Sources of hepatic triglyceride accumulation during high-fat feeding in the healthy rat

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Hepatic triglyceride (HTG) accumulation from peripheral dietary sources and from endogenous *de novo* lipogenesis (DNL) was quantified in adult Sprague–Dawley rats by combining *in vivo* localized ^{1}H MRS measurement of total hepatic lipid with a novel *ex vivo* ^{2}H NMR analysis of HTG ^{2}H enrichment from [2]H-enriched body water. The methodology for DNL determination needs further validation against standard methodologies. To examine the effect of a high-fat diet on HTG concentrations and sources, animals (n = 5) were given high-fat chow for 35 days. HTG accumulation, measured by *in vivo* ^{1}H MRS, increased significantly after 1 week ($3.85 \pm 0.60\%$ vs $2.13 \pm 0.34\%$ for animals fed on a standard chow diet, $P < 0.05$) and was maintained until week 5 ($3.30 \pm 0.60\%$ vs $1.12 \pm 0.30\%$, $P < 0.05$). Animals fed on a high-fat diet were glucose intolerant (13.3 ± 1.3 vs 9.4 ± 0.8 mM in animals fed on a standard chow diet, for 60 min glycemia after glucose challenge, $P < 0.05$). In control animals, DNL accounted for $10.9 \pm 1.0\%$ of HTG, whereas in animals given the high-fat diet, the DNL contribution was significantly reduced to $1.0 \pm 0.2\%$ ($P < 0.01$ relative to controls). In a separate study to determine the response of HTG to weaning from a high-fat diet, animals with raised HTG ($3.33 \pm 0.51\%$) after 7 days of a high-fat diet reverted to basal HTG concentrations ($0.76 \pm 0.06\%$) after an additional 7 days of weaning on a standard chow diet. These studies show that, in healthy rats, HTG concentrations are acutely influenced by dietary lipid concentrations. Although the DNL contribution to HTG content is suppressed by a high-fat diet in adult Sprague–Dawley rats, this effect is insufficient to prevent overall increases in HTG concentrations. Copyright © 2008 John Wiley & Sons, Ltd.

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INTRODUCTION

The high fat (HF) concentration of the typical western-style diet is considered to be an important factor in the development of type 2 diabetes *mellitus* (T2DM) (1–3). In this setting, there is an initial phase, often referred to as the 'prediabetic state', which is characterized by weight gain, an increase in the body fat fraction, and the development of glucose intolerance. In addition to an overall gain in body fat, there is increased deposition of ectopic triglyceride, particularly in liver and skeletal muscle. As these lipid pools may play a key role in the development of insulin resistance (IR) at an early and possibly reversible stage of T2DM, there is great interest in the study of ectopic lipid dynamics both in order to better understand the genesis of IR and also as a clinical marker for identifying people that may be at increased risk of developing T2DM. There is particular focus on the study of ectopic hepatic triglycerides (HTGs), as HTG concentration is highly sensitive to dietary fat intake, and raised HTG is tightly associated with glucose intolerance and decreased insulin sensitivity (4,5). Increased HTG concentrations are prevalent in patients with established T2DM (4–8) and in animal models of T2DM. Rodent models are important for understanding the relationship between HTG and the development of IR and glucose intolerance in the prediabetic state because prospective studies of diet on HTG concentrations can be easily designed and the effects are seen within a few days or weeks. Non-diabetic healthy rats placed on a HF diet accumulate HTG (9,10) and this is associated with significantly impaired insulin action, reduced whole-body glucose disposal rates, and hepatic IR (11–15). However, the

time-scale and reversibility of HTG change induced by a HF diet and its associated effects on glucose homeostasis are less well defined in rats than in humans. This is because non-invasive methods such as MRS/MRI that have been developed and applied for monitoring longitudinal changes in human HTG concentrations have not yet been widely applied in rats. Localized ^{1}H MRS is a non-invasive technique that has been shown to be a

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precise tool for quantifying HTG content (16–18). With appropriate high-field MR systems (7 T or above), this measurement allows quantification of HTG in mice (19) and in the Zucker diabetic fatty (ZDF) rat model of T2DM (20) and is well suited for longitudinal measurements of HTGs in a single animal.

HTGs can be derived from plasma free fatty acids (FFAs) which are taken up via lipoprotein transport and esterified to triglycerides after hepatic uptake. HTGs can also be formed *in situ* by *de novo* lipogenesis (DNL) of fatty acids from acetyl-CoA. Given the capacity of the liver to synthesize, import, and export triglycerides, the accumulation of HTGs may arise through an imbalance between triglyceride import and export and/or imbalances between triglyceride synthesis and utilization. In this study, we quantified total HTGs by *in vivo* localized ¹H MRS measurement and resolved the lipogenic and non-lipogenic contributions by integrating the MRS measurement with a novel and simple measurement of DNL by *ex vivo* ²H NMR analysis of HTG ²H enrichment from ²H-enriched body water. We used this approach to study the effects of altering dietary fat content on both HTG concentrations and sources in healthy male Sprague–Dawley rats.

