, the rats in DM+Ex and DM+MUFA groups did not exhibit a reduction in HbA_{1c}. These results demonstrate that MUFA combined with exercise have a greater efficacy on glycemic control than either MUFA or exercise alone.

A number of studies have demonstrated hypoglycemic effect of MUFA in diabetic patients and animals. Monounsaturated fat (MUFA) may play a role in a

reduction of blood glucose in type 2 diabetic patients (Garg *et al.,* 1988, 1994) and was demonstrated to reduce the peak plasma glucose in type 2 diabetic patients when compared with high-CHO diet (Rasmussen *et al*., 1993). Kotake *et al.* (2004) examined the short-term effects of a high-MUFA diet on normal and genetically mice (high-MUFA supplied 38% of the calories as fat, 26% from MUFA). They found that high-MUFA was lowering the triglyceride by the mechanism of the reduced hepatic triglyceride production and ameliorated abnormality in serum glucose levels, as well as impaired glucose tolerance of diabetic mice. A high-MUFA enteral formula can suppress post-prandial hyperglycemia and does not excessively stimulate insulin secretion in patients with type 2 diabetes mellitus, therefore, promising as a means of improving the health of diabetic patients and preventing development of cardiovascular diseases (Yokoyama *et al*., 2008).

Many investigations have proposed: the possible mechanisms of action of MUFA on glycemic control as follows:

1. Reversal of the decreased the enzyme activity of glucose metabolism in diabetic rats

Glucokinase is the key enzyme in the catabolism of glucose, which phosphorylates glucose to glucose-6-phosphate. It was revealed that MUFA significantly increased the activity of glucokinase in the liver of STZ-diabetic rats, which may be associated with reduced blood glucose (Ramesh *et al.,* 2006).

2. Increased GLP-1 (glucagon-like peptide-1) secretion

It was found that MUFA appeared to be powerful stimulators of GLP-1 secretion both in enterocytes culture from rats and *in vivo* in Zucker (genetically obese) rats (Rocca *et al*., 1995, 2001).

Paniagua *et al.* (2007) evaluated the effects of three weight-maintenance diets, which included SFA, MUFA and carbohydrate, on carbohydrate metabolism, lipid metabolism and insulin level in type 2 diabetic patients. They found that MUFA–rich diet did not cause a difference in weight-maintenance and could improve homeostasis model analysis-insulin resistance (HOMA-ir) and fasting pro-insulin levels. Ingestion of a

virgin olive-oil-based breakfast decreased postprandial glucose and increased insulin level, and increased HDL-C and glucagon-like peptide 1 (GLP-1), which secreted from gut endocrine cells in response to nutrient ingestion. The biological activities of GLP-1 include stimulation of glucose-dependent insulin secretion and inhibition of food intake. Therefore, the findings that GLP-1 lowers blood glucose and that GLP-1 may restore β-cell sensitivity suggests that activation of GLP-1 signaling may become a useful strategy to treat diabetic patients as compared with CHO-rich diet (Paniagua *et al.,* 2007).

EFFECTS ON KETONE METABOLISM: SERUM FFA, SERUM β-HYDROXYBUTYRATE, β-HYDROXYBUTYRATE DEHYDROGENASE ACTIVITY AND 3-KETOACYL-CoA TRANSFERASE ACTIVITY

Under condition of carbohydrate deprivation or insulin lack and glucagon excess, there is an increase in FFA and ketone body levels.

The ketone bodies was shown in two acidic forms as β-hydroxybutyrate and acetoacetate and metabolic end dead form as acetone, a nonmetabolized side product, resulting from increased lipolysis to compensate the fall in available carbohydrate. The concentration of ketone bodies in blood represents the balance between the rates of hepatic production and peripheral tissues utilization. The ketone bodies were increased as a result of an increased synthesis and/or impairment of utilization.

