A high-MUFA diet alone does not affect ketone body metabolism, but reduces glycated hemoglobin when combined with exercise training in diabetic rats

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Background: Monounsaturated fat (MUFA) also has glucose-lowering action, but its effect on ketone bodies is unknown.

Objectives: To examine the effects of high-MUFA diet alone or in combination with exercise training, which can improve glucose and ketone body metabolism, in a rat model of diabetes.

Methods: Wistar rats were administered streptozotocin to induce diabetes and then randomly divided into five groups: sedentary rats fed a regular diet (1), a high-saturated-fat diet (2), a high-MUFA diet (3); and exercise-trained rats fed a regular diet (4), and a high-MUFA diet (5). Training was by a treadmill twice daily, 5 days/week. At 12 weeks, glucose, glycated hemoglobin (HbA1c), insulin, nonesterified fatty acids (NEFA), and β-hydroxybutyrate levels were measured in cardiac blood. Activity of the overall ketone synthesis pathway was determined in liver and 3-ketoacyl-CoA transferase activity determined in gastrocnemius muscle.

Results: A high-MUFA diet tended to lower plasma glucose without affecting other biochemical variables. Training did not change glucose metabolism, but significantly reduced serum NEFA. Only the high-MUFA diet plus training significantly decreased HbA1c levels. Hepatic ketone synthesis was decreased and 3-ketoacyl-CoA transferase activity was increased by training alone or in combination with a high-MUFA diet. Changes in NEFA, β-hydroxybutyrate, and the enzymatic activities in response to training plus a high-MUFA diet were comparable to those caused by training alone.

Conclusion: A high-MUFA diet alone does not alter ketone body metabolism. Combination of a MUFA-rich diet and exercise training is more effective than either MUFA or exercise alone for lowering HbA1c.

Keywords: Beta-hydroxybutyric acid, diabetes mellitus, exercise training, glycated hemoglobin, hepatic ketone body synthesis, ketoacyl-CoA transferase, monounsaturated fatty acid, non-esterified fatty acid, peripheral ketone body utilization

Diabetes mellitus is an endocrine disorder caused by a lack of insulin or a resistance to insulin action. As a consequence, the ability of the body to use glucose as an energy source is reduced, thereby increasing glucose concentration in blood. Subsequently, the body increases lipolysis to produce free fatty acids (FFA) for fuel thus, increasing hepatic biosynthesis of acidic ketone bodies such as β-hydroxybutyrate, which then leads to elevated levels of circulating ketone bodies. β-Hydroxybutyrate is then used in peripheral tissues such as muscle.

Elevated levels of ketone bodies may evoke a serious metabolic complication known as diabetic ketoacidosis (DKA) [1].

Lifestyle interventions for the management of diabetes mellitus fundamentally involve exercise and diet. Exercise training is broadly known to have beneficial effects on glycemic control in diabetic patients by improving glucose tolerance, decreasing pancreatic β cell workload and improving insulin sensitivity [2]. Exercise training can improve glucose homeostasis and fatty acid and ketone metabolism in animal models of diabetes. These include decreased blood glucose levels [3-5], increased insulin sensitivity, increased glucose homeostasis, lowered FFA, and decreased triglyceride synthesis from the liver [4-7].
Furthermore, exercise training is reported to reduce plasma FFA and β-hydroxybutyric acid levels in a rat model of diabetes induced by streptozotocin (STZ) [8-10], through a reversal of defective activity of 3-ketoacyl-CoA transferase in muscle ketone use [9], and a decrease in the overall activity of ketone synthesis pathway in the liver [10].

Monounsaturated fatty acids (MUFA), which are abundantly found in tea seed oil, olive oil, and canola oil, have also been shown to benefit glycemic control in diabetic patients and animals [11-14]. According to meta-analyses of studies in patients with type 2 diabetes, consumption of a high-MUFA diet appears to lower fasting plasma glucose and glycated hemoglobin (HbA1c), an index of glycemic control [11, 12]. Additionally, a high-MUFA enteral formula can suppress postprandial hyperglycemia without excessively stimulating insulin secretion in patients with type 2 diabetes mellitus [13]. A study in diabetic mice showed that a high-MUFA diet with a controlled caloric intake could ameliorate impaired glucose tolerance [14]. The mechanism of glucose-lowering action by MUFAs is proposed to be through the reversal of decreased activity of glucokinase, a key enzyme in the catabolism of glucose [15], and through the activation of glucagon-like peptide-1 secretion [16, 17], which stimulates glucose-dependent insulin secretion and inhibits food intake.

