Original Article

A Choline-Deficient Diet Exacerbates Fatty Liver but Attenuates Insulin Resistance and Glucose Intolerance in Mice Fed a High-Fat Diet

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Liver fat accumulation is proposed to link obesity and insulin resistance. To dissect the role of liver fat in the insulin resistance of diet-induced obesity, we altered liver fat using a choline-deficient diet. C57Bl/6 mice were fed a low-fat (10% of calories) or high-fat (45% of calories) diet for 8 weeks; during the final 4 weeks, diets were either choline deficient or choline supplemented. In choline replete animals, high-fat feeding induced weight gain, elevated liver triglycerides (171%), hyperinsulinemia, and glucose intolerance. Choline deficiency did not affect body or adipose depot weights but amplified liver fat accumulation with high-fat diet (281%, *P* < 0.01). However, choline deficiency lowered fasting plasma insulin (from 983 ± 175 to 433 ± 36 pmol/l, *P* < 0.01) and improved glucose tolerance on a high-fat diet. In mice on 30% fat diet, choline deficiency increased liver mRNA levels of the rate-limiting enzyme in phosphatidylcholine synthesis and of enzymes involved in free fatty acid esterification, without affecting those of de novo lipogenesis or fatty acid oxidation. We conclude that liver fat accumulation per se does not cause insulin resistance during high-fat feeding and that choline deficiency may shunt potentially toxic free fatty acids toward innocuous storage triglyceride in the liver. *Diabetes* 55:2015–2020, 2006

It has been proposed that the extent of "ectopic " fat accumulation in nonadipose tissues such as liver and muscle is a key determinant of insulin resistance in obesity. Nonalcoholic fatty liver disease is a common feature of obesity and is strongly associated with insulin resistance (1–4). In patients with and without type 2 diabetes, liver fat content is closely correlated with fasting insulin levels, insulin requirements, and hepatic insulin sensitivity, independent of obesity (5,6). In the absence of obesity, excessive hepatic fat accumulation in lipodystrophy syndromes is associated with severe insulin resistance (7–10). It is argued that the accumulation of fat within the liver causes hepatic insulin resistance (6,11) and that peripheral insulin resistance and glucose intolerance then follow (12–14). However, some investigators have postulated that liver fat accumulation is a result, rather than cause, of peripheral insulin resistance in obesity; elevated plasma concentrations of glucose and fatty acids may promote hepatic fatty acid and triglyceride uptake and synthesis and impair β-oxidation, leading to hepatic steatosis (2,15).

There are experimental paradigms in which liver fat accumulation may not be associated with insulin resistance. A methionine and choline–deficient diet (MCDD) in rodents impairs synthesis of phosphatidylcholine, which is an essential part of the outer phospholipid component of lipoproteins. This leads to reduced secretion of liver triglyceride as VLDL, resulting in accumulation of liver triglycerides (16). Mice receiving a MCDD have lower fasting insulin and glucose levels and normal glucose tolerance, suggesting enhanced hepatic insulin sensitivity (17). However, MCDD also causes significant weight loss (18), and the relative influence of weight loss and liver fat accumulation has not been addressed. Moreover, in mice with steatohepatitis induced by MCDD, insulin receptor substrate phosphorylation in the liver was impaired, rather than enhanced, after a bolus of insulin administered into the portal vein (19), possibly because the inflammatory signals associated with hepatitis might confound effects on insulin sensitivity. A choline-deficient diet (CDD) also reliably induces fatty liver in rodents in the absence of methionine deficiency, although the mechanisms are less clear and may differ from those in MCDD (20). Unlike the MCDD model, fatty liver induced by a CDD is not associated with weight loss or significant steatohepatitis. The effects of a CDD on glucose/insulin homeostasis have not been explored.

We used a CDD in mice to manipulate liver fat content without affecting adipose fat stores to dissect the role of liver fat accumulation in the insulin resistance and glucose intolerance of diet-induced obesity.

RESEARCH DESIGN AND METHODS

In vivo protocols. All animal procedures were carried out under the terms of the U.K. Animals (Scientific Procedures) Act 1986. Twelve-week-old male C57Bl/6 mice (Charles River UK, Margate, Kent, U.K.) were maintained under controlled conditions of light and temperature.

One experiment was designed to test the effects of choline deficiency on the insulin resistance and glucose intolerance induced by high-fat feeding in mice. Twelve animals were fed a low-fat diet (10% calories as fat), and 12 were

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Acsl, acyl-CoA synthetase long chain; Agpat, lysophosphatidic acid acyltransferase; AUCgl, area under the glucose curve; CDD, choline-deficient diet; Cpt1a, carnitine palmitoyl transferase 1a; DAG, diacylglycerol; Dgat, DAG *O*-acyltransferase; FFA, free fatty acid; MCDD, methionine and choline–deficient diet; mGpat, mitochondrial glycerol phosphate acyltransferase; SREBP, sterol regulatory element–binding protein.