EXPERIMENTAL

Rat studies

Male Sprague–Dawley rats (180–220 g) were housed in a room on a 12-h light/dark cycle (8 am to 8 pm light) under constant temperature (22–25°C) and with *ad libitum* access to food and water. Animals were divided into three distinct study groups.

Study 1

Animals were studied for 20 days. They were divided into two subgroups fed on either a HF diet (n = 5) with 45% of the calories coming from fat, 35% from carbohydrate and 20% from protein (E15744-34; Ssniff Specialdiäten GmbH, Soest, Germany) or a standard chow (SC) diet (2.7% fat, 60% carbohydrate and 16% protein) (n = 6). On days 8 and 15, the two subgroups were assessed in terms of HTG content, and, on the last day of the experiment, blood was withdrawn from 6 h-fasted animals for determination of blood glycemia and FFA and triglyceride content. These animals were challenged by a glucose tolerance test consisting of an intraperitoneal injection of glucose (1.5 mg glucose/g body weight), and blood glycemia was assessed for 60 min after the glucose load, in predetermined intervals. Blood glucose concentration was measured with a standard gluc-

ometer, and plasma insulin, FFA and triglyceride concentrations were assessed with biochemical assay kits commercially available from Linco Research (Billerica, MA, USA), Wako Chemicals (Richmond, VA, USA) and Sigma (St Louis, MO, USA), respectively.

Study 2

A group of animals (n = 5) was maintained for 35 days on a HF diet. On the last day, they were assayed for HTG content by ¹H MRS and compared with a group of animals fed on a SC diet (n = 4). On the afternoon of day 35, animals were given an intraperitoneal injection of 8 mL 99.9% ²H₂O (Sigma-Aldrich, St Louis, MO, USA) in physiological saline. At this point, the drinking water was also enriched with 3% ²H₂O to maintain constant body water enrichment until the end of the study. On day 37, animals were killed at 2 pm, livers were collected and freeze-clamped for lipid analysis, and blood was withdrawn and immediately centrifuged for plasma separation. Plasma was quickly stored at −80°C. Fasting plasma glucose, insulin, FFAs and triglycerides were measured.

Study 3

Five rats were maintained on a HF diet for 7 days and then switched back to a SC diet. On days 1, 8 and 15 of the experiment at 2 pm, liver ¹H MRS was performed to assess HTG concentrations in each animal.

¹H MRS

In vivo MRI and ¹H MRS studies were performed on a 7 T Bruker Pharmascan system using a whole-body coil for radiofrequency transmitting and signal receiving. Animals were placed in the prone position, kept at 37°C, and anesthetized with isoflurane anesthetic, with maintenance under 1–1.5% (v/v) isoflurane/oxygen during the imaging and spectroscopy experiments. Transverse images of the liver were used to ensure accurate positioning of the $5 \times 5 \times 5$ mm voxel in the liver (Fig. 1). Single-voxel volume-localized ¹H MR spectra were obtained using a point-resolved spectroscopy sequence ($TR = 1000$ ms, $TE = 28$ ms) without water saturation and with 128 average scans (20). Spectra were analyzed using the NMR data processing program, MestREC (Mestrelab Research, Santiago de Compostela, Spain), where peak areas for all resonances were obtained and the lipid resonance corresponding to the methylene $[(CH_2)_n]$ arising from aliphatic fatty acid chains of triglycerides was quantified with reference to water resonance (19,20).
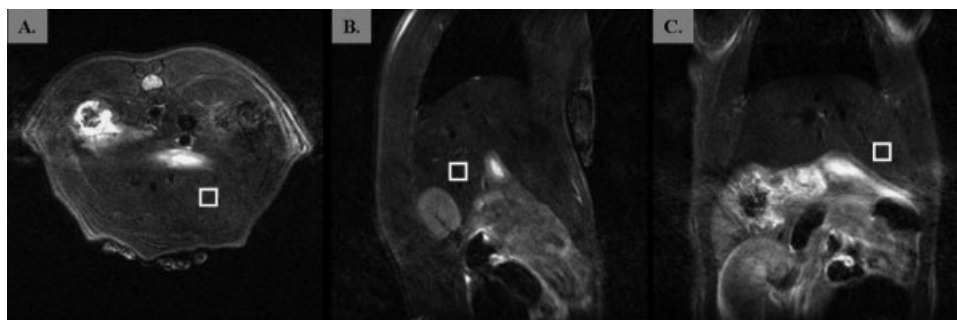

Figure 1. Axial (A), sagittal (B) and coronal (C) liver T_2-weighted MR images used for hepatic localization and voxel placement.