Ketone bodies are synthesized in liver mitochondria by four-step enzymatic pathway. Two molecules of acetyl-CoA derived from FFA are used to generate into acetoacetyl-CoA by acetyl-CoA thiolase. Acetoacetyl-CoA is then converted by HMG-CoA synthase into HMG-CoA, which is further converted into acetoacetate by HMG-CoA lyase. Finally, acetoacetate is converted into acetone by acetoacetate decarboxylase, and into β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase. In this study, the activity of β-hydroxybutyrate dehydrogenase in liver mitochondria was evaluated by using spectrophotometrically method.

Ketone bodies are utilized in peripheral tissue by three-step enzymatic pathway. β-hydroxybutyrate is converted into acetoacetate and then acetoacetyl-CoA by β-hydroxybutyrate dehydrogenase and 3-ketoacyl-CoA transferase, respectively. Finally

acetoacetyl-CoA is converted into two molecules of acetyl-CoA by acetyl-CoA thiolase. Avetyl-CoA is transferred to cytoplasm for oxidative phosphorylation in Kreb's cycle to generated energy. In this study, the activity of 3-ketoacyl-CoA transfarase in mitochondria gastrocnemius muscle was evaluated by using spectrophotometrically method.

STZ-induced diabetes is associated with a significant increase in plasma FFA and β-hydroxybutyric acid levels in sedentary rats (Lang *et al*., 2002). It also shows that exercise training can normalize plasma FFA levels and markedly reduce β-hydroxybutyric acid levels in these diabetic rats.

Previously, Winder *et al.* (1974) demonstrated that homogenates of gastrocnemius muscles from endurance-trained rats oxidized 3-β-hydroxybutyrate two to three times as rapidly as homogenates from sedentary rats. Also, Beattie and Winder (1985) showed that trained rats exhibited lower plasma β-hydroxybutyric acid levels during and after a bout of exercise than their sedentary counterparts. Ohmori *et al.* (1990) showed that trained rats had lower circulating levels of β-hydroxybutyric acid during exercise than sedentary rats. They also demonstrated that ketone body uptake by perfused hindlimb was greater in trained than in sedentary rats. Studies conducted in skeletal muscle of non-diabetic animals have demonstrated that 3-β-hydroxybutyrate dehydrogenase, 3-ketoacyl CoA-transferase, and acetoacetyl-CoA thiolase activities were increased by training (Winder *et al*., 1974).

It has been demonstrated by hepatic catheterization in severe uncontrolled diabetes that up to 80-90% of the FFA taken up by the liver is converted to ketone bodies (Owen *et al.* 1977; Wahren *et al*., 1975). STZ-induced diabetes in the rat was associated with an 80% increase in FFA levels and a 700% increase in β-hydroxybutyrate. This major difference between the rises in FFA and β-hydroxybutyric acid is most likely explained by the more efficient liver in synthesizing ketone bodies from FFA (Midaoui *et al.,* 2006,).

Quant *et al.* (1989) reported that the activity of HMG-CoA synthase from rat liver extracts doubled in alloxan-induced diabetes. Williamson *et al.* (1968) have also

demonstrated that the HMG-CoA synthase activity was increased by 90% in whole liver homogenate from alloxan-diabetic rats. They have reported that HMG-CoA lyase and acetoacetyl-CoA thiolase were increased by 35% and 4%, respectively, in whole homogenate of livers from alloxan-diabetic rats. Midaoui *et al.* (2006) evaluated the overall activity of the hepatic ketone body synthesis pathway, as measured by β-hydroxybutyrate dehydrogenase in relation to the production of β-hydroxybutyrate from acetoacetate.

The impact of long-term untreated experimental diabetes mellitus on the activity of 3-ketoacyl-CoA transferase, the key enzyme in the peripheral utilization of ketone bodies (Nosadini *et al.*, 1989) was studied. This enzymatic activity was found to elevate at the level of the gastrocnemius muscle. Using *in vivo* intravenous infusion of ketone bodies, Bässler *et al.* (1972) observed that the blood levels of ketone bodies rose exponentially with the infusion rate in diabetic rats comparatively with the linear rise observed in normal rats. The result suggested that the same rate of ketone body production by the liver would lead to much higher blood levels of ketone bodies in diabetic than in normal rats. Beatty *et al.* (1959) previously showed that the uptake of acetoacetate by muscle preparations *in vitro* was lower in diabetic than in normal rats. Furthermore, Bässler *et al.* (1973) showed that the 3-ketoacyl-CoA transferase activity was decreased by 40% (units/g) in skeletal muscle of diabetic rats.

