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Dietary fat type alters glucose metabolism in isolated rat hepatocytes

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Dietary fat type can influence the regulation of carbohydrate metabolism in multiple tissue types. The influence of feeding high-fat (40% of kilocalories) diets containing either menhaden oil (MO) or coconut oil (CO) on hepatic glycogenolytic and gluconeogenic capacities was studied in isolated rat hepatocytes. Estimates of both glycogenolytic and gluconeogenic capacities were performed on hepatocytes isolated from fed and fasted animals, respectively. In MO-fed animals, both basal and hormone-stimulated rates of glucose production were significantly greater than those in CO-fed animals. However, both groups displayed a similar maximal increase in glucose production above basal for glucagon and epinephrine (2.3- and 1.9-fold, respectively). Basal rates of adenosine 3′,5′-cyclic phosphate (cAMP) production were not different between groups whereas glucagon-stimulated cAMP production was increased twofold in the MO-fed group. In both MO and CO groups, the addition of 10 nM insulin reduced glucose production in fed animals to similar absolute rates. In animals fasted for 24 hours, gluconeogenic capacity was estimated using 10 mM pyruvate, lactate, or glycerol. Glucose production from all substrates was significantly greater in CO-fed animals. In addition to increased gluconeogenic rates, maximal phosphoenolpyruvate carboxykinase (PEPCK) activity was increased in the CO-fed group. Insulin reduced glucose production in both dietary groups, but the absolute rate of glucose production was 28% greater in the CO-fed group relative to the MO-fed group. In summary, dietary fat type can markedly influence the regulation of hepatic glucose metabolism in multiple metabolic pathways. MO feeding promoted glycogenolysis and sensitivity to insulin whereas CO feeding favored gluconeogenesis and reduced insulin sensitivity. (J. Nutr. Biochem. 10:709–715, 1999)

Keywords: fish oil; coconut oil; gluconeogenesis; glycogenolysis; insulin resistance

Introduction

The absolute quantity of fat consumed in the diet can directly influence the regulation of glucose metabolism in liver, skeletal muscle, and adipose tissue.[1] What is less understood is how different types of dietary fat, when consumed isocalorically, alter the regulation of glucose metabolism.[2–5] Dietary fat type has been shown to alter numerous processes involved in the regulation of carbohydrate metabolism. These effects are diverse and range from the molecular level,[6] where specific fatty acids can initiate gene transcription, to the whole body level, where differences in substrate oxidation and metabolic rate have been reported.[7] Of particular interest are the effects of dietary menhaden oil (MO) on the regulation of carbohydrate metabolism. Mounting evidence suggests that high-fat diets rich in MO prevent the development of insulin resistance compared with diets high in saturated or n-6 polyunsaturated fats.[3,4] In hepatocytes, skeletal muscle, and adipocytes a MO-rich diet can increase the binding affinity of insulin to the insulin receptor, and in skeletal muscle and adipose tissue a MO-rich diet increases tissue insulin sensitivity.[8–10] Although MO protects against the development of insulin resistance in animal models, in human clinical trials, MO diets can worsen or have no effect on glycemic control in diabetic populations.[11,12]

The regulation of hepatic glucose production appears to be particularly sensitive to dietary nutrients. With the feeding of high-fat n-6 polyunsaturated or saturated fats, the appearance of hepatic insulin resistance and increased hepatic glucose output precedes the development of defects

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Table 1 Diet ccmposition

Ingredient	G/kg
Casein	239
Corn starch	179
Sucrose	247
Cellulose	60
Vitamin mix (AIN-76)	12
Mineral mix (AIN-76)	41
DL-α-tocopherol	4
DL-methionine	4
Choline bitartrate	2
Corn oil	12
Treatment oil	205

All diet components purchased from ICN Biochemicals (Costa Mesa, CA USA).