HTG extraction protocol

Hepatic lipids from freeze-clamped livers were separated by a Folch extraction (21) adding 20 mL chloroform/ethanol (2:1, v/v) for each gram of liver powder with 15 min of stirring at room temperature. After centrifugation, the supernatant was collected and added to 0.9% (w/v) NaCl (5:1, v/v) with vigorous agitation. Finally, after centrifugation, the lower lipid-containing phase was collected, evaporated to dryness, and then dissolved in chloroform with pyrazine as an internal standard for NMR analysis.

^{1}H and ^{2}H NMR analysis

^{1}H and ^{2}H NMR spectra were acquired at 11.75 T with a Varian Unity Spectrometer equipped with a 5 mm broadband 'switchable' probe with z-gradient (Varian, Palo Alto, CA, USA). Proton-decoupled ^{2}H NMR spectra were acquired without field-frequency lock at 25°C using a 90° pulse, a 2.0 s acquisition time and a 2.0 s pulse delay. ^{2}H enrichment of the aggregate triglyceride methyl hydrogens was quantified by comparing the composite HTG methyl ^{2}H signal with that of an internal pyrazine-d$_4$ standard. The fraction of HTG derived from DNL was estimated as HTG [^{2}H]methyl/^{2}H body water enrichment. ^{2}H body water enrichment was determined in each plasma sample using ^{2}H NMR spectroscopy (22).

Estimation of HTG synthesis by DNL by analysis of acyl methyl hydrogen ^{2}H enrichment from ^{2}H-enriched body water

During DNL, the methyl hydrogens of the acyl moiety are directly derived from those of acetyl-CoA and do not participate in the desaturation and chain elongation reactions that occur during lipogenesis. To our knowledge, there have been no direct measurements of acetyl-CoA enrichment from ^{2}H-enriched body water. Assuming that pyruvate is the main source of lipogenic acetyl-CoA and given that the exchange of pyruvate methyl protons with those of water is 80–95% complete (23,24), the enrichment of body water is a good approximation for that of the immediate acetyl-CoA precursor.

Assuming that body water enrichment is equal to that of the acetyl-CoA precursor, the fraction of triglyceride acyl groups derived from DNL (HTG lipogenic fraction) is simply the triglyceride enrichment divided by body water enrichment:

$$\text{HTG lipogenic fraction } (\%) = 100$$
$$\times \frac{^2\text{H} - \text{acyl methyl enrichment}}{^2\text{H} - \text{body water enrichment}}$$

In the ^{2}H NMR spectrum, methyl signals from palmitoyl (C$_{16}$) and stearoyl (C$_{18}$) chains co-resonate (25); hence the NMR measurement reports the methyl enrichment from both C$_{16}$ and C$_{18}$ acyl moieties. A significant portion of stearoyl acyl units are derived by an independent chain elongation reaction of palmitoyl-CoA (26). However, as the chain is elongated at the carboxyl end of the acyl moiety, the methyl hydrogens of these elongated acyl moieties still represent the original lipogenic C$_{16}$ moiety. Therefore, the ^{2}H-enrichment level represents the total population of acyl units (C$_{16}$ plus C$_{18}$) derived from lipogenesis.

Data analysis

Data are presented as mean ± SEM. Statistical differences were determined using the paired or unpaired bilateral t test, where $P < 0.05$ was considered to be significant.

RESULTS

Study group characteristics

Table 1 shows the mean weight gain for the 20-day and 35-day study groups in addition to fasting blood glucose, plasma insulin, FFA and triglyceride concentrations. Animals on the HF diet initially showed a greater weight gain than the controls, but after 35 days of HF diet, these animals showed a smaller weight gain than animals on a SC diet for the same time period. HF diets have previously been shown to reduce appetite in rodents (27), and therefore we can speculate that the lower net weight gain in the HF-fed animals was probably due to diminished food intake. Fasting blood glucose, plasma insulin, FFA and triglyceride concentrations were not significantly modified in animals on the HF diet compared with those maintained on SC. However, plasma glucose disposal after a glucose challenge was less efficient in HF-fed animals than in their SC counterparts (Fig. 2). In post-absorptive SC-fed animals, blood glucose concentrations peaked at 15 min and returned to basal values (9.4 ± 0.8 mM)

Table 1. Biochemical and physical characteristics of the different groups of animals studied