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TABLE 1
Energy composition of experimental diets

	Experiment 1				Experiment 2	
	LoCS	LoCD	HiCS	HiCD	CS	CD
kcal/g	3.85	3.85	4.73	4.73	4.47	4.47
Protein (kcal%)	20	20	20	20	15	15
Carbohydrate (kcal%)	70	70	35	35	55	55
Fat (kcal%)	10	10	45	45	30	30
Choline bitartate (g/kg)	2	0	2	0	4.3	0

CD, choline deficient; CS, choline supplemented; Hi, high fat; Lo, low fat.

fed a high-fat diet (45% calories as fat; Research Diets, New Brunswick, NJ; Table 1) for 8 weeks. During the last 4 weeks, one-half the animals in each group (n = 6) received a CDD, whereas the other half (n = 6) received a diet supplemented with choline. Animals were weighed, and food intake per cage was measured each day. After 7 weeks of the low- or high-fat diets (i.e., after 3 weeks of the CDD and choline-supplemented diet), an intraperitoneal glucose tolerance test was performed after an overnight fast. After 8 weeks, animals were killed by decapitation between 0900 and 1100 after ad lib feeding overnight. Trunk blood was obtained, organs were weighed, and liver was snap-frozen on dry ice. The following adipose tissue depots were dissected by the same investigator: *1*) epididymal, *2*) inguinal subcutaneous, and *3*) intra-abdominal, comprising retroperitoneal (by first separating the perirenal fat and then dissecting the retroperitoneal pad en bloc) and mesenteric (by cutting the intestine below the duodenal-jejunum junction and stripping the fat by gently pulling the intestinal loops apart).

A second experiment was designed to examine changes in hepatic gene expression induced by choline deficiency. Mice were allocated (n = 6 per group) to receive either a CDD as described and standardized by Lombardi (Dyets, Bethlehem, PA) or an isocaloric choline supplemented diet (Table 1) for 3 weeks. Both diets contained 30% calories as fat. Daily body weights and food intake per cage were recorded. After 3 weeks, animals were killed by decapitation between 0900 and 1100 after ad lib feeding overnight. Trunk blood was obtained, organs were weighed, and liver was snap-frozen on dry ice for subsequent analyses.

Intraperitoneal glucose tolerance test. Mice were fasted overnight and then injected intraperitoneally with 2 mg/g D-glucose (25% [w/v] stock solution in saline). Blood samples were taken by tail nicking into EDTA microtubes (Sarstedt, Leicester, U.K.) at 0 (before injection and within 1 min of disturbing the cage) and at 15-, 30-, 60-, and 90-min intervals after the glucose bolus. Insulin resistance and glucose tolerance were assessed by fasting plasma insulin and glucose and the area under the glucose curve (AUCgl) after the intraperitoneal glucose tolerance test.

Ex vivo assays

Plasma assays. Glucose concentrations were determined using a hexokinase assay (ThermoTrace, Noble Park, Australia), insulin was measured with an ultra-sensitive mouse ELISA kit (CrystalChem, Chicago, IL), triglycerides were measured enzymatically with the Infinity triglycerides liquid stable reagent (ThermoTrace), and plasma free fatty acids (FFAs) were measured with the Wako NEFA C Test kit (Wako Pure Chemicals, Osaka, Japan).

Liver triglyceride. For quantitative analysis, two 100-mg fragments from the tip of the right lobe of the liver were homogenized in 20 volumes of propan-2-ol (VWR International, Lutterworth, U.K.), then shaken in an orbital shaker for 45 min, and centrifuged at 3,000 × g for 10 min, and the supernatant was assayed for triglyceride using a commercial triglyceride kit (ThermoTrace).

Quantitative real-time PCR. Liver (~50 mg) was homogenized in 1 ml TRIzol (Life Technologies, Gaithersburg, MD), and total RNA was extracted following the manufacturer's protocol. RNA was quantified using spectrophotometric analysis at OD_{260}. RNA integrity was checked by agarose gel electrophoresis. Oligo(deoxythymidine)-primed cDNA was synthesized from 0.5-μg RNA samples using the Promega Reverse Transcription System (Promega, Southampton, Hants, U.K.). Using the ABI PRISM 7900 Sequence Detection System (PE Applied Biosystems, Cheshire, U.K.), transcript level quantification was performed with commercially available real-time PCR primer-probe sets (Taqman Assays-on-Demand Gene Expression Products; Applied Biosystems) for CTP:phosphocholine cytidylyltransferase-α (Pcyt1a, assay ID Mm0044774_m1), acyl-CoA synthetase long chain 1 (Acsl1, assay ID Mm00484217_m1), Acsl4 (assay ID Mm00490331_m1), Acsl5 (assay ID Mm01261100_m1), mitochondrial glycerol phosphate acyltransferase (mGpat, assay ID Mm00833328_m1), lysophosphatidic acid acyltransferase 1 (Agpat1, assay ID Mm00479700_m1), Agpat2 (assay ID Mm00458880_m1), diacylglycerol (DAG) *O*-acyltransferase 1 (Dgat1, assay ID Mm00515643_m1), Dgat2 (assay ID Mm00499530), peroxisome proliferator–activated receptor-α (assay ID Mm00440939), carnitine palmitoyl transferase 1a (Cpt1a, assay ID Mm00550438_m1), sterol regulatory element–binding protein-1c (SREBP-1c; assay ID Mm00550338_m1), acetyl-CoA carboxylase 1 (assay ID Mm01304289_m1), fatty acid synthase (assay ID Mm00662319_m1), and PEPCK (assay ID Mm00440636_m1). Cyclophyllin A was used as the endogenous reference control for all transcripts. The relative amount of mRNA was determined from standard curves generated for both the target and endogenous reference using serial dilutions of cDNA.