Table 2 Diet fatty acid composition

	MO	CO
8:0	nd	2.12
10:0	nd	6.0
12:0	nd	45.09
14:0	7.41	19.23
16:0	19.09	10.72
16:1*n*7	9.21	ND
18:0	3.97	10.82
18:1*n*9	12.9	ND
18:2*n*6	4.6	6.32
20:2*n*6	1.87	ND
18:3*n*3	1.59	ND
20:5*n*3	16.05	ND
22:6*n*3	15.79	ND

Results are presented as percentage of total fatty acids. Fatty acids with less than 1% are not shown. Data is the average of three separate injections of methyl esters prepared from the oil mixtures used to prepare the diets.
MO–menhaden oil. CO– coconut oil. ND–none detected.

in glucose uptake in skeletal muscle or adipose tissue.[1] In addition, high protein,[13] fructose,[5] and sucrose diets[4] also cause rapid (within 1 week) changes in hepatic glucose metabolism. These changes include increases in gluconeogenesis, decreases in insulin inhibition of hepatic glucose production, and increases in the indirect pathway of glycogen synthesis. Several studies have examined the effect of dietary fat type on hepatic gluconeogenesis. Using BHE/cdb rats, Kullen et al.[14,15] demonstrated that gluconeogenesis was greater in animals fed a beef tallow diet versus a MO diet. The proposed mechanism leading to lower gluconeogenesis[15] in the MO group was a decrease in cellular ATP content. Whether MO diets decrease gluconeogenesis and adenosine triphosphate (ATP) content compared with saturated fat diets in a normal strain of rats has not been determined. The effect of dietary fat type on hormone-stimulated glycogenolysis in liver has received little or no attention, although there is evidence suggesting that the glucagon signaling pathway, which regulates hepatic glycogen breakdown and to a lesser extent gluconeogenesis, is upregulated with MO feeding.[16]

Given the limited information available as to the effects of dietary fat type on hepatic glycogenolysis and gluconeogenesis, the goals of the present study were to estimate both the glycogenolytic and gluconeogenic capacities of isolated hepatocytes in rats fed diets enriched in MO, which contains large amounts of long chain polyunsaturated n-3 fatty acids, or coconut oil (CO), which contains mainly saturated fatty acids, and to determine if insulin inhibition of these pathways is altered as a result of feeding different dietary fat types.

Material and methods

Animals and feeding

Male Sprague-Dawley rats, aged 35 to 40 days, were obtained from an institutional breeding stock, weighed, and divided into two groups. Each group received a nutritionally complete semipurified diet (*Table 1*) that contained 40%, 40%, and 20% of total kilocalories from fat, carbohydrate, and protein, respectively. The only difference in the diets was the source of the fat: One group received MO and the other group CO. Each diet contained 5% of the total fat (w/w) as corn oil to prevent essential fatty acid deficiency. Fatty acid composition of the diets was determined by gas chromatography (*Table 2*). Diets were prepared fresh weekly, gassed with nitrogen, and stored at −20°C until use. Animals had free access to food and water and were housed in wire bottom cages in a room that was maintained at 25°C on a 12-hour light/dark cycle. Animals consumed the experimental diet for 4 weeks. Food consumption was monitored daily and animal weight was recorded weekly. All procedures for animal use were in accordance with the guiding principles in the care and use of laboratory animals of Arizona State University.

Hepatocyte isolation

Hepatocytes were obtained by collagenase perfusion of the liver as described by Berry and Friend[17] and modified by Exton and Blackmore.[18] Briefly, rats were anesthetized with an intramuscular injection of ketamine (50 mg/kg), xylazine (10 mg/kg), and acepromazine (5 mg/kg). The abdominal cavity was then opened and the portal vein cannulated. The liver was perfused with calcium free Krebs-Ringers bicarbonate buffer containing 11 mM glucose and 5 mM pyruvate that had been equilibrated with 95% oxygen (O_2) and 5% carbon dioxide (CO_2) at 37°C and pH 7.4. Once the liver was cleared of blood, 50 mL of the initial perfusate was allowed to drain to waste. Collagenase (0.2 mg/mL, Type A, Boehringer Mannheim, Petersburg, VA USA) was then added to the perfusion system and recirculated until the liver was appropriately digested (approximately 10–12 min). The liver was carefully removed and the capsule gently peeled off. The liver was then shaken in 50 mL of Krebs-Ringers bicarbonate buffer containing 2.5 mM calcium, 1% gelatin (Difco, Detroit MI USA), and 5 mM glucose to release hepatocytes. Cells were washed three times in the Krebs-Ringers buffer with gelatin and suspended at 50 mg/mL (wet weight). The initial quality of the cell preparation was assessed by trypan blue exclusion (0.2% final concentration) and measurement of cellular ATP content.[19]