	Study 1 (Day 20)		Study 2 (Day 35)	
	SC diet	HF diet	SC diet	HF diet
Number of rats	6	5	4	5
Weight gain (g)	110 ± 3	131 ± 3*	191 ± 7	138 ± 8*
Blood glucose (mM)	7.7 ± 0.2	8.4 ± 0.4	8.3 ± 0.6	9.7 ± 0.3
Insulin (ng/mL)	—	—	0.27 ± 0.07	0.34 ± 0.11
FFAs (mM)	9.23 ± 0.81	12.31 ± 1.19	—	—
Triglycerides (mg/dL)	76.4 ± 5.0	70.4 ± 7.3	—	—

Data are presented as mean ± SEM.
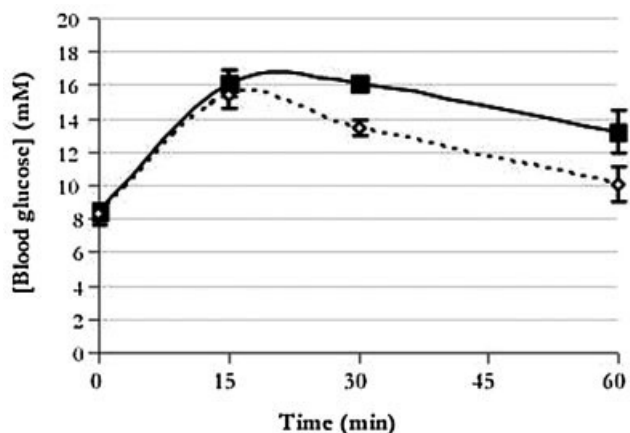*$P < 0.05$, relative to the control group for each study.
—, Not determined.

Figure 2. Glucose challenge profile over a 60 min period after the glucose load. Filled squares, animals fed a HF diet (n = 5); open diamonds, rats given a SC diet (n = 6).

within 60 min of a glucose challenge. In comparison, post-absorptive HF-fed rats had attained higher plasma glucose concentrations 15 min after the challenge. Subsequently, plasma glucose concentrations failed to return to basal concentrations by 60 min (60 min plasma glucose = 13.3 ± 1.3 mM; $P < 0.05$ compared with SC-fed animals).

HTG content

Single-voxel ^{1}H MRS was validated *in vivo* against the 'gold standard' histological detection of HTG content, providing good correlation parameters (17,19). The spectroscopic data reported here using a 7 T system allows the confident quantification of lipid, $(CH_2)_n$, and H_2O signals. Liver ^{1}H MR spectra obtained from an animal on day 1 and day 8 of the HF diet are shown in Fig. 3. The resonances from methylene protons of triglyceride acyl chains appear between 1.0 and 1.6 ppm and are shown in more detail in the inset. The composite acyl $(CH_2)_n$ signal was used to

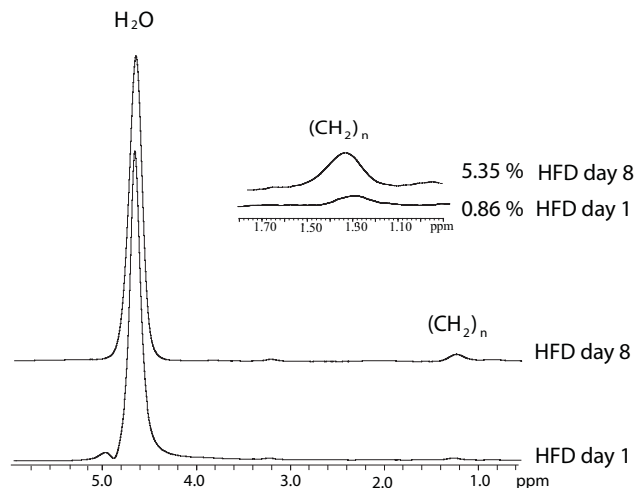**Figure 3.** Hepatic ^{1}H MR spectra of an animal maintained on a HF diet (HFD), at days 1 and 8. H_2O is the water signal, and $(CH_2)_n$ corresponds to the methylene protons of triglyceride acyl chains, which are shown expanded in the inset for the respective spectra.

calculate intracellular triglyceride content because of its higher signal intensity compared with the acyl methyl (CH_3) resonance.

Eight days of a HF diet promoted a significant increase in HTG concentrations, expressed as percentage of liver weight, compared with animals fed a SC diet ($3.85 \pm 0.60\%$ vs $2.13 \pm 0.34\%$, $P < 0.05$). Interestingly, when the animals were continued on the HF diet, HTG concentrations did not show any further increases over SC-fed rats ($3.98 \pm 0.86\%$ vs $1.55 \pm 0.18\%$, $P < 0.05$ on day 15; $3.30\% \pm 0.60$ vs $1.12 \pm 0.30\%$, $P < 0.05$ on day 35). In a group of rats that were fed for 7 days on a HF diet and then subsequently weaned on to a SC diet for 7 days, HTG concentrations increased approximately threefold after the change to the HF diet, attaining comparable concentrations ($3.33 \pm 0.51\%$) to those observed at the same stage for the other HF groups. Seven days after weaning on to the SC diet, HTG concentrations had returned to basal values ($0.76 \pm 0.06\%$) (Fig. 4).