Statistics. Data are expressed as means ± SE. Groups were compared using unpaired Student's t tests or two way ANOVA with factors of dietary fat (high or low) and dietary choline (supplemented or CDD) with post hoc Tukey's multiple comparisons tests.

RESULTS

Effect of choline deficiency on high-fat diet–induced obesity, fatty liver, insulin sensitivity, glucose tolerance, and serum lipids. Among choline-supplemented animals, the high-fat diet induced gains in body weight (Fig. 1), adipose tissue depot weights, and liver triglyceride content (Table 2) compared with the low-fat diet. The CDD did not influence body weight (Fig. 1) or adipose tissue depot sizes (Table 2). However, a CDD increased liver triglyceride content in animals on both low- and high-fat diets (Table 2).

Among choline-supplemented animals, high-fat feeding induced insulin resistance and glucose intolerance, with an elevation in fasting plasma glucose and insulin levels and elevated plasma glucose during the intraperitoneal glucose tolerance test (Fig. 2). These effects of high-fat

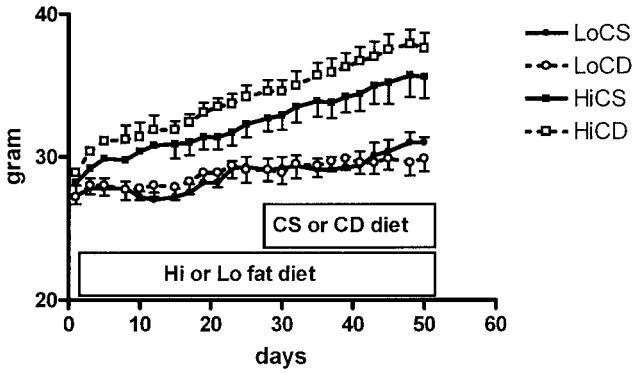

FIG. 1. Effects of high-fat diet and CDD on body weight. Twelve animals were fed a low-fat diet (10% calories as fat; Lo), and 12 were fed a high-fat diet (45% calories as fat; Hi) for 8 weeks. During the last 4 weeks, one-half of the animals in each group (n = 6) received a CDD (CD), whereas the other one-half (n = 6) continued on a diet supplemented with choline (CS). Data are expressed as means ± SE. Total weight change was compared by two-way ANOVA with post hoc Tukey's test. Animals on a high-fat diet gained significantly more weight than those on a low-fat diet (HiCS vs. LoCS, P = 0.001). A CDD (CD) had no effect on weight gain.

TABLE 2
Effect of high-fat diet and choline deficiency on body weight, fat depot size, and plasma biochemistry

	LoCS	LoCD	HiCS	HiCD
Total wt gain (g)	3.77 ± 0.76	2.97 ± 0.45	9.0 ± 0.99*	8.15 ± 0.99
Adipose depot sizes (% body wt)				
Subcutaneous	1.0 ± 0.1	0.8 ± 0.1	1.7 ± 0.2*	1.6 ± 0.1
Epididymal	1.9 ± 0.2	1.4 ± 0.1	4.2 ± 0.3†	4.3 ± 0.2
Intraabdominal	1.3 ± 0.1	1.1 ± 0.1	2.4 ± 0.2†	2.4 ± 0.1
Liver wt (g)	4.0 ± 0.4	4.1 ± 0.5	4.2 ± 0.2	4.0 ± 0.3
Liver triglyceride (μmol/g)	13.4 ± 2.6	31.6 ± 7.2‡	36.8 ± 4.8*	59.5 ± 2.1§
Nonfasting plasma results				
Glucose (mmol/l)	7.9 ± 0.9	7.8 ± 0.4	8.9 ± 0.6	8.4 ± 0.5
Insulin (pmol/l)	301 ± 11	241 ± 47	983 ± 18†	433 ± 36§
Triglycerides (mmol/l)	0.8 ± 0.2	0.5 ± 0.0	1.1 ± 0.2	1.0 ± 0.2
Free fatty acids (mmol/l)	0.8 ± 0.1	0.7 ± 0.2	0.9 ± 0.2	1.0 ± 0.1
Fasting plasma results				
Glucose (mmol/l)	6.1 ± 0.2	5.6 ± 0.3	7.5 ± 0.3*	6.8 ± 0.2
Insulin (pmol/l)	26.9 ± 3.7	23.4 ± 1.8	337.6 ± 71†	116.2 ± 17.4§
Triglycerides (mmol/l)	0.7 ± 0.1	0.4 ± 0.2	0.8 ± 0.2	0.8 ± 0.1
Free fatty acids (mmol/l)	1.4 ± 0.1	1.4 ± 0.3	1.9 ± 0.2¶	2.1 ± 0.2
Glucose tolerance test AUCgl (min · mmol^{-1} · l^{-1})	622.7 ± 33.6	603.0 ± 56.0	861.4 ± 26.8†	691.4 ± 14.0§