Given that the major effect of MUFA is to improve glucose metabolism, another possible effect of MUFA may involve ketone body metabolism, including ketone body production and utilization. However, the effect of a high-MUFA diet on FFA level and ketone body metabolism is not fully understood. Furthermore, there is still no information regarding the effects of combining high-MUFA diet and exercise training on ketone body metabolism and glycemic control. Therefore, the purpose of the present study was to investigate the effects of a high-MUFA diet alone or in combination with exercise training on ketone body metabolism and glycemic control. Therefore, the purpose of the present study was to investigate the effects of a high-MUFA diet alone or in combination with exercise training on ketone body metabolism and glycemic control in a rat model of diabetes. The STZ 55 mg dose used in this study is considered to produce a model representative of type 1 diabetes [18]. Results obtained from this study may have important implications for diabetes treatment and DKA prevention.

**Materials and methods**

**Reagents**

The following chemicals were obtained from Sigma Chemicals, Saint Louis, MO, USA: acetyl-CoA, acetyl phosphate, β-NADH, citrate buffer, dithiothreitol, iodoacetamide, MOPS (3-(N-morpholino) propanesulfonic acid), phosphotransacetylase (pTA), STZ, succinyl-CoA, Tris(hydroxymethyl)amino-methane, and Triton X-100.

**Rat preparation**

Male Wistar rats weighing 180–220 g obtained from the National Laboratory Animal Center, Mahidol University, Nakornpathom, Thailand were used in this study. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Research Council of Thailand. All care was taken to minimize animal suffering. The animal protocols were approved by the committee of Animal Care and Use, Faculty of Medicine, Chulalongkorn University. Rats were allowed to acclimatize to the laboratory environment for five to seven days after arrival. Two rats were housed per stainless steel cage in a room held at 25 ± 1°C under standard conditions with a 12 h light–dark cycle. They were allowed regular dry rat chow and water ad libitum before the experiment. Body weight was recorded once a week. Daily caloric intake was monitored in the morning (10:00 AM to 11:00 AM).

Diabetes mellitus was induced in 4 h fasted rats with STZ dissolved in 0.1 mol/L citrate buffer (pH 4.5) and intraperitoneally injected as a single dose of 55 mg/kg [18]. Two days later, after a 4 h fast, glucose concentration in the tail blood of the STZ-injected animals was assessed by using a glucometer (Accu-Chek Advantage; Boehringer Mannheim, Mannheim, Germany). Only animals with glucose values between 250–300 mg/dL were recruited into the study.

**Experimental design**

One day after verification of a diabetic-like condition, rats were then randomly divided into five groups, consisting of 7 to 8 animals per group: (1) sedentary rats fed a regular diet ad libitum (370 kcal/100 g diet, 11% of total calories as fat) as a control group (CON group, n = 7), (2) sedentary rats fed a high-saturated fat diet ad libitum (456 kcal/100 g diet, 42% of total calories as fat) (SFA group, n = 8), (3) sedentary rats fed a high-MUFA diet ad libitum (456 kcal/100 g diet, 42% of total calories as fat) (MUFA group, n = 8), (4) exercise-trained rats fed a regular diet ad libitum (EX group, n = 7), and (5) exercise-
trained rats fed a high-MUFA diet ad libitum (MUFA+EX group, n = 8).

The experimental period lasted twelve weeks. Animals were transferred into a quiet cage for final body weight assessment on the morning of the last day of experimentation. At 36–38 h after the most recent exercise bout and after 4 h fasting, all rats were killed using an intraperitoneally injected overdose of sodium pentobarbital (100 mg/kg). Cardiac blood (10 mL) was taken for determination of metabolic parameters. Thoracic abdominal walls were opened by midline incision. Liver and right gastrocnemius muscle were removed, weighed, cut into pieces and submerged in liquid N2 for 2 min and then kept frozen at −80°C for further analysis of overall activity of the hepatic ketone synthesis pathway, and 3-ketoacyl CoA-transferase activity, as a marker of muscle ketone utilization, respectively.