Study 1: Measurement of glycogenolysis in fed animals

For estimates of glycogenolytic capacity, hepatocytes were isolated from fed rats between 6:30 and 8:00 AM on the experimental day. Before being used in incubations, hepatocytes were equilibrated at 37°C while being gassed with 95% O_2 and 5% CO_2 for

Table 3 Animal and isolated cell characteristics

	Study 1		Study 2	
	MO	CO	MO	CO
Animal weight (g)	294 ± 10	284 ± 13	298 ± 13	301 ± 11
Cell ATP (nmol/mg)	2.75 ± 0.28	2.57 ± 0.25	2.44 ± 0.11	2.42 ± 0.15
Initial glycogen (μg/mg)	37.2 ± 2.29	35.92 ± 2.66	5.55 ± 0.52*	5.83 ± 0.91*
Trypan blue exclusion	94.4 ± 1.9	94.55 ± 2.3	92.32 ± 2.1	93.48 ± 2.0

Trypan blue exclusion is percentage of cells excluding the dye immediately prior to the start of incubation. $n = 8$ per diet group in study 1 and 6 per diet group in study 2. All data are presented as mean ± SE. *Significantly different from glycogen values in study 1.
MO–menhaden oil. CO–coconut oil. ATP–adenosine triphosphate.

20 minutes. Cells (50 mg/mL) were then incubated in Krebs-Ringers bicarbonate with 5 mM glucose and 1% gelatin with or without the addition of glucagon, epinephrine, or insulin in plastic 25 mL Erlenmeyer flasks for 1 hour with continuous gassing (95% O_2/5% CO_2). Incubations were terminated by the addition of 500 μL of 0.6 M perchloric acid to 500 μL of cell suspension. Cellular debris was pelleted by brief centrifugation and the supernatant removed and neutralized by addition of a small quantity of 1 M $KHCO_3$. The supernatant was analyzed for glucose enzymatically.[20] Glucose production is expressed as nmol glucose produced in 1 hour per milligram of cell wet weight, with values corrected for glucose in the medium at time 0 of the incubations. Initial glycogen levels at the beginning of the incubations were determined in 100 mg/mL aliquots of cell suspension by measuring glucose[20] after enzymatic digestion of glycogen to glucose.[21]

Study 2: Measurement of gluconeogenic capacity in fasted animals

Animals used for estimating gluconeogenic capacity were fasted for 24 hours to deplete hepatic glycogen stores prior to hepatocyte isolation. Isolated hepatocytes were incubated for 1 hour with the gluconeogenic precursors lactate, pyruvate, or glycerol at a final concentration of 10 mM. In addition, insulin (10 nmol) was added to incubations containing 10 mM pyruvate to assess the ability of insulin to inhibit gluconeogenesis. Glucose and glycogen were measured as described in study 1.

Measurement of cAMP

Before being used to assess rates of 3′,5′-cyclic phosphate (cAMP) production, hepatocytes were gassed with 95% O_2 and 5% CO_2 for 20 minutes at 37°C. Cells (50 mg/mL) were then incubated for 10 minutes in Krebs-Ringers bicarbonate buffer (pH 7.4) containing either no addition (basal) or 1 nM glucagon. After 10 minutes, incubations were stopped using ice-cold ethanol (final concentration 66% v/v) and cAMP was assayed using a radioimmunoassay (Amersham, Piscataway, NJ USA).

Enzyme activities

Livers from rats fasted for 6 to 8 hours were removed under general anesthesia. Ten percent homogenates were prepared at 0°C in a medium containing 250 mM sucrose and 20 mM Hepes (pH 7.4) on ice. Homogenates were centrifuged at 130,000 × g for 30 minutes. The resulting supernatant was used to assay phosphoenolpyruvate carboxykinase (PEPCK) enzyme activity. PEPCK activity was assayed enzymatically as described by Petrescu et al.[22] Glucose-6-phosphatase activity was assayed on the microsomal containing fraction of liver homogenate by measuring the hydrolysis of inorganic phosphate from glucose-6-phosphate.[23]

Statistical analysis

Repeated measures analysis of variance was used to examine differences between groups and studies 1 and 2. If the overall F was significant, comparisons between means were made using a student Newman Kuels test. Student's *t*-tests were used to compare between groups in a study on a single variable. A *P*-value of less than 0.5 was considered significant for all comparisons.