Data are means ± SE. Data were compared by two-way ANOVA with post hoc Tukey's test. ¶$P < 0.05$, *$P < 0.01$, †$P < 0.001$ for low-fat (Lo), choline-supplemented (CS) diet vs. high-fat (Hi), choline-supplemented diet; §$P < 0.01$ for high-fat, choline-supplemented diet vs. high-fat CDD (HiCD); ‡$P < 0.05$ for LoCS vs. low-fat CDD (LoCD).

feeding were substantially attenuated in animals receiving a CDD, despite similar obesity and fatty liver accumulation. Animals on the choline-deficient high-fat diet had lower fasting insulin and lower plasma glucose during the intraperitoneal glucose tolerance test. Fasting glucose showed trends toward improvement that did not reach statistical significance (Table 2).

There were no statistically significant changes in serum triglyceride with high-fat diet or CDD in fed or fasted state. High-fat diet increased fasting plasma FFAs, but there was no significant effect of a CDD (Table 2).

Effect of CDD on hepatic mRNA levels. Effects of CDD on hepatic gene expression in mice receiving a moderate fat diet and the roles of the various genes in lipid metabolism are shown in Fig. 3. Choline deficiency caused a significant increase in hepatic expression of Pcyt1a mRNA, the rate-limiting enzyme of phosphatidylcholine synthesis, and of the mRNA levels of enzymes proximal to Pcyt1a in phosphatidylcholine synthesis and common to triglyceride synthesis, including Acsl1 and Acsl4, mGpat, and Agpat1. Choline deficiency also increased mRNA levels of Dgat2 (but not Dgat1), an enzyme responsible for the final esterification step of DAG to triacylglycerol. In contrast, mRNA for enzymes that regulate de novo lipogenesis (fatty acid synthase and acetyl-CoA carboxylase) and access of fatty acids to mitochondrial oxidation (Acsl5 and Cpt1a) were unchanged by choline deficiency, as were transcription factors (peroxisome proliferator–activated receptor-α and SREBP-1c) that regulate expression of these genes. mRNA levels of PEPCK, the rate-limiting enzyme of gluconeogenesis, were reduced by one-half in animals on a CDD.

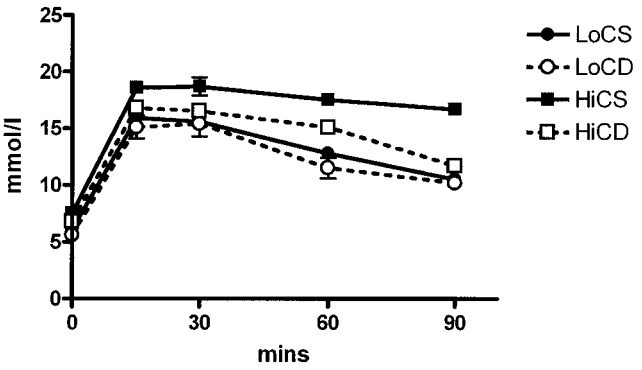

FIG. 2. Plasma glucose after intraperitoneal glucose tolerance test. Mice were fasted overnight and then injected intraperitoneally with 2 mg/g D-glucose, and blood glucose was measured at the indicated times; Lo, low-fat diet, 10% calories as fat; Hi, high-fat diet, 45% calories as fat; CS, choline supplemented; CD, choline deficient. Data are expressed as means ± SE and were analyzed by comparing the AUCgl by two-way ANOVA with post hoc Tukey's test. High-fat feeding led to glucose intolerance (AUCgl, LoCS 622.7 ± 33.6 vs. HiCS 861.4 ± 26.8 min · mmol^{-1} · l^{-1}, $P < 0.01$). A CDD significantly improved high-fat diet–induced glucose intolerance (AUCgl, HiCS 861.4 ± 26.8 vs. HiCD 691.4 ± 14.0 min · mmol^{-1} · l^{-1}, $P < 0.01$).