High-MUFA and high-SFA diet preparations

High fat diets were prepared every two days. High-MUFA and high-SFA diets thoroughly mixed 80 g of regular dry feed (081 CP mice feed, Perfect Companion Group Co, Samutprakarn, Thailand) with 20 mL of extra-virgin olive oil (Sabroso, Oleoforfait, Cordoba, Spain) and palm oil (Tesco, Oleen Co, Samutsakorn, Thailand), respectively. Olive and palm oils were purchased from local markets and stored at 25°C. This custom blend supplied 456 kcal of energy per 100 g diet (42% of total calories as fat; which were 25% and 16.4% from MUFA and SFA, respectively).

Exercise training protocol

The exercise training protocol used in the present study was modified from that used by El Midaoui et al. [9]. A progressive training program involved a moderate-intensity exercise performed on a motorized treadmill (SportsArt 1190; Woodinville, WA, USA) with 8% slope twice daily (between 10:00 AM and 12:00 PM, and between 17:00 PM and 19:00 PM), 5 days/week for twelve weeks. The rats began running at a speed of 22 m/min for 10 min during the first week. Speed and duration were gradually increased to 26 m/min for 50 min during the final two weeks (Table 1).

**Table 1. Exercise training program conducted on a motorized treadmill**

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**Assay of the overall activity of hepatic ketone body synthesis pathway**

Ketone body synthesis occurs in liver mitochondria by a 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 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase into hydroxymethylglutaryl-CoA, which is further converted into acetoacetate by hydroxymethylglutaryl-CoA lyase. Finally, acetoacetate is converted into β-hydroxybutyrate by β-hydroxybutyrate dehydrogenase. When each molecule of β-hydroxybutyrate is generated from acetoacetate, one molecule of NADH is used. In the present study, the overall activity of hepatic ketone body synthesis pathway in liver mitochondria was determined according to the methods previously described by El Midaoui et al. [12], by measurement of change in optical density of NADH after acetyl-CoA addition to a medium containing liver extracts using a UV–visible spectrophotometer (UV-1601PC; Shimadzu, Kyoto, Japan) at 340 nm at 30 °C in a 1-cm light path. One unit of enzyme activity causes 1 μmol/min of NADH transformation into NAD.

To prevent first-step reaction reversal resulting from free CoA produced by acetyl-CoA hydrolase present in the liver, acetyl phosphate and pTA (from Bacillus stearothermophilus; Sigma) was added to the incubation medium to convert free CoA to acetyl-CoA, as previously described by Lynen et al. [19]. Although there was a possibility of contamination of the mitochondrial enzyme preparation by cytoplasmic HMG-CoA synthase, this enzyme contamination would pose no problem because of the lack of Mg++ [20].

**Assay of 3-ketoacyl CoA-transferase activity in peripheral ketone body utilization pathway**

The use of ketone bodies in peripheral tissue involves a three-step enzymatic pathway. β-Hydroxybutyrate is converted into acetoacetate, then into acetoacetyl-CoA by β-hydroxybutyrate dehydrogenase, and 3-ketoacyl-CoA transferase, respectively. The latter enzyme uses succinyl-CoA as the CoA donor. Acetoacetyl-CoA is finally converted by acetyl-CoA thiolase into two molecules of acetyl-CoA before transfer to the Krebs cycle and oxidative phosphorylation to generate energy. In this study, the activity of 3-ketoacyl CoA-transferase, a key enzyme in the peripheral utilization of ketone bodies [21], was determined spectrophotometrically in gastrocnemius muscle according to the method of Williamson et al. [22], as modified by Rebrin et al. [23], by using a UV–visible spectrophotometer (UV-1601PC; Shimadzu), at 313 nm at 25°C in a 1-cm light path for 2 min. The assay is based on the determination of the rate of acetoacyl-CoA formation from succinyl-CoA and acetoacetate.

All enzymatic assays were carried out in duplicate and the average value was used for calculations.

**Statistical analysis**

All data are presented as means and SD. Comparisons among groups of animals used a one-way ANOVA and differences in pairs of means among groups were made using Tukey post hoc analysis. P ≤ 0.05 was considered significant.

**Results**

**Physical and physiological characteristics**

As shown in Table 2, when compared with control, final body weight and gastrocnemius weight were significantly increased in the SFA group, but were comparable among the MUFA, EX, MUFA+EX, and CON groups. Liver and muscle weight per body weight did not differ significantly among all groups. Daily caloric intake of rats fed diet rich in MUFA (MUFA and MUFA+EX groups) were significantly decreased when compared with those fed regular diets in the CON and EX groups.