Contribution of DNL to HTG concentrations

The enrichment of HTG methyl hydrogens from ^{2}H-enriched body water provides a measure of the contribution of DNL to the total HTG pool. The triglyceride methyl hydrogens are directly traceable to the original acetyl-CoA precursor, and they are also well resolved in the ^{2}H NMR spectrum of HTGs isolated by a simple Folch extraction, as illustrated in Fig. 5. The intensity of the methyl ^{2}H-NMR signal reflects both the ^{2}H enrichment at that site and the quantity of triglycerides in the NMR tube. Therefore, with constant triglyceride amounts, differences in ^{2}H-NMR signal intensities between two samples reflect different levels of ^{2}H enrichment. The spectra shown in Fig. 5 represent 175 μmol portions of HTGs extracted from an SC-fed and an HF-fed animal (A and B, respectively). The two samples had equivalent amounts of triglycerides, as shown by the equal triglyceride ^{1}H NMR signal intensities of their ^{1}H NMR spectra (Fig. 5). However, the ^{2}H-NMR signal intensities were ~10-fold higher in the SC-fed animal than in the HF-fed animal, indicating that the extent of ^{2}H incorporation into the triglyceride of the SC-fed animal was much greater than that of its HF-fed companion. From the ^{1}H and ^{2}H NMR data, triglyceride methyl ^{2}H enrichment levels were estimated as described in the Experimental section, and, by relating these enrichment values to that of body water, the fraction of HTG derived from lipogenesis was derived (Table 2). Body water enrichment was slightly but significantly increased in the HF-fed animals relative to the SC-fed animals, which may reflect a higher body lipid fraction in the former group. In animals given a SC diet, $10.9 \pm 1.0\%$ of total HTGs was derived from lipogenesis over a 48 h period, and the remaining $89.1 \pm 1.0\%$ was either obtained from dietary sources or was present before the administration of deuterated water. With the HF diet, the lipogenic fraction was reduced approximately 10-fold, accounting for only $1.0 \pm 0.2\%$ of total HTGs ($P < 0.01$ compared with the lipogenic contribution in SC-fed animals).