DISCUSSION

We have shown that a CDD increases liver fat content without affecting body weight or peripheral fat pad weight in mice. However, on a low-fat diet, this fatty liver accumulation was not associated with insulin resistance or glucose intolerance. Moreover, although CDD amplified the liver fat accumulation induced by a high-fat diet, it improved both insulin sensitivity (lowering fasting insulin and reducing hepatic PEPCK mRNA levels) and glucose tolerance. These data suggest that hepatic fat accumulation per se does not cause insulin resistance in diet-induced obesity. We have explored the mechanism of the insulin- sensitizing effect of CDD. Unlike the methionine CDD, the insulin-sensitizing effect of CDD could not be attributed to weight loss. Nor were there changes in serum fats to suggest alterations in FFA flux from peripheral tissues. However, within the liver, we show that a CDD results in increased mRNA levels of the rate-limiting enzyme of phosphatidylcholine synthesis and of enzymes involved in FFA esterification to triglyceride. This occurs without changes in expression of enzymes involved in de novo lipogenesis or fatty acid oxidation and without changes in the major transcription factors that regulate these processes. These data suggest that the insulin-

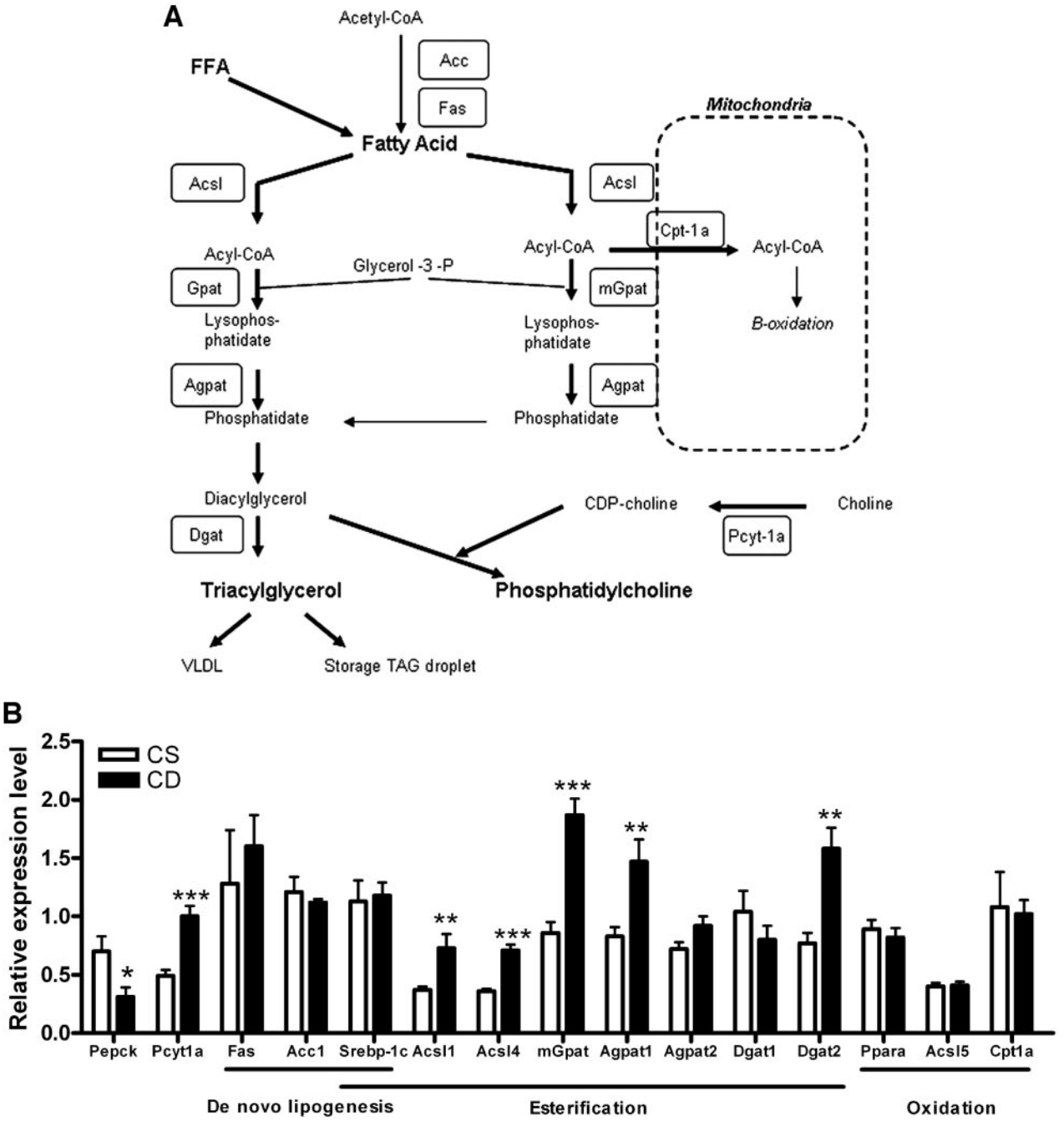

FIG. 3. Effects of choline deficiency on hepatic mRNA levels for genes involved in fatty acid and phosphatidylcholine metabolism. *A*: Pathways of fatty acid synthesis, esterification, and oxidation and of phosphatidylcholine synthesis. *B*: mRNA levels, relative to cyclophyllin A, of hepatic enzymes and transcription factors involved in triglyceride and phosphatidylcholine synthesis. *Acc*I, acetyl-CoA carboxylase 1; Fas, fatty acid synthase; PPARα, peroxisome proliferator–activated receptor-α. Animals were fed a standardized CDD (CD) or an isocaloric choline-supplemented diet (CS) for 3 weeks. Data were compared by *t* test and are expressed as means ± SE; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