**Blood biochemical parameters**

Blood biochemical parameters are shown in Table 3. Plasma glucose and insulin levels among all groups did not differ significantly. However, glucose levels in MUFA and MUFA+EX groups were reduced by 21.8% and 24.0%, respectively, compared with controls. A significant decrease in HbA1c was found only in MUFA+EX compared with the CON group.

Compared with controls, NEFA showed a significant decrease in EX and MUFA+EX groups and β-hydroxybutyrate levels were decreased in EX and MUFA+EX groups by 47.1% and 55.8%, respectively, but the reductions were not significant.
As illustrated in Figure 1, there was a significant decrease in hepatic ketone body synthesis pathway activity of EX and MUFA+EX groups compared with controls. Rats in the MUFA group showed a significant decrease in this activity compared to those in SFA group.

Ketoacyl CoA-transferase activity

Figure 2 shows a significant increase in enzymatic activity of muscle ketone body use in both EX and MUFA+EX groups compared with the control group.

Discussion

The main finding of this study was that in STZ-induced diabetic rats, neither fatty acid nor ketone body metabolism parameters were changed following consumption of a high-MUFA. A combination of MUFA-rich diet and exercise training was more effective than either MUFA or exercise alone for lowering HbA1C without affecting plasma insulin levels. Reduction in circulating levels of ketone bodies was mainly induced by exercise training, mediated by a decrease in total hepatic ketone body synthesis activity as well as increased muscle ketone body use as measured by 3-ketoacyl CoA transferase activity.

The strength of the present study is that all rats were fed ad libitum thus allowing self-regulation of energy intake resembling free-living conditions. Additionally, the high-SFA diet group provided same calories from fat as the high-MUFA group. Both were included in the experiment to confirm that any metabolic parameter changes resulted from MUFA effects, not from energy intake or high fat content. Limitations of the study are that the energy derived from olive and palm oils as well as MUFA and SFA is
Figure 1. Overall activity of hepatic ketone body synthesis pathway in diabetic rats after the 12 week intervention with high-MUFA diet and exercise training
Data are means ± SD, expressed per gram of liver. CON = sedentary rats fed regular diet (n = 7); SFA = sedentary rats fed high-SFA diet (n = 8); MUFA = sedentary rats fed high-MUFA diet (n = 8); EX = exercise-trained rats fed regular diet (n = 7); MUFA+EX = exercise-trained rats fed high-MUFA diet (n = 8); *P < 0.05 vs. CON; bP < 0.05 vs. SFA

Figure 2. Activity of 3-ketoacyl CoA-transferase as a marker of muscle ketone body utilization pathway in diabetic rats after the 12 week intervention with high-MUFA diet and exercise training
Data are means ± SD, expressed per gram of muscle. CON = sedentary rats fed regular diet (n = 7); SFA = sedentary rats fed high-SFA diet (n = 8); MUFA = sedentary rats fed high-MUFA diet (n = 8); EX = exercise-trained rats fed regular diet (n = 7); MUFA+EX = exercise-trained rats fed high-MUFA diet (n = 8); *P < 0.05 vs. CON; bP < 0.05 vs. SFA; cP < 0.05 vs. MUFA
calculated on the basis of nutritional facts labeled on the product, therefore giving approximate, but not precise caloric values and fasting time before taking blood samples for animal biochemical measurements. Because each animal was given free access to diet and water before fasting for 4 h, some animals may have fasted longer than 4 h. It should also be noted that in determination of ketogenic activity, the final ketogenic reaction catalyzed by β-hydroxybutyrate dehydrogenase consumes one mole of NADH per mole of acetoacetate reduced to β-hydroxybutyrate. However, β-hydroxybutyrate dehydrogenase is anchored to the inner leaflet of the mitochondrial membrane. Thus, disruption of mitochondrial membranes may artificially diminish rates of NADH consumption.

Consumption of high MUFA appeared to affect energy intake and energy expenditure. In line with some previous studies in dairy cows [24, 25], rats fed diets high in SFA and MUFA tended to consume fewer calories than those fed a regular diet. Mechanisms involved in fat-induced depression of food intake have been proposed to include the reducing equivalents produced by fatty acid oxidation [26], feedback satiety signals [27], and increased post-feeding satiety hormones such as plasma cholecystokinin and pancreatic polypeptide concentrations [28]. Despite a slight decrease in caloric intake, SFA rats weighed more than controls. This may be the result of effects of SFA on decreased fat oxidation and daily energy expenditure [29]. Conversely, replacing dietary SFA with MUFA was reported to increase physical activity and resting energy expenditure through its effect on mitochondrial function [30]. Accordingly, MUFA-rich diets did not promote weight gain in diabetic rats as discovered in the present study. Similar results were also seen in other studies in obese subjects consuming energy-controlled high-MUFA diets [31] and in insulin-resistant subjects after isocaloric replacement of SFA by supplements of virgin olive oil rich in MUFA [32]. Taking these findings together, it is possible that MUFA may modulate energy balance or even tend to promote a negative energy balance in diabetic rats. The modulation of energy balance is found to help reverse diabetes by normalizing β cell function and insulin sensitivity in patients with type 2 diabetes [33].