DISCUSSION

Effect of diet on HTG concentrations

When rats were placed on a HF diet for 5 weeks, HTG concentrations increased around threefold within the first week but then stabilized over the remaining 4 weeks of the study. Our results resemble previous studies of HTG concentrations in rats
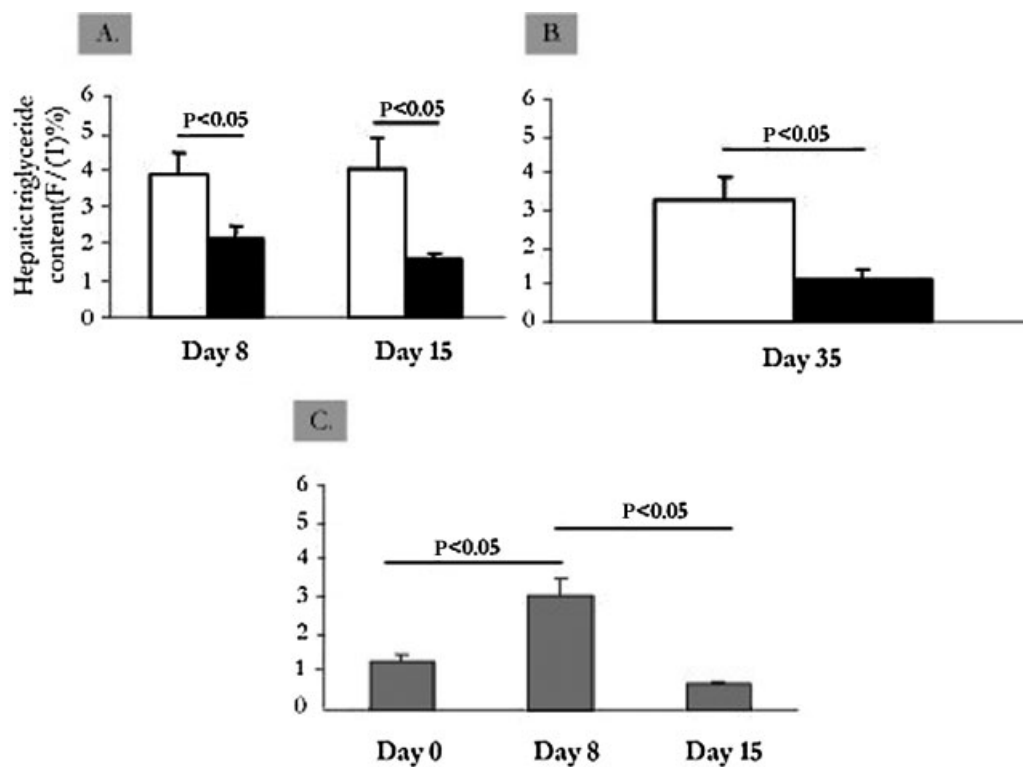
Figure 4. HTG concentrations presented as mean ± SEM. (A) Study 1: HTG content on day 8 and day 15 of rats maintained for 20 days on a HF diet (n = 5) and compared with a control group given a SC diet (n = 6). (B) Study 2: HTG concentrations of rats maintained for 35 days on a HF diet (n = 5) and compared with a control group given a SC diet (n = 4). (C) Study 3: HTG content of a group of animals maintained for 7 days on a HF diet and then switched back to a SC diet (n = 5). White columns, HF diet; black columns, SC diet; grey columns, HF diet followed by SC diet.
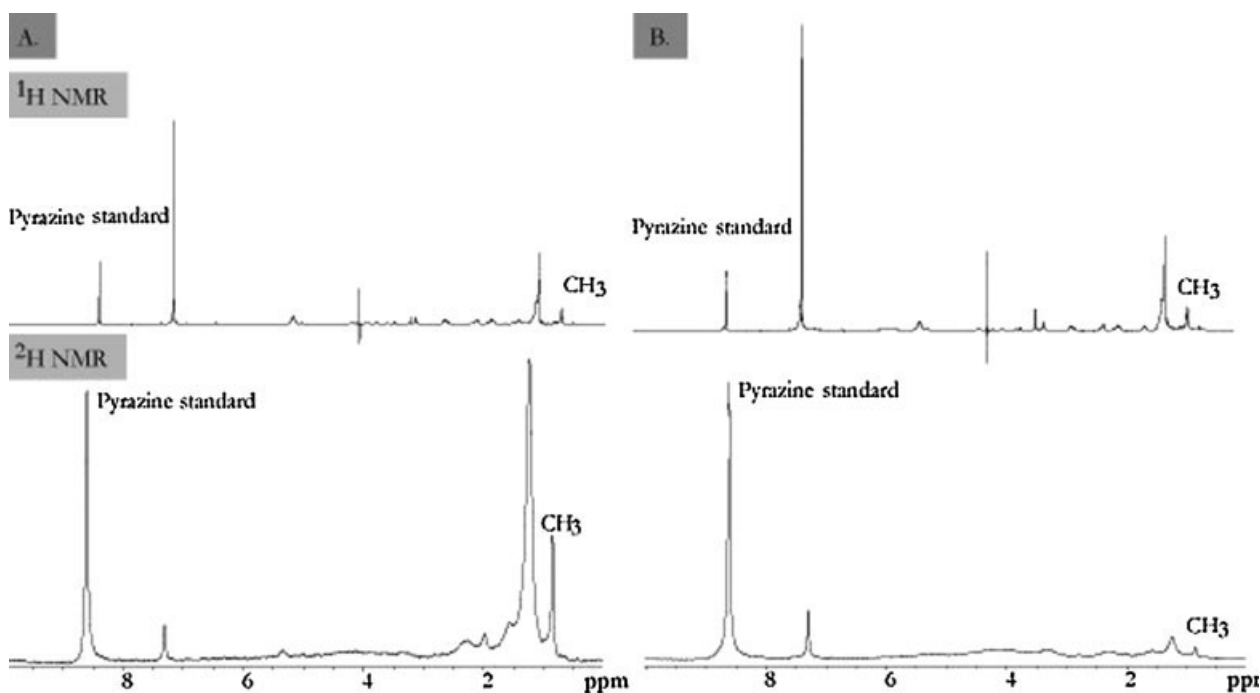
Figure 5. ^{1}H and ^{2}H NMR spectra of extracted HTGs from (A) rat on SC diet for 35 days and (B) rat fed on HF diet for 35 days. Pyrazine and triglyceride methyl signals are indicated.

