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Increased diacylglycerol acyltransferase activity is associated with triglyceride accumulation in tissues of diet-induced insulin-resistant hyperlipidemic hamsters

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Abstract

Over-accumulation of triglyceride (TG) in insulin-sensitive tissues is associated with the development of insulin resistance. We investigated whether enhanced de novo lipogenesis via diacylglycerol acyltransferase (DGAT) may contribute to the over-accumulation of TG in various tissues (liver, adipose, muscle, and intestine) using 2 well-characterized hyperlipidemic, insulin-resistant hamster models. In general, a marked increase in TG accumulation was noted in most tissues. Interestingly, the increase in TG accumulation corresponded to an increase in microsomal DGAT activity which ranged from 114% to 575% in all of the examined tissues (n = 7 per group). To delineate the mechanism for the increase in DGAT activity, we measured the expression of DGAT-1 and DGAT-2 messenger RNA by relative reverse transcriptase polymerase chain reaction (RT-PCR). In general, DGAT gene expression changed with DGAT-1 changing the most in the liver and adipose tissue, whereas DGAT-2 showed responses mainly in muscle and intestine. The increases in messenger RNA expression were not remarkable (averaging 35%; n = 4 per group) indicating that posttranscriptional mechanism(s) may play a larger role in regulating DGAT activity. In summary, the data suggest that elevated DGAT activity/expression and the subsequent increase in de novo lipogenesis could in part induce the insulin-resistant state.

1. Introduction

The accumulation of triglyceride (TG) in insulin-sensitive tissues has been associated with insulin resistance and type 2 diabetes [1,2]. The term used to describe this phenomenon has been referred to as lipotoxicity. It is thought that lipotoxicity or the accumulation of TG in insulin-sensitive tissues impairs insulin action in their respective organs causing insulin resistance [1,2]. De novo lipogenesis is known to be one possible mechanism resulting in the accumulation of TG in insulin-sensitive tissues [2]. Activation of the lipogenic transcriptional factor, sterol regulatory element-binding protein-1 that regulates their target enzymes, fatty acid synthase (FAS), and acetyl CoA carboxylase (ACC), appears to be a major contributory factor underlying the increase in lipogenesis [3]. Surprisingly, few studies have examined diacylglycerol acyltransferase (DGAT), the enzyme that catalyzes the final step in TG synthesis, as another possible mechanism for increased lipogenesis and impaired insulin action [4,5]. Part of the reason may be that progress in understanding DGAT has only emerged since the cloning of DGAT-1 and DGAT-2 in recent years. Although some studies have recently supported the idea that over-expression of DGAT may play a role in insulin resistance [6,7], these studies have not examined the relationship of TG accumulation with DGAT activity and gene expression in various insulin-sensitive tissues. Therefore, the aim of our study was to characterize intracellular TG mass, DGAT activity, and the expression of DGAT-1 and DGAT-2 genes in various insulin-sensitive tissues (liver, adipose, muscle, and intestine) using 2 well-characterized diet-induced hyperlipidemic, insulin-resistant hamster models—the high fructose–fed hamster and the high fat-fed hamster [8,9]. Such study could provide a greater insight as to the role of DGAT in the development of

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insulin resistance and to which DGAT genes may be of greater importance in the development of insulin resistance.

2. Research design and methods

2.1. Animals

Male Golden Syrian hamsters (110-120 g) (*Mesocricetus auratus*, Charles River, Wilmington, Mass) were housed individually on alternating 12-hour light, 12-hour dark cycle with free access to food and water. After about a week of acclimatization, animals were randomly divided in 3 groups of 7. Each group was placed either on a control diet (standard chow), fructose-enriched diet (60% fructose, 22% casein; pelletted; Dyets Inc, no. 161506, Bethlehem, Pa), or fat-enriched diet (6.8% corn oil, 30% beef tallow, 24.6% casein, 20.9% cornstarch; powdered; Dyets Inc, no. 100742) for 3 weeks. All hamsters were weighed weekly and all surgical procedures were done under isoflurane (4% in 100% oxygen by mask). Euthanasia was conducted under isoflurane gas and cutting the diaphragm via a thoractomy as to create negative pressure. Before collection of blood and tissues, hamsters were fasted overnight for 16 hours. Tissues collected were liver, epididymal white adipose tissue, soleus muscle, and small intestine. The small intestine was washed free of lumenal debris and cut into 2 sections (proximal and distal). All excised tissues were rinsed with cold phosphate-buffered saline, cut into several sections, and placed in cold phosphate-buffered saline. One section was used immediately for microsomal isolation, another section was placed in RNAlater (Ambion Inc, Austin, Tex) and stored at −80°C for RNA isolation, and the remaining sections were stored at −80°C. The study was approved by our Institutional Animal Care and Use Committee.

