Glucose Contribution to In Vivo Synthesis of Glyceride-Glycerol and Fatty Acids in Rats Adapted to a High-Protein, Carbohydrate-Free Diet

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Triacylglycerol (TAG) synthesis from all carbon sources and from glucose carbon was evaluated in rats fed a high-protein, carbohydrate-free (HP) diet or control diet by determining simultaneously in the same animal the rate of incorporation of ^3H_2O and of ^{14}C-glucose into the two TAG moieties in the carcass, liver, and retroperitoneal and epididymal adipose tissue. Incorporation rates of ^3H_2O into TAG-fatty acids (FAs) in the two adipose tissues and in liver were reduced in HP rats to about 20% and 50%, respectively, of the rates in control rats. In the two experimental groups, glucose was a poor precursor for FA synthesis, contributing only 22.8% of whole-body (carcass plus liver) total FA synthesis in control rats and even less (14%) in HP rats. In contrast to the reduction in FA synthesis, incorporation of ^3H_2O into TAG-glycerol in HP rats did not differ significantly or was even higher (in epididymal tissue) versus the control level. In all tissues of both HP and control rats, the rate of ^{14}C-glucose incorporation into TAG-glycerol was much higher than the rate of incorporation into FA. Glyceroneogenesis, estimated by subtracting TAG-glycerol synthesis from glucose from the rate obtained with ^3H_2O, was significantly increased in adipose tissue from HP rats, with almost all of the glycerol formed by this route being used to esterify preformed FAs. It is suggested that the increased adipose tissue glyceroneogenesis is important for esterification of diet-derived FA and preservation of body fat stores in rats adapted to the HP diet.

PREVIOUS STUDIES from this laboratory[1,2] have shown that in vivo lipogenesis, assessed by the rate of incorporation of tritium from ^3H_2O into tissue fatty acids (FAs), is markedly reduced in the carcass, liver, and adipose tissue from rats adapted to a high-protein, carbohydrate-free (HP) diet. The diet affects only the synthesis of FAs from triacylglycerols (TAGs), with no change observed in phospholipid-FA synthesis.[2] Despite the reduced lipogenic activity in both liver and adipose tissue, body fat stores of rats fed the HP diet are remarkably well maintained, with carcass FAs amounting to about 85% to 90% of values in control rats after 30 days on the diet.[1] The relatively small loss of body fat in HP rats could be due, among other factors, to a diet-induced reduction in overall body energy expenditure. In fact, we have shown[3] that the thermogenic capacity of brown adipose tissue, which has an important role in the regulation of energy balance in small rodents,[4] is reduced in rats adapted to the HP diet. Another factor that could contribute to body fat preservation would be a more efficient utilization of diet lipid for storage purposes, a process that requires an adequate supply of α-glycerophosphate. We have recently shown[5] that in the fed state, adipose tissue from rats fed the HP diet has a high glyceroneogenic capacity, as evidenced by increased levels of phosphoenolpyruvate carboxykinase activity and by enhanced rates of in vitro incorporation of ^{14}C from pyruvate into TAG when the tissue is incubated in the absence of glucose. However, in the presence of physiological concentrations of glucose, the contribution of pyruvate to adipose tissue glycerol synthesis was negligible in both HP and control rats, with almost all of the glyceride-glycerol deriving from the hexose.[5] This finding casts doubt on the importance of glyceroneogenesis in in vivo conditions, as HP-adapted rats have a high gluconeogenic activity and are able to maintain normal blood glucose levels.[6]

The purpose of the present study was to estimate in vivo in rats adapted to the HP diet the contribution of glucose carbon and of carbon from other sources to the synthesis of FA and glycerol moieties of TAGs in the carcass, liver, and adipose tissue.