Results

Animal characteristics

After 4 weeks on the experimental diets there were no differences in body weight between diet groups in either study 1 or study 2 (*Table 3*) and there were no differences in body weight between studies 1 and 2. Food consumption averaged 14.7 ± 1.2 g/day for MO and 15.2 ± 1.5 g/day for CO over the entire dietary treatment period and no differences were found between diet groups or between studies. Both epididymal and retroperitoneal fat pad masses were greater in the CO- versus MO-fed animals: Epididymal fat pad masses were 5.38 ± 0.66 g and 3.44 ± 0.67 g for the CO and MO groups, respectively, and retroperitoneal fat pad masses were 6.22 ± 0.58 g and 4.03 ± 0.27 g for CO and MO groups, respectively.

Study 1: Estimates of glycogenolysis

Hepatocytes used to estimate glycogenolysis had cellular ATP levels greater then 2.5 nmol/mg, and in all experiments greater than 90% of the cells excluded trypan blue (*Table 3*), indicating high quality cell preparations. Dose response curves for glucose production in hepatocytes stimulated with either glucagon or epinephrine are illustrated in *Figure 1* and *Figure 2*, respectively. Glucagon stimulated maximal glucose release at 1 nM with no further increase at higher concentrations, and epinephrine stimulated maximal glucose release at 0.1 μM. These responses are similar to those seen in isolated perfused liver experiments[24] and indicate that glucagon and epinephrine receptor function remained intact following the collagenase isolation procedure. Basal glycogenolysis was measured by incubating hepatocytes for 1 hour without hormone. Basal rates of glycogenolysis were lower in hepatocytes from CO-fed rats (*Figures 1 and 2*). Addition of glucagon (*Figure 1*) or epinephrine (*Figure 2*) stimulated glucose release in a dose-dependent manner in both groups, with greater absolute glucose release occurring

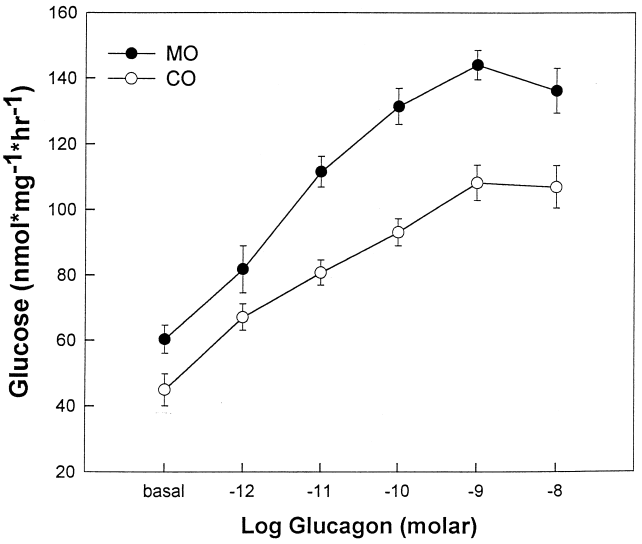

Figure 1 Dose response curves for the dependence of glucose production on glucagon concentration in isolated hepatocytes from fed animals. Values presented are the mean ± SE for hepatocytes prepared from eight animals for each group. The menhaden oil (MO) group was significantly different ($P < 0.05$) from the coconut oil (CO) group at basal and all hormone concentrations.

in the MO-fed group for any given glucagon or epinephrine concentration. It is important to note that although the absolute rate of glucose release was significantly higher in the MO group, the relative magnitude of response to a given hormone concentration was similar in both groups. Both groups exhibited approximately 2.3-fold stimulation of glucose production above basal at maximal glucagon concentration and approximately 1.9-fold stimulation of glucose production with maximal epinephrine concentrations.