2.2. Plasma analyses

Blood was withdrawn by cardiac puncture under anesthesia using isoflurane. Blood was collected in an EDTA-coated tube and centrifuged at 3000 × *g* for 10 minutes at 4°C to obtain plasma. Glucose, cholesterol, and TG levels were determined on a clinical chemistry analyzer (VITROS DT60, Ortho-Chemical Diagnostics, Rochester, NY) from freshly collected plasma. Free fatty acid (FFA) was determined using a NEFA C kit from Wako Chemicals USA, Inc (Richmond, VA) using also fresh plasma, whereas plasma insulin level was determined by radioimmunoassay (Linco Research, St Louis, Mo) using a frozen aliquot.

2.3. Triglyceride mass

Triglyceride mass was measured essentially as described by Brown et al [10]. Briefly, 100 μg of frozen tissue were minced and homogenized in 2 mL of sucrose buffer (0.3mol/L sucrose, 25 nmol/L 2-mercaptoethanol, and 10 mmol/L EDTA, pH 7.0) and mixed with chloroform. The organic phase was separated, dried, and the lipids resuspended in 100 μL of ethanol. Triglyceride mass was performed according to the manufacturer procedure (Triglyceride GPO-PAP Method, Randox, San Diego, Calif).

2.4. Microsomal DGAT activity

Microsomes were initially isolated as described [11]. Briefly, 300 mg of fresh tissue were minced and homogenized in 4 mL of TEA buffer (0.1 mol/L triethanolamine, 20 mmol/L EDTA, and 2 mmol/L dithiothreitol, pH 7.4) using a Potter-Elvehjem homogenizer. The homogenate was centrifuged twice at 12 000 × *g* for 10 minutes at 4°C to remove cellular debris and mitochondria. The supernatant was removed and centrifuged at 100 000 × *g* for 60 minutes at 4°C using a Beckman L7-55 ultracentrifuge. The microsomal pellet was then rinsed with the same buffer as above and stored at −80°C until ready to be assayed for DGAT activity. Frozen microsomes were resuspended in 1 mL of TEA buffer (0.1 mol/L triethanolamine, 20 mmol/L EDTA, and 10 mmol/L dithiothreitol, pH 7.8) and allowed to stand for 60 minutes on ice. After the addition of 4 mL of buffer, microsomes were centrifuged at 100 000 × *g* for 60 minutes at 4°C to further purify the fraction. The microsomal pellet was rinsed, resuspended in 100 μL of Tris buffer, pH 7.8, and protein concentration was determined as described below. Esterification of diacylglycerol was measured by using labeled palmitoyl-CoA as described by Grigor and Bell [12] with minor modifications. Briefly, the assay was performed in a total volume of 120 μL of Tris buffer (175 mmol/L, pH 7.8) containing bovine serum albumin fatty acid–free (0.14 mg/mL), $MgCl_2$ (4 mmol/L), and 0.25 μCi/mL [palmitoyl-1-^{14}C]palmitoyl-CoA (40-60 mCi/mmol, Perkin Elmer Life Science Research Products, Boston, Mass). After a 10-minute preincubation at 23°C, the reaction was started by the addition of microsomes (80 μg protein/mL) and 1,2-diacylglycerol (800 μmol/L) for 10 minutes. The reaction was terminated by the addition of hexane and the TG formed was extracted and subjected to thin-layer chromatography in chloroform/acetic acid (96:4). The assay was linear up to 1000 μg/mL of proteins (data not shown).