Effect of Exercise on ketone metabolism

In this study, it was found that in DM+Ex+regular diet group, serum FFA was significantly decreased, while serum β-hydroxybutyrate tended to decrease but not significantly. β-hydroxybutyrate dehydrogenase activity was significantly decreased and 3-ketoacyl-CoA transferase activity was significantly increased when compared with control (DM+regular diet) group. The present findings were also consistent with those of Midaoui *et al*. (2005, 2006). They found that physical training in diabetic rats significantly decreased FFA, β-hydroxybutyric acid and activity of β-hydroxybutyrate dehydrogenase,

while significantly increased activity of 3-ketoacyl-CoA transferase when compared with sedentary diabetic rats.

It has been suggested that the reduction in plasma β-hydroxybutyric acid levels observed in trained diabetic rats is probably explained, in part, by the reversal of the decrease in 3-ketoacyl CoA-transferase activity present in sedentary diabetic rats (Midaoui *et al*., 2005). Furthermore, physical training could decrease β-hydroxybutyric acid in part by the decrease in plasma FFA levels and in part by the decrease in overall activity of the hepatic ketone body synthesis pathway, as measured by HMG-CoA synthase (Midaoui *et al*., 2006). It was also compatible with observations that physical training is associated with an increase in the sensitivity of carnitine pamitoyltransferase (CPT-I) to malonyl-CoA (Starritt *et al*., 2000), which would be expected to decrease the oxidation of FFA and the production of ketone bodies. Therefore, physical training has been shown to increase the metabolism of ketone bodies, resulting in an overall lower concentration of β-hydroxybutyric acid (Midaoui *et al*., 2006).

Effect of MUFA on ketone metabolism

In this study, it was demonstrated that serum FFA and serum β-hydroxybutyrate were unchanged in DM+MUFA group. β-hydroxybutyrate dehydrogenase activity tended to decrease but not significantly. The activity of 3-ketoacyl-CoA transferase did not significantly difference when compared with control (DM+regular diet) group.

In the present study, MUFA did not affects ketone metabolism, although there was decreased in blood glucose levels. It is suggested that ketone bodies levels are not necceserily dependent on glucose levels. However, the effect of MUFA on plasma FFA is controversial. A study in type 2 diabetic patients also revealed that plasma FFA did not significantly differ after 6-hour post consumption of high-MUFA meals (Manning *et al.,* 2004). In addition, serum FFA in type 2 diabetic patient receiving high-MUFA enteral formula did not significantly differ from those receiving high-carbohydrate enteral formula (Yokoyama *et al.,* 2008). It is suggested that MUFA did not affect serum FFA.

A study showed that the high-MUFA could lower serum FFA levels in C57BL/6J (the genetically diabetic) mice and decreased mobilization of FFA from adipose tissue might occur, which was associated with the lowering triglyceride production. It has been reported that oleic acid inhibits hormone-sensitive lipase (HSL), the enzyme regulating the release of lipids from adipose tissue into the bloodstream as FFA and has a major role in determining circulating levels (Severson and Hurley, 1984, Jepson and Yeaman, 1992). It seems that triglyceride lowering effect of MUFA occurred as a consequence of a decrease in FFA levels at least in part because FFA are delivered to the liver and stimulate hepatic triglyceride production (Lewis, 1997; Kohout *et al*., 1971).

Effect of MUFA plus exercise on ketone metabolism

When compared with control group, FFA was found to decrease significantly to the same extent in DM+MUFA+Ex and DM+Ex+regular diet groups, but not in DM+MUFA group. This result clearly demonstrates that the lowering effect of exercise training on serum FFA. The effect of exercise training on a decreased serum FFA as shown in this study is consistent with the study of Midaoui *et al.* (2005, 2006).