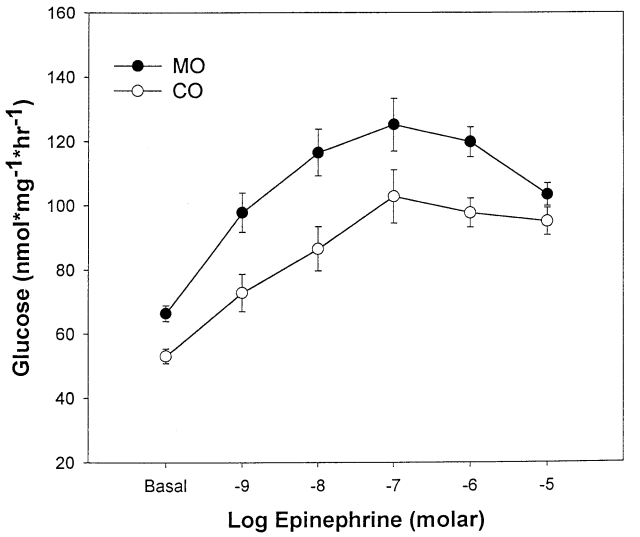

Figure 2 Dose response curves for dependence of glucose production on epinephrine concentration in isolated hepatocytes from fed animals. Values presented are the mean ± SE for hepatocytes prepared from six animals for each group. The menhaden oil (MO) group was significantly different ($P < 0.05$) from the coconut oil (CO) group at basal and all hormone concentrations.

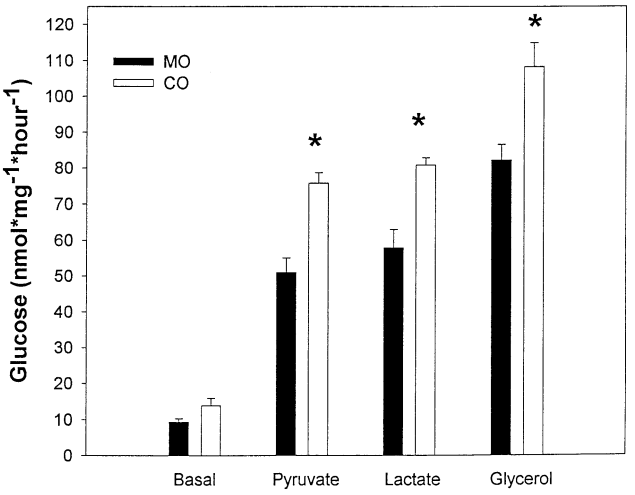

Figure 3 Glucose production via gluconeogenesis in hepatocytes isolated from animals fasted for 24 hours. Isolated hepatocytes were incubated with 10 mM pyruvate, lactate, or glycerol. Values presented are the mean ± SE for hepatocytes prepared from six animals for each group. *Significantly different ($P < 0.05$) from the other diet group. MO, menhaden oil; CO, coconut oil.

Initial glycogen levels were not different between the groups (*Table 3*), indicating that the observed differences in the rates of glucose output were not the result of differences in initial glycogen content.

Study 2: Gluconeogenic capacity

To estimate gluconeogenic capacity, rats were fasted for 24 hours prior to hepatocyte isolation. Similar to hepatocytes isolated from fed animals, hepatocytes isolated from fasted animals had normal ATP levels and greater than 90% trypan blue exclusion, again indicative of a high quality cell preparation. Fasting for 24 hours significantly reduced liver glycogen levels compared with fed animals (*Table 3*) and initial glycogen levels were not different between dietary groups. When 10 mM pyruvate, lactate, or glycerol was added to incubations as gluconeogenic precursor, glucose production in hepatocytes from CO-fed rats was significantly elevated compared with that in MO rats (*Figure 3*). Basal rates of glucose production (i.e., no gluconeogenic precursor added) were not significantly different between groups.

Insulin inhibition of glucose production

To assess the degree of insulin resistance induced by the high-fat diets, hepatocytes from fed animals were incubated with glucagon (1 nM) or epinephrine (0.1 μM) in the presence of 10 nM insulin. As expected, glucose release was decreased in both groups with the addition of insulin. The absolute rate of glucose release from glycogen was not different between MO and CO groups when insulin was added (*Figure 4*), but the relative magnitude of the reduction was greatest in the MO group (*Figure 5*). Gluconeogenesis in the presence of insulin (10 nM) expressed in absolute terms (i.e., nmol/mg/hr; *Figure 4*) was significantly greater in CO-fed than in MO-fed animals, whereas