2.5. Measurement of DGAT mRNA abundance

Relative reverse transcriptase polymerase chain reaction (RT-PCR) was performed to determine the levels of DGAT-1 and DGAT-2 gene expression. Total RNA was isolated using RNAzol B (Tel-Test, Friendswood, Tex) according to the manufacturer procedure with a slight modification for adipose tissue. Adipose homogenates were repeatedly passed through a cannula and centrifuged to remove the lipid layer. RNA concentrations were determined by spectrophotometry. Isolated total RNA was reversed transcribed using 100 U/μL SuperSCRIPTII RNase H$^-$ reverse transcriptase (Invitrogen Life Technologies, Carlsbad, Calif) primed with 0.3 μg/μL random primers (Invitrogen). Diacylglycerol acyltransferase messenger RNA (mRNA) was amplified in an Eppendorf Mastercycler (Eppendorf Scientific, Inc, Westbury, NY) using 10 pmol each of mouse DGAT-1–specific primers (sense 5′ -GGCCTTCTTCCAC-

GAGTACC-3′; antisense 5′-GGCCTCATAGTTG-AGCACG-3′) or mouse DGAT-2–specific primers (sense 5′-AAGAAGTTTCCTGGCATAAG-3′; antisense 5′-AAA-GATCACCTGCTTGTATA-3′), and Taq DNA polymerase (Eppendorf Scientific, Inc) according to the following thermal cycle: 30 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C for 31 (DGAT-1) or 32 (DGAT-2) cycles (determined to lie within the linear amplification phase). 18S rRNA was used as an internal standard and was co-amplified using primers and competimers from Ambion Inc using a 2:8 primer/competimer ratio. Amplified products (216 base pairs [bp] for DGAT-1, 304 bp for DGAT-2, and 488 bp for 18S rRNA) were run on 2% agarose gels and visualized by staining with ethidium bromide. Band intensities were analyzed densitometrically using the Gel Doc Gel Documentation System (Bio-Rad Laboratories, Inc, Hercules, Calif) and DGAT mRNA levels were normalized with respect to 18S rRNA.

2.6. Other methods

Protein content was measured according to Bradford [13] (D_c Protein Assay; BioRad) using bovine serum albumin as the standard.

2.7. Statistical analysis

Statistical differences were analyzed by using Student *t* test with the level of significance set at .05.

3. Results

3.1. Metabolic effects of fructose and fat feeding in Golden Syrian hamsters

Table 1 shows the mean body weight changes observed in control, fructose- and fat-fed hamsters before and after a 3-week feeding period. An increased trend in body weight was observed in all groups over the 3-week feeding period; however, this did not reach statistical significance ($P > .05$ vs initial weight, n = 7 per group). Tissue weight was also measured at the end of the study. The weight of liver and adipose tissue increased significantly in both models (28% and 27% in fructose-fed hamsters and 33% and 40% in fat-fed animals, respectively, $P < .05$ vs control chow, n = 7 per group). The weight of muscle and intestine (proximal and distal) was similar in the groups fed the specific diets ($P > .05$ vs control chow, n = 7 per group). Consequently, both fructose- and fat-enriched diets increased liver and adipose tissue weight apparently by increasing fat deposition.

Table 1
Effects of fructose- and fat-enriched diets on body and tissue weight

Parameters	Chow	Fructose	Fat
Initial body weight	123.7 ± 7.4	127.0 ± 10.3	129.9 ± 11.7
Final body weight	125.0 ± 9.5*	134.0 ± 12.1*	141.0 ± 14.3*,**
Liver	3.6 ± 0.5	4.6 ± 0.7**	4.8 ± 0.4**
Adipose	3.0 ± 0.5	3.8 ± 0.5**	4.2 ± 1.0**
Muscle	5.4 ± 0.8	4.7 ± 0.3	5.7 ± 0.5
Proximal intestine	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1**
Distal intestine	0.8 ± 0.1	0.9 ± 0.1	1.1 ± 0.1**

Data are given as means ± SD of 7 animals per group expressed in grams.
* $P > .05$ vs initial body weight.
** $P < .05$ vs control chow diet.