The present study demonstrates a significant decrease in serum FFA in DM+MUFA+Ex. Although only in this group was serum β-hydroxybutyrate significantly decreased, a synergistic effect of exercise training and MUFA could not clearly be demonstrated ketone bodies. β-hydroxybutyrate dehydrogenase activity was significantly decreased and 3-ketoacyl-CoA transferase activity was significantly increased when compared with control (DM+regular diet) group. Because, MUFA alone did not affect plasma FFA, ketone bidies and enzymatic activity of ketone synthesis and utilization. It is suggested that effect on an improvement of ketone metabolism in diabetic rats in this study is mainly due to exercise training.

CONCLUSION

The present findings can be summarized as follows (figure 5-1):

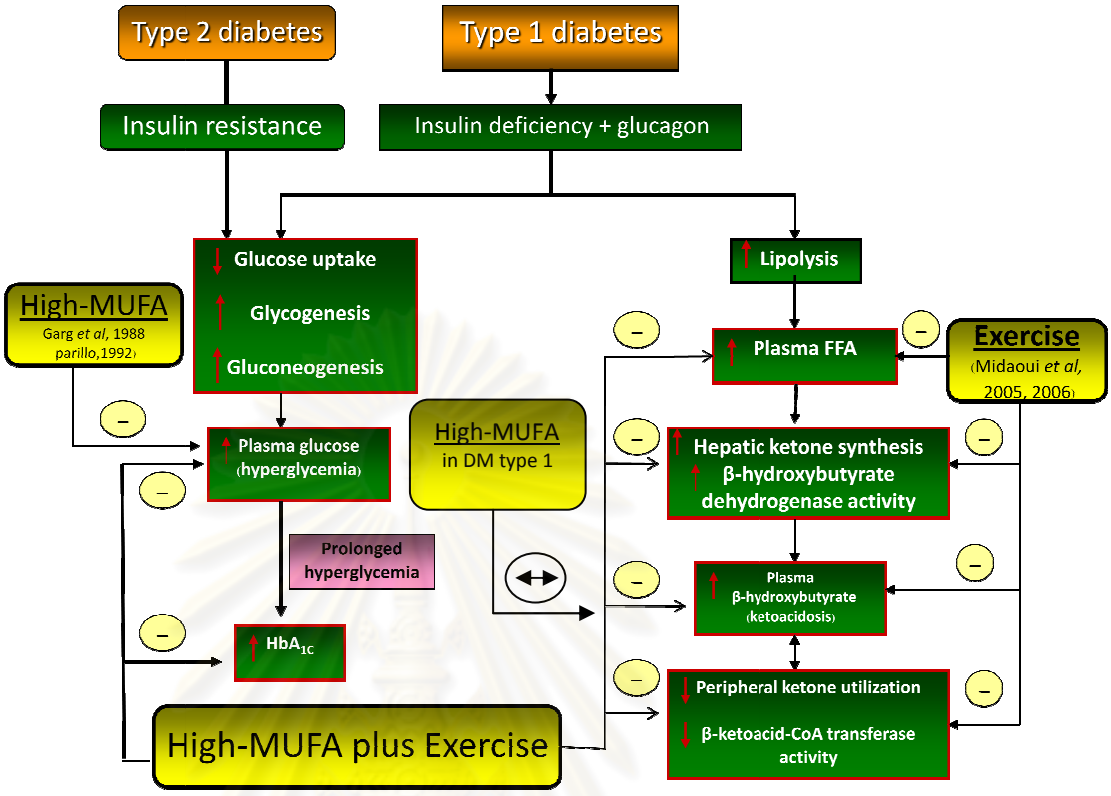

Figure 5-1 Summary of the effects of MUFA and MUFA plus exercise on ketone metabolism and glycemic control in diabetic rats.

1. MUFA reduced plasma glucose levels but did not lower serum FFA and ketone bodies levels. However, MUFA tended to decrease hepatic ketone synthesis but did not significantly increase muscle ketone utilization.