Table 2. ^{2}H body water enrichments and 48 h DNL fractional synthetic rates for animals fed HF (n = 5) and SC (n = 4) diets for 35 days

	SC diet	HF diet
^{2}H body water enrichment (%)	1.59 ± 0.04	1.76 ± 0.03*
^{2}H triglyceride methyl enrichment (%)	1.78 ± 0.15	0.18 ± 0.03*
HTG lipogenic fraction (%)	10.9 ± 1.0	1.0 ± 0.2**
HTG non-lipogenic fraction (%)	89.1 ± 1.0	99.0 ± 0.2**

Data are presented as mean ± SEM.
*$P < 0.05$ and
**$P < 0.01$ compared with the SC diet.

on a HF diet, in which HTGs, measured by the 'gold standard' post-mortem enzymatic assay, increased by 200% in the first 2 weeks (9). In a separate study, a threefold increase in HTGs was found after 3 days of a HF diet (10), whereas others only showed a ~30% increase in HTGs after 4 weeks of a diet containing 40% of the total calories as lard (33). A threefold increase in HTGs is a relatively modest change compared with HTG concentrations in patients with non-alcoholic steatohepatitis or in rat models of diet-induced non-alcoholic steatohepatitis. Normal human HTG concentrations are similar to those of the rat (1–2%), whereas patients with non-alcoholic steatohepatitis, with or without T2DM, have HTG concentrations in the range 10–50%. In rats on a choline-deficient diet, HTGs accumulate to ~40% of liver mass (28).

Our studies showed that elevated HTG concentrations induced by HF feeding quickly reverted to basal values when the rats were weaned on to a SC diet, demonstrating the reversibility of this process and the high sensitivity of HTGs to diet modification. Our findings are analogous to recent observations in patients with T2DM, where raised HTG concentrations were restored to normal values in a relatively short time by dietary intervention (8). Alterations in rat HTG concentrations were not accompanied by changes in plasma triglycerides, suggesting that the liver may play a role as a systemic buffer in the face of dietary fat overload. Our observations also show that an increase in HTGs is among the earliest observable changes in whole-body lipid status when rats are challenged by HF feeding and demonstrate the utility of localized ^{1}H MRS for non-invasively monitoring this change. Although single-voxel ^{1}H MRS was validated *in vivo* against histological and enzymatic assays of HTG content (17,19), possible caveats include the presence of focal steatosis (32), in which HTG concentrations measured by localized MR might differ from the mean value measured by post-mortem enzymatic assay.

Sources of HTG

The fatty acid components of HTGs can be derived from outside the liver or can be synthesized within it by DNL. Although the contribution of DNL to systemic fatty acid synthesis has been determined in both rats and humans by various tracer methods, there are no reports on its contribution to HTG concentrations. Although DNL rates are quite low in relation to total body lipid

mass, they assume much higher significance in relation to the much smaller HTG pool size and could therefore play an important role in the regulation of HTG concentrations.

The association between DNL flux assessed by whole-body fatty acid synthesis rates, HF feeding, and HTG has been explored in both healthy rats and T2DM models. Placement of both healthy rats and the lean littermates of ZDF rats on a HF diet resulted in a reduction in DNL activity (29), whereas, in the obese ZDF rats, with increased HTG content (20), DNL rates were unchanged (30). As HTG concentrations reflect the balance between fatty acid import, DNL, and triglyceride export, the relationship between DNL fluxes and the contribution of DNL to HTG concentrations is not known. Our studies indicate that DNL contributions to HTG content are modest for healthy animals on a SC diet, but are essentially negligible when they are placed on a HF diet. Therefore, the increase in HTG concentrations during HF feeding is almost entirely driven by uptake and esterification of plasma FFA.

The effects of diet and pathophysiology on DNL in rats have strong parallels with observations of DNL in humans. In humans, the fractional contribution of DNL to hepatic lipid synthesis was non-invasively inferred from the analysis of triglyceride derived from the very-low-density fraction of plasma lipoprotein (31,32). In healthy humans, a HF diet for 25 days resulted in a sharply reduced DNL contribution to very-low-density lipoprotein triglyceride compared with subjects on a low-fat diet (32). These observations bear a strong resemblance to those from our rat studies and indicate that, for both healthy rats and humans, DNL is highly attenuated by short-term HF feeding. The effects of longer-term HF diets on DNL rates have not been characterized in humans. In subjects with non-alcoholic fatty liver disease, a common end point of excessive long-term dietary fat intake, DNL contributions were found to be chronically raised under both fed and fasting conditions (33). These observations suggest that the regulation of DNL by dietary substrates was not functional, and resemble the absence of dietary DNL regulation observed in obese ZDF rats. Overall, these studies indicate a strong similarity in DNL between rats and humans and support the use of rat models for investigating the effects of diet, endocrine status and interventions on DNL.