Table 2
Effects of fructose- and fat-enriched diets on plasma biochemical parameters

Parameters	Chow	Fructose	Fat
Glucose (mg/dL)	182 ± 18	186 ± 21	178 ± 34
Cholesterol (mg/dL)	83 ± 16	144 ± 18*	126 ± 11*
TGs (mg/dL)	114 ± 21	162 ± 43*	170 ± 70*
FFA (mEq/L)	0.36 ± 0.07	0.46 ± 0.08*	0.43 ± 0.08*
Insulin (ng/mL)	0.25 ± 0.05	0.37 ± 0.06*	0.49 ± 0.10*

Data are given as means ± SD of 7 animals per group.
* $P < .05$ vs control chow diet.

3.2. Effects of fructose- and fat-enriched diets on plasma biochemical parameters

Various plasma biochemical parameters were examined to assess the extent of the hyperlipidemia and insulin resistance upon high-fructose and high-fat feeding in our animal model. Fructose- and fat-enriched diets given to hamsters over a 3-week period significantly increased plasma levels of cholesterol, TG, FFA, and insulin by 73%, 42% 28%, and 48% (fructose group, $P < .05$ vs control chow, n = 7 per group), and 52%, 49%, 19%, and 96% (fat group, $P < .05$ vs control chow, n = 7 per group), respectively (Table 2). Glucose levels remained essentially unchanged in fructose- and fat-fed hamsters ($P > .05$ vs control chow, n = 7 per group).

3.3. Effect of fructose- and fat-enriched diets on TG mass in various tissues

We next examined the effect of fructose- and fat-enriched diets given to hamsters over a 3-week period on TG mass in various tissues. As shown in Table 3, high-fructose diet significantly increased TG mass in liver and adipose tissue by 84% and 31%, respectively ($P < .05$, n = 7 per group). Similarly, a high-fat diet increased TG mass in both liver and adipose tissue, in addition to muscle, by 137%, 45%, and 43%, respectively ($P < .05$, n = 7 per group). However, TG mass in the intestine showed nonsignificant variations

Table 3
Effect of fructose- and fat-enriched diets on TG mass in various tissues

Parameters	Chow	Fructose	Fat
Liver	6.8 ± 1.5	12.5 ± 2.3*	16.1 ± 5.6*
Adipose	560.3 ± 13.6	736.1 ± 32.4*	811.5 ± 45.2*
Muscle	5.7 ± 2.3	6.5 ± 1.5	9.3 ± 2.7*
Proximal intestine	1.0 ± 0.8	2.2 ± 1.7	1.6 ± 0.8
Distal intestine	1.7 ± 1.2	0.9 ± 0.7	1.5 ± 0.5

Data are given as means ± SD of 7 animals per group expressed in milligrams per gram of tissue.
* $P < .05$ vs control chow diet.

with an increased trend for the proximal segment and a decreased trend for the distal segment in both fructose- and fat-fed hamsters ($P > .05$ vs control chow, n = 7 per group). Triglyceride mass in the muscle of fructose-fed hamsters also showed nonsignificant changes ($P > .05$ vs control chow, n = 7 per group).

3.4. Effect of fructose- and fat-enriched diets on DGAT activity in various tissues

To delineate the mechanism for the increase in TG mass, DGAT activity was measured. Microsomes (as a source of DGAT) were isolated from various tissues and the rate of incorporation of [^{14}C]palmitoyl CoA into TG was measured in vitro. The assay was performed in the presence of 4 mmol/L of $MgCl_2$, which is known to measure both DGAT-1 and DGAT-2 [14]. As shown in Fig. 1, fructose- and fat-enriched diets significantly increased DGAT activity in all of the examined tissues ($P < .05$, n = 7 per group). The highest increases were observed in adipose tissue and the proximal intestine (424% and 213% in fructose-fed hamsters; 575% and 523% in fat-fed animals, respectively). In liver, muscle, and distal intestine, DGAT activity increased between 120% and 200%. The results compared well with our results on TG mass, with the exception of the intestine.