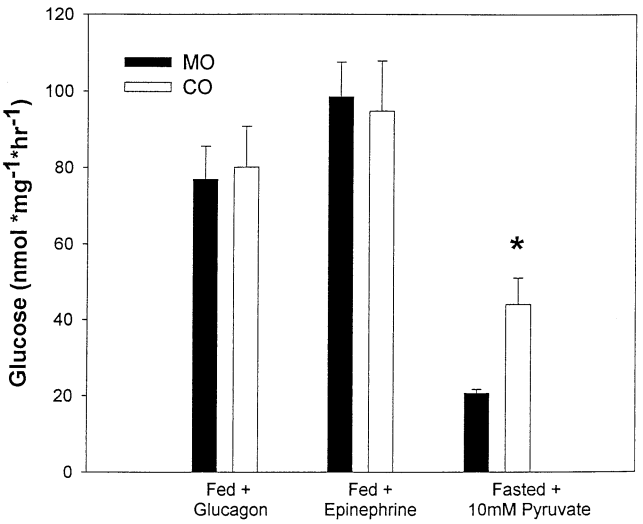

Figure 4 Glucose production from fed and fasted animals in the presence of insulin. For fed animals glucose release was measured in incubations containing either 1 nM glucagon or .1 mM epinephrine with the addition of 10 nM insulin. In hepatocytes isolated from fasted animals, glucose release was measured in incubations containing 10 mM pyruvate + 10 nM insulin. Values presented are the mean ± SE for hepatocytes prepared from eight animals for each group for glucagon and six animals for each group for epinephrine and pyruvate. *Significantly different ($P < 0.05$) from the other diet group. MO, menhaden oil; CO, coconut oil.

the relative decrease compared with the no insulin condition (*Figure 5*) was significantly less in the CO-fed animals.

cAMP production

Basal rates of AMP production were 0.33 ± 0.005 pmol/mg/10 min and 0.34 ± 0.019 pmol/mg/10 min in CO and MO, respectively, and not different between groups. When hepatocytes were stimulated with 1 nM glucagon, cAMP production was approximately twofold higher in the MO-fed group than in the CO-fed group. Rates of cAMP production were 6.36 ± 0.91 pmol/mg/10 min in the MO-fed group and 3.43 ± 0.38 pmol/mg/10 min in the CO-fed group.

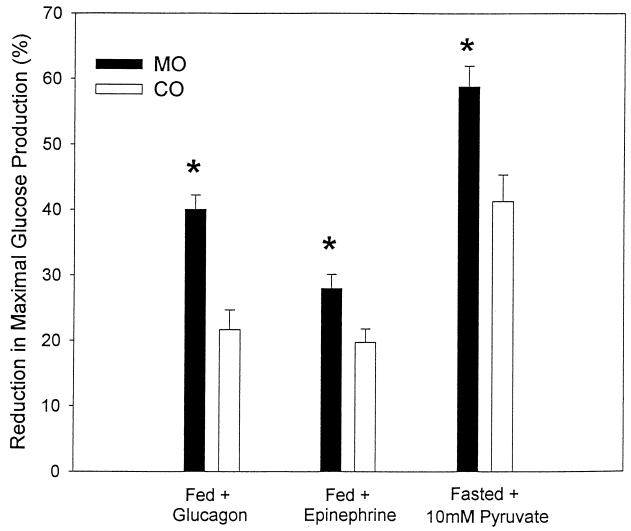

Figure 5 Relative decrease in glucose production from fed and fasted animals in the presence of 10 nM insulin compared with the maximal value without insulin for similar condition. Values presented are the mean ± SE for hepatocytes prepared from eight animals for each group for glucagon and six animals for each group for epinephrine and pyruvate. *Significantly different ($P < 0.05$) from the other diet group. MO, menhaden oil; CO, coconut oil.

Gluconeogenic enzyme activities

The maximal activity of PEPCK was significantly increased in the livers of animals fed the CO diet. The maximal activity for PEPCK was 10.8 ± 0.93 nmol/min/mg protein in CO-fed animals and 7.5 ± 0.82 nmol/min/mg protein in MO-fed animals. Glucose-6-phosphatase activity was 18 ± 2.5 nmol Pi/min/mg in MO-fed animals and 13.5 ± 2.9 nmol Pi/min/mg in the CO-fed animals and not significantly different between groups.