2. This exercise training program reduced serum FFA and tended to reduce ketone bodies. The exercise training also exerted an inhibitory effect on hepatic ketone synthesis and stimulatory effect on muscle ketone utilization.

3. MUFA plus exercise was more effective than either MUFA or exercise alone for improvement of glycemic control and reduction in blood level of ketone bodies by decreasing the activity of the hepatic ketone body synthesis pathway as measured by β-hydroxybutyrate dehydrogenase activity as well as increasing the activity of muscle ketone body utilization pathway as measured by 3-ketoacyl CoA transferase activity.

This study suggested that MUFA and proper exercise training program when used in combination potentially ameliorate metabolic abnormalities including glucose,

lipid and ketone metabolism in diabetic patients. This will lead to prevention of diabetic complication such as diabetic ketoacidosis (DKA), micro- and macrovascular complications.

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APPENDIX

Nutrition Fact of olive oil

Serving Size: 1 Table spoon = 15 ml

Serving Per Container: about 35

Amount Per Serving

Calories: 120 kcal Calories from fat: 120 kcal

% Dairy Value: 22

Total Fat 14 gram

Saturated Fat	2	g
Trans Fat	0	g
PUFA	1.5	g
MUFA	**10**	**g**
Cholesterol	0	mg
Total carbohydrate	0	g
Protein	0	g
Sodium	0	g

Nutrition Fact of palm oil

Serving Size: 1 Table spoon = 15 ml

Serving Per Container: about 33

Amount Per Serving

Calories: 130 kcal Calories from fat: 130 kcal

Total Fat 15 gram

Saturated Fat	**7**	**g**
PUFA	1.5	g
MUFA	6.5	g
Cholesterol	0	mg
Total carbohydrate	0	g
Protein	0	g
Sodium	0	g

กลุ่มที่	ตัวที่	BW day o (g)	Final BW (g)	Blood glucose (mg/dL) day 0 by glucostrip	Plasma glucose (mg/dL) by BRIA lab	HbA1C (%)	FFA (mmol/L)	β-hydroxy butyrate (nmol/μL)	Insulin (ng/m)
1 Control (DM+ regular diet)	1	294	310	252	420	7.9	0.98	3.09	2.03
	2	256	291	250	440	10.0	0.91	11.41	1.96
	3	266	340	300	412	7.6	-	4.55	1.93
	4	292	335	299	479	8.5	0.54	-	1.61
	5	277	288	284	428	7.6	0.72	3.14	1.63
	6	270	381	257	397	7.9	0.87	5.52	1.91
	7	252	238	258	423	7.9	0.91	5.49	1.96
2 DM+ SFA	1	300	400	252	446	8.4	0.86	-	1.89
	2	288	404	288	432	7.1	0.60	2.86	1.98
	3	264	378	284	289	6.5	0.84	-	1.87
	4	259	420	254	428	6.8	0.90	2.46	1.95
	5	256	397	300	400	6.3	-	2.99	1.93
	6	272	394	251	292	6.4	0.85	3.50	1.90
	7	260	361	293	411	8.0	0.86	4.60	1.99
	8	258	360	287	426	7.7	0.89	4.31	-
3 DM+ MUFA	1	259	257	259	453	7.0	0.68	2.45	1.88
	2	254	391	298	415	7.8	0.62	2.48	1.92
	3	245	361	262	247	6.9	0.62	2.01	1.50
	4	279	420	298	176	5.2	0.79	1.96	-
	5	275	406	265	320	6.2	-	6.01	1.96
	6	282	393	296	472	7.9	0.76	4.30	1.98
	7	278	427	285	205	7.2	0.46	-	1.95