3.5. Effect of fructose- and fat-enriched diets on DGAT mRNA expression in various tissues

To characterize the increase in DGAT activity, we studied the gene expression of DGAT-1 and DGAT-2 mRNA in response to high-fructose and high-fat diet. Total RNA was extracted from hamster tissues, and relative RT-PCR was performed to detect the transcript levels of DGAT-1 and DGAT-2 genes. Fig. 2A is the ethidium bromide–stained gel of a representative experiment performed on 2 hamsters with a bar graph summarizing the data of 4 hamsters for DGAT-1 (Fig. 2B). High-fructose and high-fat diets showed significant increases in DGAT-1 mRNA expression in liver and adipose tissue (61% and 35% in fructose-fed animals; 55% and 51% in fat-fed animals, respectively, $P < .05$ vs control chow, n = 4). High-fat diet, but not high-fructose diet, increased DGAT-1 mRNA expression in hamster muscle by 135% ($P < .05$ vs control chow, n = 4). Both high-fructose and high-fat diets showed no effect on DGAT-1 mRNA expression in the intestine (proximal and distal). Fig. 3A is the ethidium bromide–stained gel of a representative experiment performed on 2 hamsters with a bar graph summarizing the data of 4 hamsters for DGAT-2 (Fig. 3B). In contrast to DGAT-1, high-fructose and-high fat diets showed

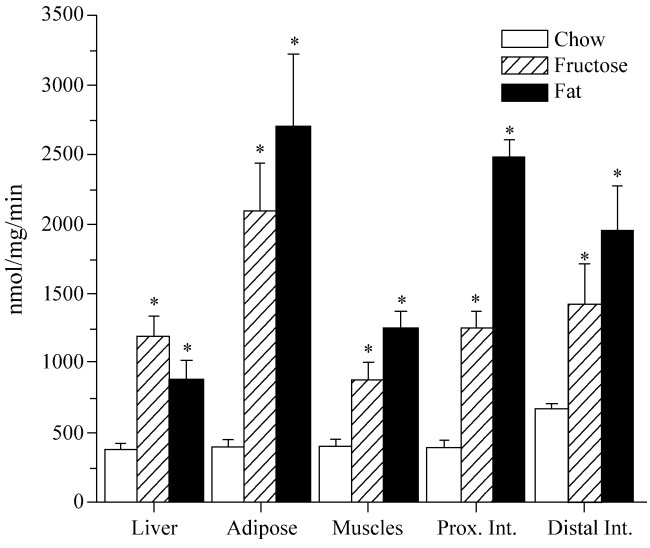

Fig. 1. Effect of fructose- and fat-enriched diets on microsomal DGAT activity. Animals were divided into 3 groups—a control chow diet, fructose-enriched diet, or fat-enriched diet—for 21 days. Microsomes were isolated from various tissues (liver, adipose, muscle, and intestine) and DGAT activity was determined by esterification of diacylglycerol using [^{14}C]palmitoyl CoA. The amount of [^{14}C]TG was determined by thin-layer chromatography and scintillation counting. Data are expressed as nanomoles of [^{14}C]palmitoyl CoA incorporated into TG per milligram of microsomal protein per minute. Values represent the mean ± SD of 7 animals per group. Asterisk indicates $P < .05$ vs control chow diet.