sensitizing effects of a CDD may result from shunting of FFA into metabolically innocuous triglyceride stores.

Choline is required for synthesis of phosphatidylcholine, an essential part of VLDL. In liver, phosphatidylcholine is synthesized through two routes: *1*) the CDP-choline pathway, for which the rate-limiting enzyme is Pcyt1a and the substrate is dietary choline; and *2*) methylation of phosphatidylethanolamine catalyzed by phosphatidylethanolamine *N*-methyltransferase, which requires dietary methionine. MCDDs or CDDs reliably induce a fatty liver in animals. However, the mechanisms operating in MCDD and CDD may differ. MCDD-fed animals accumulate fat in the central, perivenous zone of the liver (zone 3) (21), whereas animals on CDD first accumulate fat in the periportal zone (zone 1) before it spills over into the other areas (22). Deficiency of both methionine and choline impairs hepatocyte secretion of VLDL both in vivo and in vitro (16). However, choline deficiency alone is associated with compensatory upregulation of Pcyt1a activity and phosphatidylethanolamine *N*-methyltransferase expression and activity (20,23,24), such that phosphatidylcholine supplies are only slightly reduced and VLDL secretion is not impaired in mice (20). Here, we show that mRNA levels of Pcyt1a are increased by CDD and that fasting

serum triglyceride levels are not significantly reduced by CDD in mice. Although VLDL secretion was not quantified, there is a close correlation of fasting triglyceride and VLDL levels so that our data accord with previous studies suggesting that CDD increases Pcyt1a activity sufficiently to maintain VLDL secretion (23,24).

If reduced VLDL excretion is not the basis for fatty liver in CDD, then what is its mechanism? The pathways of fatty acid synthesis, oxidation, and esterification are reviewed in Fig. 3*A*. We show that CDD increases the mRNA levels of enzymes predominantly involved in triglyceride synthesis without affecting transcripts of enzymes involved in FFA synthesis or FFA oxidation. Acsl1, -4, and -5 are the predominant isoforms in the liver (25); it has been suggested that Acsl1 and -4 favor entry of fatty acids into synthetic pathways, whereas Acsl5 favors fatty acid oxidation (26). The increased mRNA levels of Acsl1 and -4 but not Acsl5 with CDD are therefore consistent with enhanced triglyceride storage and reduced oxidation. Mitochondrial Gpat is one of at least three Gpat isoforms present in the liver (27). Although it is expressed on the mitochondrial surface, expression of mGpat has been shown to lead to triglyceride synthesis, which might be explained by mGpat competing with CPT1a for Acyl CoA on the mitochondrial surface (28). Again, increased mGpat mRNA with CDD is consistent with enhanced triglyceride synthesis. As shown in Fig. 3*A*, increased Agpat expression with CDD is also consistent with enhanced triglyceride synthesis, although the significance of an increase of only Agpat1 and not Agpat2 mRNA is not known. Yamazaki et al. (29) showed that Dgat1 and Dgat2 overexpression led to different liver phenotypes, with Dgat2 overexpression leading to increased triglyceride synthesis in the liver but without increased VLDL secretion and with Dgat1 overexpression increasing VLDL secretion. The selective increase in Dgat2 mRNA with CDD is therefore consistent with fatty liver accumulation.

These pathways of phosphatidylcholine and triglyceride synthesis are inter-related, because DAG is common to both (Fig. 3*A*). Furthermore, enzymes for both pathways are subject to common transcriptional regulation, for example by the lipogenic transcriptional factor SREBP, which upregulates both mGpat and Pcyt1a (30–33). It may be that activation of DAG synthesis is a compensatory mechanism, acting together with upregulation of Pcyt1a expression, to preserve phosphatidylcholine synthesis during dietary choline deficiency. However, in extreme circumstances, accumulated DAG is directed toward triglyceride synthesis in the presence of limited amounts of CDP-choline (34).