	8	262	227	299	390	7.5	0.92	14.14	1.88
4 DM+ Ex+ regular diet	1	267	435	255	389	7.5	0.54	2.57	1.54
	2	296	372	265	231	5.5	0.41	2.25	1.90
	3	250	373	260	336	8.1	0.48	2.13	1.55
	4	232	292	257	413	8.7	0.59	3.43	1.61
	5	288	324	276	439	8.3	0.55	-	1.52
	6	266	417	297	388	6.9	0.62	3.35	1.64
	7	267	395	295	372	8.0	0.49	3.74	1.90
5 DM+ MUFA+ Ex	1	295	360	300	411	7.5	0.44	1.92	1.94
	2	263	337	294	358	5.7	-	2.06	1.96
	3	282	430	250	281	5.3	0.30	3.55	1.98
	4	270	374	250	207	5.0	-	2.08	1.93
	5	246	302	270	390	6.8	0.67	1.37	1.91
	6	251	370	250	193	5.0	0.79	1.98	1.90
	7	255	350	299	390	7.5	0.61	3.62	1.58
	8	232	271	300	375	6.7	0.29	2.87	1.26

กลุ่ม	**ตัวที่**	Enzymatic activity of ketone synthesis (µmoles of NADH/min/g of liver)			Enzymatic activity of ketone utilization (µmoles of acetoacetyl-CoA/min/g of muscle)		
		ครั้งที่ 1	**ครั้งที่ 2**	Mean	**ครั้งที่ 1**	**ครั้งที่ 2**	Mean
1 Control (DM+ regular diet)	1	0.766	0.906	0.864	0.048	0.036	0.042
	2	0.975	0.767	0.871	0.067	0.070	0.068
	3	0.689	0.754	0.722	0.073	0.066	0.069
	4	1.456	1.183	1.320	0.083	0.083	0.083
	5	0.417	0.488	0.453	0.074	0.080	0.077
	6	0.627	0.606	0.617	0.080	0.075	0.077
	7	0.845	0.702	0.774	0.083	0.073	0.078

2 DM+ SFA	1	1.463	1.325	1.394	0.080	0.083	0.081
	2	0.529	0.683	0.606	0.039	0.059	0.049
	3	0.766	0.940	0.853	0.102	0.087	0.094
	4	0.777	0.785	0.781	0.092	0.089	0.090
	5	1.200	1.139	1.170	0.086	0.093	0.089
	6	1.031	1.017	1.024	0.089	0.080	0.084
	7	0.675	0.586	0.631	0.083	0.087	0.085
	8	1.477	1.403	1.440	0.077	0.073	0.075
3 DM+ MUFA	1	0.294	0.313	0.304	0.0492	0.090	0.069
	2	0.257	0.285	0.271	0.094	0.126	0.110
	3	0.836	0.683	0.760	0.080	0.077	0.079
	4	0.397	0.488	0.443	0.100	0.107	0.104
	5	0.673	0.519	0.596	0.096	0.103	0.099
	6	0.155	0.118	0.137	0.089	0.093	0.091
	7	1.031	1.031	1.031	0.074	0.065	0.070
	8	0.247	0.233	0.240	0.086	0.098	0.092
4 DM+ Ex+ regular diet	1	0.238	0.120	0.179	0.090	0.078	0.084
	2	0.221	0.147	0.184	0.089	0.084	0.087
	3	0.143	0.113	0.128	0.112	0.119	0.116
	4	0.689	0.546	0.618	0.105	0.086	0.096
	5	0.556	0.696	0.626	0.128	0.125	0.127
	6	0.269	0.280	0.275	0.093	0.080	0.087
	7	0.391	0.495	0.443	0.103	0.109	0.106
5 DM+ MUFA +Ex	1	0.278	0.488	0.383	0.129	0.110	0.120
	2	0.351	0.234	0.293	0.090	0.099	0.095
	3	0.143	0.117	0.130	0.130	0.127	0.129
	4	0.278	0.488	0.383	0.113	0.094	0.104
	5	0.285	0.265	0.275	0.112	0.113	0.113
	6	0.501	0.650	0.576	0.127	0.123	0.125

	7	0.257	0.293	0.275	0.113	0.104	0.109
	8	0.347	0.208	0.278	0.119	0.112	0.116

BIOGRAPHY

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