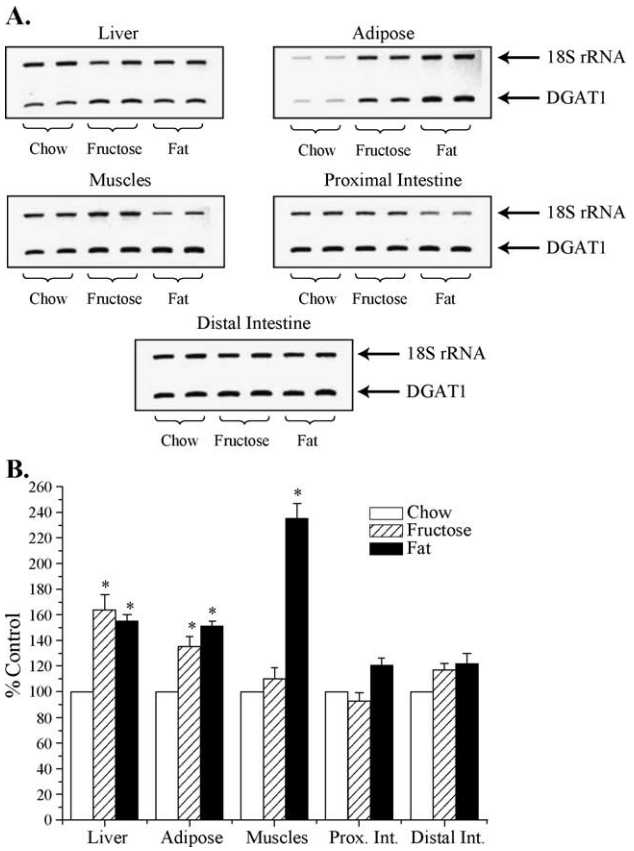

Fig. 2. Effect of fructose- and fat-enriched diets on DGAT-1 mRNA levels. Animals were treated as described in Fig. 1. Total RNA was extracted from various tissues, mRNA levels analyzed by relative RT-PCR, and the gel stained with ethidium bromide. Band intensities were scanned densitometrically and DGAT-1 mRNA levels were normalized with respect to the 18S rRNA internal control. (A) is a representative stained gel showing the signals for DGAT-1 and 18S rRNA in different tissues of 2 hamsters whereas (B) is a bar chart summarizing the data of 4 hamsters. Values in (B) represent the mean ± SD of 4 animals per group. Asterisk indicates $P < .05$ vs control chow diet.

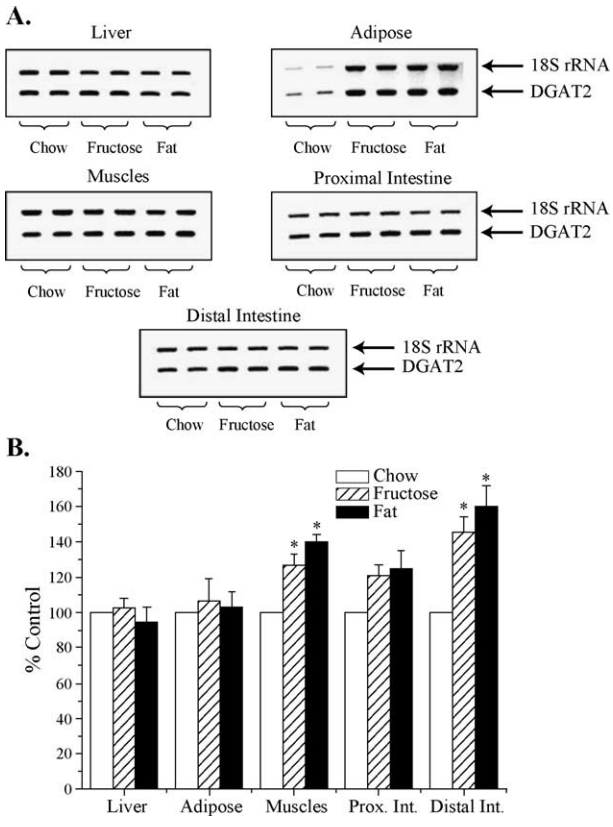

Fig. 3. Effect of fructose- and fat-enriched diets on DGAT-2 mRNA levels. Animals were treated as described in Fig. 1 and relative RT-PCR was performed as described in Fig. 2. (A) is a representative stained gel showing the signals for DGAT-2 and 18S rRNA in different tissues of 2 hamsters whereas (B) is a bar chart summarizing the data of 4 hamsters. Values in (B) represent the mean ± SD of 4 animals per group. Asterisk indicates $P < .05$ vs control chow diet.

significant increases in DGAT-2 mRNA expression in muscle and distal intestine (27% and 46% in fructose-fed animals; 35% and 60% in fat-fed animals, respectively, $P < .05$ vs control chow, n = 4). Both high-fructose and high-fat diets showed no effect on DGAT-2 mRNA expression in liver, adipose tissue, and proximal intestine.