Discussion

Both the quantity and the type of fat consumed in the diet have been demonstrated to alter the regulation of carbohydrate metabolism. The dietary model used in this study was to feed animals high-fat diets (40% of kilocalories) containing either a highly saturated fat (CO) or a highly unsaturated fat (MO). The rationale for choosing these fats was that they are vastly different in their physical properties and chemical structure and have been shown to alter the regulation of carbohydrate metabolism both in vitro and in vivo. MO prevents the development of insulin resistance in skeletal muscle, adipose, and liver when fed in a high-fat diet compared with other saturated and unsaturated fats.[2–5] Feeding animals high-fat diets containing either MO or CO for 4 weeks resulted in animals that had equal body weights and food consumption at the end of the feeding period, although epididymal and retroperitoneal fat pad masses were approximately 30% greater in the CO-fed animals. Other studies also have demonstrated similar effects of feeding saturated fat versus MO diets on fat pad mass.[25,26]

The main focus of these experiments was to examine the effect of feeding MO or CO on glucose production in isolated hepatocytes from fed (estimate of glycogenolytic capacity) or fasted (estimate of gluconeogenic capacity) animals. Isolated hepatocytes are an ideal experimental model for such studies because dose response curves for hormones, as well as estimates of gluconeogenic capacity using multiple precursors and hormone additions, can be performed on cells obtained from a single animal. The high level of trypan blue exclusion, normal cellular ATP levels, and hormonal responses similar to those observed in liver perfusion experiments[24] all indicate that the hepatocyte preparations retained qualities similar to those of intact tissue.

In these experiments basal, glucagon-stimulated, and epinephrine-stimulated glucose production was elevated in hepatocytes from MO-fed compared with CO-fed animals. Based on a study by Lee and Hamm,[16] which reported that glucagon-stimulated cAMP production rates were elevated in membranes isolated from animals fed a MO diet, we initially predicted that glucose output for any given glucagon concentration would be greater in the MO-fed group than in the CO-fed group. This prediction was based on the

fact that glucagon is a potent stimulator of cAMP production and glycogenolysis.[24,27] In the present study, which used an intact cell preparation, cAMP production was elevated with glucagon stimulation but basal levels were not different between groups. This is in contrast to glycogenolysis where both basal and glucagon-stimulated glucose production were elevated in the MO-fed group. The elevated basal rates of glycogenolysis in the MO-fed animals compared with the CO-fed animals and similar dose response characteristics to hormone stimulation in both groups suggest that cAMP may not be responsible for the observed differences in glucose release. In addition, given the fact that glycogenolytic capacity increased similarly in response to both glucagon, which acts to elevate cAMP concentration, and epinephrine, which elevates intracellular calcium levels, the observed differences in glucose output may reflect multiple cellular responses to these diets.

There are several possible mechanisms that may explain the increases in gluconeogenesis associated with high saturated fat diets. In vivo, increased delivery of gluconeogenic substrates (e.g., fatty acids and glycerol) from peripheral tissues to the liver can increase gluconeogenesis. There is significant evidence demonstrating that increased plasma free fatty acid (FFA) concentration can decrease insulin supression of gluconeogenesis in vivo.[28] It is well known that MO diets decrease plasma FFA concentrations.[29,30] Thus, in vivo decreases in FFA in MO-fed animals could contribute to a greater hepatic insulin sensitivity. Because these experiments were done on isolated cells, delivery of substrate in our system was equivalent and FFA was not present in these experiments. Thus, any effect of increased plasma FFA on the decreased ability of insulin to suppress gluconeogenesis (GNEO) that may have been present in vivo was also present in vitro in the CO-fed group. In the present study the maximal activity of PEPCK was increased in the livers of animals fed the CO diet compared with the MO diet. In addition, increases in visceral fat mass, as were present in the CO-fed group, have been demonstrated to increase both PEPCK and glucose-6-phosphatase activity.[31] In the present study only PEPCK activity was increased although there was a tendency for increased glucose-6-phosphatase in CO-fed animals as well. Thus, it is clear that in contrast to the CO diet a MO diet protects against increases in gluconeogenic enzyme content and capacity for gluconeogenesis.