A combination of MUFA-rich diet and moderate exercise training was employed in this study to determine the effect on glucose and ketone body metabolism in a diabetic condition. The results showed that diabetic rats in the EX group did not exhibit any improvement in glucose metabolism, while their sedentary counterparts had an average glucose level of more than 400 mg/dL at the end of the 12-week experiment. This finding is consistent with previous investigations, which indicated that exercise training improved glucose homeostasis in rats with mild STZ-induced diabetes [34-36], but not in those with a severe diabetic state [6, 9, 37, 38]. In this study, MUFA diet tended to reduce fasting plasma glucose levels, but no additional reduction was seen in combination with exercise. Nevertheless, the results obtained did not support the action of MUFA on increased insulin secretion because there was no demonstrable change in the levels of plasma insulin. Interestingly, only exercise-trained rats fed a MUFA-rich diet showed a significant reduction in HbA1c to nearly normal levels over the 12 week experimental period. We propose that the mechanisms underlying this interaction between MUFA and training on glucose homeostasis might involve glucose uptake and metabolism and modulate energy balance.

With respect to changes in circulating NEFA and ketone body metabolism, this exercise training program was able to reduce serum NEFA and tended to reduce β-hydroxybutyrate levels. Because fasting plasma glucose concentrations were not affected by training, reduction in NEFA therefore, appears to be primarily the result of the cumulative effects of each exercise bout on fatty acid oxidation without ketone body conversion. Moreover, exercise training also exhibited an inhibitory effect on hepatic ketone synthesis and a stimulatory effect on muscle ketone use. These observations confirm the previous reports in diabetic rat model that exercise training can lower ketone body concentration through a combination of lower NEFA levels, decreased hepatic ketone synthesis [10], and enhanced muscle ketone utilization pathway [9]. However, ketone body use referred to here is ketone body oxidation, i.e. the process by which ketone bodies are cleaved to liberate acetyl-CoA for entry into the TCA cycle and terminal oxidation. Anabolic fates of ketones also include incorporation into lipids and sterols after activation by cytoplasmic acetoacetyl CoA synthetase. These pathways also may influence circulating ketone body concentrations.

The sedentary rats fed high-MUFA diet finding reflecting no change in NEFA is in concordance with human studies. In type 2 diabetic patients, no significant difference in plasma FFA was found after
6 h post-consumption of high-MUFA meals [39]. In addition, post-prandial circulating FFA level in type 2 diabetic patients receiving high-MUFA enteral formula did not significantly differ from those who received high-carbohydrate enteral formula [13]. However, in normal C57BL/6J mice, high-MUFA diet could lower serum FFA levels and decreased mobilization of FFA from adipose tissue might occur, which was associated with the lowering triglyceride production [14] via an inhibition of hormone-sensitive lipase [32]. In the present study, although an ad libitum MUFA-rich diet alone tended to decrease the levels of fasting plasma glucose, it affected neither serum NEFA levels nor ketone body metabolism. The changes in blood concentrations of NEFA and β-hydroxybutyrate as well as enzymatic activities of hepatic ketone synthesis and muscle ketone utilization pathway in trained rats fed high-MUFA diet, however, were the same extent as those fed regular diet. Therefore, the amelioration of ketone metabolism observed in the present study is mainly the result of exercise training.

In conclusion, MUFA tends to reduce fasting plasma glucose levels without affecting ketone body metabolism. Exercise training can improve ketone body metabolism, but MUFA did not provide additional benefits on ketone body metabolism. When used in combination, an ad libitum MUFA-rich diet and exercise training benefits HbA1c levels in a rat model of diabetes induced by STZ. Further investigation is needed to elucidate the mechanism by which MUFA interacts with exercise training in glucose metabolism in the diabetic condition. Translational research towards a clinical application is also warranted.

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The authors hereby declare that they have no competing interests.

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