4. Discussion

There is strong evidence to support that fat diversion from adipose to non-adipose tissue such as liver and muscle, which are not adapted to TG storage, may lead to insulin resistance and type 2 diabetes [15]. When TG over-accumulate in non-adipose tissues, metabolites including fatty acids, ceramide, and diacylglycerol may enter deleterious nonoxidative pathways and compromise cellular function [2]. Hence, the clinical condition of diabetes has been described as a lipotoxic disease. However, it remains to be seen whether lipotoxicity is a causative factor or merely a marker in the development of type 2 diabetes.

Much of the evidence for the association between lipid accumulation and insulin resistance come from animal studies. Rats fed a high-fat diet developed insulin resistance as TG accumulated in the muscle [16]. More recently, the association has been demonstrated in the obese rodent model of diabetes, the Zucker diabetic fatty rat [3,17]. In our studies, we sought to examine the association using the fructose- and high fat–induced hyperlipidemic, insulin-resistant golden Syrian hamster model system. Golden Syrian hamsters are known to be a useful species for lipid research because of their lipid metabolism resembling that of human beings [18,19]. Therefore, our hamster model may be appropriate for evaluating human-like dyslipidemia associated with insulin resistance. As previously reported [8,9], high-fructose feeding and high-fat feeding for a 3-week period is known to induce hyperlipidemia and hyperinsulinemia with the development of whole body insulin resistance in the Syrian golden hamster. In the present study, we found that our hamsters fed a high fructose– or high fat–enriched diet for 3 weeks also developed hyperlipidemia and insulin resistance. The increases in plasma cholesterol and TG levels were associated with hyperinsulinemia without a change in plasma glucose levels. Upon induction of hyperlipidemia/insulin resistance, a notable observation in our study was the increased accumulation of TG in various insulin-sensitive tissues, including liver, adipose tissue, and muscle. This corresponded to an increase weight in liver and adipose tissue. The lipid excess observed in our study confirms the association between lipid accumulation and insulin resistance.

An area of current investigation is the mechanism(s) accounting for the increase in intracellular accumulation of TG. Mechanisms or factors leading to TG accumulation are not clear, but may be derived from increased lipogenesis and/or reduced fatty acid oxidation [20]. Increased availability of fatty acyl CoAs may increase intracellular TG content by providing excess substrate for TG biosynthesis. In our study, a small but significant increase in FFA levels were noted which may explain the increased TG accumulation. Similarly, increased plasma very low-density lipoprotein levels as reported by others in our model system [8,21] could also contribute to an increase in FFA flux to insulin-sensitive tissues available for TG biosynthesis. Alternatively, enhanced lipogenic enzyme activity may provide another molecular basis for the accumulation of TG in various insulin-sensitive tissues. Zhou et al [3] and Lee et al [17] showed increases in de novo TG synthesis in pancreatic islet cells of Zucker diabetic fatty rat via a number of lipogenic enzymes including FAS, ACC, and glycerol-3-phosphate acyltransferase. The group concluded the increases in these enzymes contributed to the fat overload that leads to pancreatic dysfunction and diabetes in their animal model. Although these studies support enhanced de novo lipogenesis via FAS, ACC, and glycerol-3-phosphate acyltransferase in the pathogenesis of insulin resistance, very few studies have examined DGAT, a key enzyme in the synthesis of TG, as another possible factor. Using a transgenic DGAT-1 mice and isolated pancreatic

islet cells over-expressing DGAT-1, Chen et al [5] and Kelpe et al [4] recently supported the lipotoxicity-insulin resistance hypothesis. However, these studies were focused only on adipose tissue and pancreatic islet cells and did not investigate other insulin-sensitive tissues. Also, these studies were not performed from insulin-resistant animals. Thus, we followed our study by examining TG accumulation and DGAT activity in a number of tissues, including liver, adipose tissue, muscle, and intestine, using our diet-induced insulin-resistant hamster model. Such undertaking may provide a greater understanding of the role of DGAT in the development of insulin resistance including which DGAT gene may be of greater importance in the accumulation of TG in tissues of insulin-resistant animals.