Deuterated water is a highly convenient tracer for quantifying DNL, as steady-state enrichment in plasma is achieved within minutes of an intraperitoneal injection bolus (unpublished data), which can then be maintained indefinitely by providing a maintenance level of ^{2}H$_2$O enrichment in drinking water. However, the measurement relies on some untested assumptions. We considered that pyruvate was the main source of lipogenic acetyl-CoA and that the exchange of pyruvate methyl protons with those of the water is essentially complete (23,24). Given this, the ^{2}H enrichment of body water is assumed to be equal to that of acetyl-CoA and hence can be used as the precursor enrichment. To the extent that the methyl hydrogens of acetyl-CoA are not fully exchanged with those of body water, the DNL contribution will be underestimated. With mass spectrometry measurements of ^{2}H-lipid enrichment, a correction factor, known as the N value, corresponding to the average number of deuterium atoms incorporated into palmitate, is applied to account for incomplete exchange (34). For *in vivo* rat studies, N was determined to be 22, corresponding to an exchange fraction of 22/31, or ~75% complete. Acetyl-CoA precursors, such as pyruvate, exchange extensively with bulk water, hence fatty acyl hydrogens derived directly from acetyl-CoA, including the

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terminal methyl hydrogens, may have a somewhat higher enrichment level (24,25). Assuming a 75% rather than a 100% extent of ^2H incorporation into acetyl-CoA, our DNL estimates would increase from 10.9% to 14.3% for the SC-fed rats and from 1.0% to 1.3% for the HF diet group. The other key assumption behind our estimates of lipogenic and non-lipogenic contributions to HTG content is that the HTG pool turned over completely during the 72 h exposure to ^2H_2O. To the extent that the HTG pool was incompletely turned over, our measurements give an underestimate of the DNL contribution. In healthy humans, 9.2 g triglycerides per day are exported as very-low-density lipopoteins (35). Assuming a liver mass of 1.5 kg and HTG concentrations of 1%, giving a HTG pool size of 15 g, the daily output represents ~60% of HTGs, hence the pool is completely replaced in less than 2 days. Given the faster basal metabolic rate of the rat, we assume that, under our study conditions, the HTG pool was completely turned over within the 72 h period of ^2H-body water enrichment. However, triglyceride turnover in steatotic livers may be longer, given the increased pool size. Hence, under these conditions, the period of body water ^2H enrichment may need to be extended.

Relationship between HTGs and glycemic status

In animal models, glycemic control and IR are highly associated with the accumulation of triglycerides in liver and skeletal muscle (13,14). These sites show a selective contribution to the pathogenesis of IR and hyperglycemia in that the accumulation of intramyocellular lipid in skeletal muscle impairs the action of insulin on the whole-body disposal of glucose, and increased HTG is associated with impairment of endogenous glucose production. During the transition to HF feeding, the HTG pool – because of its relatively small mass and high turnover – is likely to be modified faster and to a larger extent than intramyocellular lipid. Therefore, at this early stage, the reduced ability of insulin to suppress post-absorptive endogenous glucose production is likely to play an important role in the development of glucose intolerance. Studies of healthy animals after 3 days on a HF diet showed that, although insulin-stimulated glucose disposal and basal endogenous glucose production were not different from control animals maintained on a SC diet, under hyperinsulinemic clamp conditions there was impaired suppression of endogenous glucose production, indicative of hepatic IR (10). In our study, the extent of glucose intolerance induced by 35 days of HF feeding was relatively modest, as seen by comparable fasting blood glucose and insulin concentrations to those in animals maintained on the SC diet. A previous study reported enhanced IR and glucose intolerance after 4 weeks of a HF diet (36). Seven weeks of a HF diet in healthy rats was also associated with higher blood glucose 2 h after the oral glucose challenge (37). The slight loss of glucose tolerance that we observed in our studies may reflect the relatively small increment in HTG concentrations compared with that observed in T2DM rats with severe glucose intolerance (20). In comparison with the clamp measurement, a glucose tolerance test does not provide direct measurements of insulin action on glucose production and disposal fluxes and therefore may not resolve hepatic and peripheral IR. However, as with ^1H MRS analysis of HTG content, a glucose tolerance test can, in principle, be repeated in the same animal and may therefore be useful for relating longitudinal changes in HTGs to alterations in glycemic status for individual animals.

CONCLUSIONS

In healthy rats, HTG concentrations can be acutely raised or lowered by altering the dietary fat content, and these changes can be effectively monitored by ^1H MRS. During HF feeding, essentially all of the HTG is derived from dietary lipid with very little contribution from DNL. ^1H MRS is a non-invasive and accurate technique suitable for longitudinal studies of evaluation of HTG content in animal models and can be used to assess the effects of dietary interventions. Our novel ^2H NMR measurement of HTG ^2H enrichment from ^2H_2O, although requiring further validation in vivo, is a simple and practical approach for assessing the contribution of DNL to HTG concentrations.

Acknowledgements

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