These changes in fatty acid metabolism predict increased intrahepatic triglyceride stores but in the presence of lower intracellular FFA levels. This combination contrasts with the elevated intracellular FFA levels that are thought to occur in fatty liver in obesity and provides a plausible explanation for the insulin-sensitizing effect of CDD. In obesity, increased concentrations of FFA in hepatocytes result from increased de novo lipogenesis, decreased β-oxidation, and increased flux of FFAs from the diet and adipose tissue. Elevated FFAs are thought to lead to hepatic insulin resistance because, among other mechanisms, fatty acid metabolites such as fatty acyl CoAs or ceramides may indirectly promote phosphorylation of serine/threonine sites on insulin receptor substrates and impair insulin-induced activation of phosphatidylinositol 3-kinase (35,36). Enhancing the removal of FFAs by their esterification into triglycerides may reduce their negative impact on insulin sensitivity. For example, Listenberger et al. (37) reported that accumulation of excess fatty acids in cellular triglyceride in CHO cells protects against lipotoxicity (measured by the propensity of fatty acids to cause apoptosis), whereas impaired synthesis of triglyceride in cells from Dgat1-null mice leads to lipotoxicity. Benefits of enhanced triglyceride storage on insulin sensitivity have also been demonstrated in myocytes (38). Similarly, it has been suggested that differences in hepatic lipogenesis underlie the resistance of C57Bl/6J-*ob/ob* mice to diabetes by comparison with BTBR-*ob/ob* mice (39). Future studies will be required to define changes in hepatic flux of lipid metabolites induced by a CDD.

These observations raise the possibility that novel therapies to enhance insulin sensitivity in obesity might be based on enhancing triglyceride storage or altering hepatic FFA metabolism. However, although a reduction in FFA concentrations by CDD may explain enhanced insulin sensitivity, the associated increase in triglyceride stores may not be entirely innocuous. The fatty liver of a CDD, unlike that of a MCDD, does not lead to marked hepatitis or cirrhosis but does lead to the development of hepatocellular carcinoma (21,40). In obesity, optimal therapy is most likely to be addressed to reducing the increased FFA flux to the liver from peripheral adipose tissue.

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REFERENCES

1. Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N: Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 50:1844–1850, 2001
2. Sanyal AJ, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN: Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 120:1183–1192, 2001
3. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C: NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 35:373–379, 2002
4. Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, Cassader M, David E, Cavallo-Perin P, Rizzetto M: Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *Hepatology* 35:367–372, 2002
5. Ryysy L, Hakkinen AM, Goto T, Vehkavaara S, Westerbacka J, Halavaara J, Yki-Jarvinen H: Hepatic fat content and insulin action on free fatty acids and glucose metabolism rather than insulin absorption are associated with insulin requirements during insulin therapy in type 2 diabetic patients. *Diabetes* 49:749–758, 2000
6. Seppala-Lindroos A, Vehkavaara S, Hakkinen AM, Goto T, Westerbacka J, Sovijarvi A, Halavaara J, Yki-Jarvinen H: Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 87:3023–3028, 2002
7. Moitra J, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B, Feigenbaum L, Lee E, Aoyama T, Eckhaus M, Reitman ML, Vinson C: Life without white fat: a transgenic mouse. *Genes Dev* 12:3168–3181, 1998
8. Carr A, Samaras K, Chisholm DJ, Cooper DA: Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. *Lancet* 351:1881–1883, 1998
9. Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, Brown MS: Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. *Genes Dev* 12:3182–3194, 1998
10. Reitman ML, Arioglu E, Gavrilova O, Taylor SI: Lipoatrophy revisited. *Trends Endocrinol Metab* 11:410–416, 2000
11. Samuel VT, Liu ZX, Qu X, Elder BD, Bilz S, Befroy D, Romanelli AJ,