When the results for gluconeogenic capacity are considered together with those for glycogenolytic capacity, a possible explanation emerges as to how dietary fats might alter hepatic glucose metabolism. Glucose output from the liver results from two processes, gluconeogenesis and glycogenolysis. An important concept in hepatic glucose metabolism is autoregulation of glucose output. This concept asserts that as flux through one pathway of glucose production increases, flux through the other decreases; thus, hepatic glucose output remains constant.[32] Furthermore, this regulation occurs independently of hormonal and neural input. In the present experiments, hepatocytes from MO-fed animals had higher rates of basal, glucagon-stimulated, and epinephrine-stimulated glucose release than those fed the CO diet. Conversely, animals fed the CO diet displayed a greater gluconeogenic capacity. Estimates of glycogenolysis indicate that glucose production was elevated approximately 22% at basal concentrations and approximately 29% at maximal glucagon concentrations in the MO- versus CO-fed group, whereas gluconeogenic capacity was elevated between 25% and 28% in the CO group depending on gluconeogenic precursor. Together these results suggest that feeding a diet high in saturated fat caused a reduction in glycogenolytic capacity and an equivalent increase in gluconeogenic capacity in the CO group relative to the MO group. The net effect of these alterations in vivo would most likely be that under basal conditions hepatic glucose output would not be altered by either diet but the relative proportion that is coming from gluconeogenesis is increased by the CO diet. It could also be predicted that after a meal, when GNEO should be suppressed by insulin, the reduced ability of insulin to suppress GNEO in the CO group might result in loss of autoregulation and a relative hyperglycemia compared with the MO group. Conversely, the increased ability of glucagon to stimulate cAMP production in the MO-fed group could increase gluconeogenesis in MO hepatocytes.

One possible explanation for the intrahepatic adaptations observed in this study that deserves further investigation is the role that dietary nutrients play in altering gluconeogenic enzyme composition and the metabolic zonation of carbohydrate metabolism within the liver. In a normal liver, higher activities of the enzymes specific for glycolysis are located perivenous, with respect to blood flow, whereas those involved in gluconeogenesis are located more periportal, with respect to blood flow. Although the present study used a mixed hepatocyte preparation, results suggest that the respective diets either induce the CO hepatocytes to develop a more periportal enzymatic profile or the MO hepatocytes to develop a more perivenous enzymatic composition. Diet[33] has been demonstrated to alter the periportal-perivenous distribution of PEPCK in rats fed high-fat and high protein diets. To date the effect of dietary fat type on the periportal-perivenous distribution of the enzymes involved in glucose metabolism has not been studied and represents an intriguing area for future research.

The data presented in these studies suggest that dietary fat type causes reciprocal changes in the activities of the glycogenolytic and gluconeogenic pathways. The mechanism(s) by which fatty acids induce these changes is (are) currently unknown, although several possible explanations have been proposed. Diets enriched in specific fatty acids cause changes in membrane fatty acid composition that closely mimic the fatty acid composition of the diet.[34] Such changes in membrane structure can alter membrane protein function (e.g. hormone receptor signaling) and thus markedly influence cell processes. These diets did indeed alter plasma membrane fatty acid composition (data not shown); thus, membrane-induced changes in hormone (glucagon and insulin) signaling[9,10,16] could, in part, explain the observed results. An altered sensitivity to either glucagon or insulin could, in effect, be the initial signal that could, in turn, alter the expression of key regulatory enzymes involved in gluconeogenesis and glycogenolysis. In addition, specific fatty acids, especially long chain unsaturated fatty acids, have recently been shown to activate peroxisome proliferator-activated receptors that regulate the expression of several enzymes involved in hepatic carbohydrate and fat metabolism.[35]

In summary, the results of these studies suggest that the adaptations in hepatic glucose metabolism to diets high in MO or CO are intrahepatic in nature. Dietary fat type causes adaptations that result in a liver that is either more gluconeogenic and insulin resistant (i.e., the CO diet) or glycogenolytic and insulin sensitive (i.e., the MO diet). In addition, results of the present investigation provide further evidence to support the beneficial effects of MO diets relative to saturated fat on the development of insulin resistance. Although the ability of specific fatty acids to modulate hepatic glucose metabolism is rapidly becoming an accepted phenomenon, the mechanism(s) that underlie to these observations is (are) far from being understood.

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