Two DGAT genes have been identified. DGAT-1 gene was the first to be sequenced and shown to be related to the acyl CoA:cholesterol acyltransferase gene family [22]. DGAT-1 appears to function in fat absorption and energy homeostasis [22]. Interestingly, Farese's laboratory using DGAT-1$^{-/-}$ knockout mice showed that deficiency in DGAT-1 resulted in obesity resistance and improved glucose metabolism by reversing leptin and insulin resistance [6,7]. They further confirmed using the knockout mice that alternative mechanisms existed for synthesizing TG. In 2001, the same group cloned DGAT-2 [14] and suggested that it played a significant role in TG metabolism possibly by compensating for conditions leading to the absence of DGAT-1 [23]. DGAT-2 is a new gene family that has no homology with DGAT-1 [14]. The function of DGAT-2 remains elusive, but may have specialized functions in basal TG synthesis, storage of intracellular TG, and skin development [24].

In our study, remarkable increases in DGAT activity were noted in all examined tissues. The results corresponded well with the increase in TG mass with the exception of the intestine. The discrepancy may reflect a greater TG turnover rate in the intestine. Rizvi et al [25] recently showed increased lipase activity in the small intestine of diabetic hamsters as a mechanism of eliminating the extra dietary fat load. Alternatively, DGAT may be involved in the formation of chylomicrons; hence, TG may be secreted as chylomicrons. The net effect on intestinal TG mass may therefore be neutral despite increases observed in DGAT activity. Taken together, this is the first study to demonstrate that TG accumulation is accompanied by increased DGAT activity in an insulin-resistant animal model. Wakimoto et al [26] did show increased DGAT-2 gene expression in a number of tissues from genetically induced diabetic mice; however, no comparisons with TG mass or DGAT activity were reported. We cannot rule out other factors, be other enzymes involved in lipogenesis, fatty acid oxidation, or lipid hydrolysis as other contributing factors into why TG accumulates into various tissues in our model system.

To delineate the mechanisms for the increase in DGAT activity, gene expression of DGAT-1 and DGAT-2 was assessed by relative RT-PCR. With regard to DGAT-1, significant increases were observed for liver (both fructose and fat diets), adipose tissue (both fructose and fat diets), and muscle (fat diet only). For DGAT-2, significant increases were noted for muscle and distal intestine (both fructose and fat diets). Our results on DGAT-2 mRNA expression compared well with that of Wakimoto et al [26] using 2 genetically induced diabetic mice with the exception that DGAT-2 mRNA levels also increased in adipose tissue in their studies. Similarly, Meegalla et al [27] also observed increases in DGAT-2 mRNA levels in adipose tissue of ob/ob mice. Why we did not observe an increase in DGAT-2 mRNA levels in adipose tissue likely reflects a different animal model system (hamster vs mice and genetically induced vs diet-induced diabetes).

We generally observed small increases or no changes in DGAT-1 and DGAT-2 gene expression despite large increases in DGAT activity in all of the examined tissues. The results suggest that posttranscriptional mechanism(s) may play a larger role in regulating DGAT activity. Others have suggested this possibility as well. Although activity increased 60-fold in differentiating 3T3-L1 adipocytes, mRNA expression of DGAT-1 and DGAT-2 increased only 8- and 30-fold, indicating that DGAT regulation is predominantly posttranscriptional [28]. Yu et al [29] recently investigated the posttranscriptional regulation of DGAT-1, including protein stability and protein phosphorylation. The group showed that DGAT-1 protein mass remained constant even when proteolysis was blocked, suggesting that protein stability is not a determining factor in the regulation of DGAT activity. The same investigators also ruled out protein phosphorylation as a point of regulation when a mutation of a conserved tyrosine residue yielded a fully active enzyme [29]. The study concluded that DGAT activity was regulated primarily at the translational level [29].

In conclusion, our observation that TG mass compared well with DGAT activity upon high-fructose or high-fat feeding suggests the possibility that enhanced de novo lipogenesis via DGAT may be a key underlying factor in the development of insulin resistance in a number of insulin-sensitive tissues. Regulation in DGAT activity appeared to be mainly a posttranscriptional event. Whether DGAT-1 or DGAT-2 is predominantly involved will require further investigation.

Acknowledgments

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