Shulman GI: Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *J Biol Chem* 279:32345–32353, 2004
12. Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH: Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes* 40:1397–1403, 1991
13. Kim JK, Fillmore JJ, Chen Y, Yu C, Moore IK, Pypaert M, Lutz EP, Kako Y, Velez-Carrasco W, Goldberg IJ, Breslow JL, Shulman GI: Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. *Proc Natl Acad Sci U S A* 98:7522–7527, 2001
14. Kim SP, Ellmerer M, Van Citters GW, Bergman RN: Primacy of hepatic insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate-fat diet in the dog. *Diabetes* 52:2453–2460, 2003
15. Marchesini G, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N: Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 107:450–455, 1999
16. Yao ZM, Vance DE: The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J Biol Chem* 263:2998–3004, 1988
17. Rinella ME, Green RM: The methionine-choline deficient dietary model of steatohepatitis does not exhibit insulin resistance. *J Hepatol* 40:47–51, 2004
18. Kirsch R, Clarkson V, Shephard EG, Marais DA, Jaffer MA, Woodburne VE, Kirsch RE, Hall PL: Rodent nutritional model of non-alcoholic steatohepatitis: species, strain and sex difference studies. *J Gastroenterol Hepatol* 18:1272–1282, 2003
19. Schattenberg JM, Wang Y, Singh R, Rigoli RM, Czaja MJ: Hepatocyte CYP2E1 overexpression and steatohepatitis lead to impaired hepatic insulin signaling. *J Biol Chem* 280:9887–9894, 2005
20. Kulinski A, Vance DE, Vance JE: A choline-deficient diet in mice inhibits neither the CDP-choline pathway for phosphatidylcholine synthesis in hepatocytes nor apolipoprotein B secretion. *J Biol Chem* 279:23916–23924, 2004
21. Hartroft, WS: The sequence of pathologic events in the development of experimental fatty liver and cirrhosis. *Ann N Y Acad Sci* 57:633–645, 1954
22. Rushmore TH, Ghazarian DM, Subrahmanyan V, Farber E, Ghoshal AK: Probable free radical effects on rat liver nuclei during early hepatocarcinogenesis with a choline-devoid low methionine diet. *Cancer Res* 47:6731–6740, 1987
23. Yao ZM, Jamil H, Vance DE: Choline deficiency causes translocation of CTP:phosphocholine cytidylyltransferase from cytosol to endoplasmic reticulum in rat liver. *J Biol Chem* 265:4326–4331, 1990
24. Cui Z, Vance DE: Expression of phosphatidylethanolamine N-methyltransferase-2 is markedly enhanced in long term choline-deficient rats. *J Biol Chem* 271:2839–2843, 1996
25. Coleman RA, Lewin TM, Muoio DM: Physiological and nutritional regulation of enzymes of triacylglycerol synthesis. *Annu Rev Nutr* 20:77–103, 2000
26. Lewin TM, Kim JH, Granger DA, Vance JE, Coleman RA: Acyl-CoA synthetase isoforms 1, 4, and 5 are present in different subcellular membranes in rat liver and can be inhibited independently. *J Biol Chem* 276:24674–24679, 2001
27. Coleman RA, Lee DP: Enzymes of triacylglycerol synthesis and their regulation. *Prog Lipid Res* 43:134–176, 2004
28. Lewin TM, Wang S, Nagle CA, Van Horn CG, Coleman RA: Mitochondrial glycerol-3-phosphate acyltransferase-1 directs the metabolic fate of exogenous fatty acids in hepatocytes. *Am J Physiol Endocrinol Metab* 288: E835–E844, 2005
29. Yamazaki T, Sasaki E, Kakinuma C, Yano T, Miura S, Ezaki O: Increased very low density lipoprotein secretion and gonadal fat mass in mice overexpressing liver DGAT1. *J Biol Chem* 280:21506–21514, 2005
30. Shimomura I, Shimano H, Korn BS, Bashmakov Y, Horton JD: Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. *J Biol Chem* 273:35299–35306, 1998
31. Ericsson J, Jackson SM, Kim JB, Spiegelman BM, Edwards PA: Identification of glycerol-3-phosphate acyltransferase as an adipocyte determination and differentiation factor 1- and sterol regulatory element-binding protein-responsive gene. *J Biol Chem* 272:7298–7305, 1997
32. Kast HR, Nguyen CM, Anisfeld AM, Ericsson J, Edwards PA: CTP: phosphocholine cytidylyltransferase, a new sterol- and SREBP-responsive gene. *J Lipid Res* 42:1266–1272, 2001
33. Lagace TA, Storey MK, Ridgway ND: Regulation of phosphatidylcholine metabolism in Chinese hamster ovary cells by the sterol regulatory element-binding protein (SREBP)/SREBP cleavage-activating protein pathway. *J Biol Chem* 275:14367–14374, 2000
34. Stals HK, Mannaerts GP, Declercq PE: Factors influencing triacylglycerol synthesis in permeabilized rat hepatocytes. *Biochem J* 283:719–725, 1992
35. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI: Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 103:253–259, 1999
36. Griffin ME, Marcucci MJ, Cline GW, Bell K, Barucci N, Lee D, Goodyear LJ, Kraegen EW, White MF, Shulman GI: Free fatty acid-induced insulin resistance is associated with activation of protein kinase C τ and alterations in the insulin signaling cascade. *Diabetes* 48:1270–1274, 1999
37. Listenberger LL, Han X, Lewis SE, Cases S, Farese RV Jr, Ory DS, Schaffer JE: Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc Natl Acad Sci U S A* 100:3077–3082, 2003
38. Montell E, Turini M, Marotta M, Roberts M, Noe V, Ciudad CJ, Mace K, Gomez-Foix AM: DAG accumulation from saturated fatty acids desensitizes insulin stimulation of glucose uptake in muscle cells. *Am J Physiol Endocrinol Metab* 280:E229–E237, 2001
39. Lan H, Rabaglia ME, Stoehr JP, Nadler ST, Schueler KL, Zou F, Yandell BS, Attie AD: Gene expression profiles of nondiabetic and diabetic obese mice suggest a role of hepatic lipogenic capacity in diabetes susceptibility. *Diabetes* 52:688–700, 2003
40. Goshal AK, Farber E: Choline deficiency, lipotrope deficiency and the development of liver disease including liver cancer: a new perspective. *Lab Invest* 68:255–260, 1993