MATERIALS AND METHODS

Experimental Approach

The glucose contribution to the synthesis of glycerol and FA moieties of TAG was evaluated by determining simultaneously in the same animal the rate of incorporation of ^3H from tritiated water, which estimates total synthesis (from all carbon sources), and of ^{14}C from glucose into the two TAG fractions in the carcass, liver, and adipose tissue. The assumptions and supportive arguments for the adequacy of ^3H_2O for measurements of lipid synthesis from all carbon sources have been presented by Windmueller and Spaeth[7] and Jungas.[8] The flux of glucose carbon to tissue and carcass FA or glycerol was estimated using the semicompartmental approach described by Baker and Huebotter,[9] which is a modification of the noncompartmental approach of Shipley et al[10] and combines features of both noncompartmental and compartmental analyses. The semicompartmental analysis requires measurement of the specific activity-time curve of the precursor after a single injection of a radioactive tracer (as in the method of Shipley et al[10]) and measurement of the radioactivity in an "end product" at any point in time (60 minutes in the present study). The technique's assumptions and supportive arguments are described by Baker and Huebotter.[9] It was assumed that no appreciable turnover of ^3H- or ^{14}C-labeled product occurred during the experimental period, so the rates obtained are minimal values.

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Submitted November 5, 1997; accepted April 23, 1998.

Supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (97/0974-0) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (510923/93-3).

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0026-0495/98/4710-0009$03.00/0

Animals and Treatment

Male Wistar rats weighing initially 60 to 90 g were housed in suspended wire-bottom cages with water ad libitum in a room kept at 25° ± 2°C with a 12-hour light:dark cycle. The animals were adapted for 20 days to a HP purified diet containing 70% casein, no carbohydrate, and 8% corn oil or to a balanced control diet containing 17% casein, 66% carbohydrate, and 8% corn oil. The two diets, which were approximately isocaloric and contained equal amounts of vitamins and minerals, have been described in detail.[3] As in previous studies with the same diet,[6] after an initial period of adaptation of a few days, food ingestion and the rate of body weight increase were similar for the two groups of rats. The animals weighed 180 to 200 g when used for the experiments, which were performed between 8:30 and 10:30 AM.

In Vivo Lipogenesis Measurements

U-^{14}C-glucose (10 μCi) and $^{3}H_2O$ (5 mCi) dissolved in 0.5 mL saline were injected into fed nonanesthetized rats through a Silastic catheter (Dow Corning, Midland, MI) inserted into the right jugular vein 2 days before the experiment. After flushing the catheter with saline and with the rat free in its cage, blood samples of 0.2 mL were taken 1, 5, 15, 30, and 60 minutes after label injection for determination of ^{14}C-glucose specific activity. Immediately after obtainment of the 60-minute sample, which was also used for determination of plasma water specific activity, the animals were killed by cervical dislocation. The liver, epididymal fat pads, and retroperitoneal adipose tissue depots laying over the psoas muscle were rapidly removed and weighed. After removal of the intestinal tract, the remaining carcass was weighed, autoclaved to soften the bones, and homogenized in 300 mL water.

Isolation of tissue TAG-FA and glycerol. Total lipids from liver and adipose tissue samples and from aliquots of carcass homogenate were extracted with 2:1 chloroform:methanol by the procedure of Folch et al.[11] $^{3}H_2O$ was removed from the inferior phase (predominantly chloroform) by washing three times with a superior-phase mixture.[11] After each shaking, the tubes were briefly centrifuged to sharpen the phase boundary, and the superior phase was aspirated and discarded. The chloroform phase was evaporated to dryness under N_2, and TAGs were hydrolyzed with ethanolic KOH for 1 hour at 70°C. After extraction of nonsaponifiable lipids and acidification with 6N H_2SO_4, ^{14}C-FA and ^{3}H-FA were extracted with petroleum ether, and the extract was evaporated to dryness in a scintillation vial and dissolved in toluene-2,5-diphenyloxazole (PP0). A volume of the aqueous hydrolysate containing ^{14}C-^{3}H-glycerol was dissolved in toluene-Triton-PPO-[1,4 bis(5-phenyl-2-oxozolyl)-benzene] (POPOP).

Determination of plasma glucose and body water specific radioactivity. Plasma ^{14}C-glucose was isolated by thin-layer chromatography, and its radioactivity was measured as described by Baker et al.[12] The plasma glucose concentration was determined with glucose oxidase in a Beckman (Fullerton, CA) glucose analyzer. Body water specific activity was determined directly on aliquots of diluted (20 times) plasma dissolved in toluene-Triton-PPO-POPOP.

Radioactivity measurements. The degree of quenching in each sample was obtained to enable calculation of radioactivity in dpm. Simultaneous liquid scintillation counting (LS 7600 Beckman spectrometer) of the ^{3}H and ^{14}C content of FAs or glycerol was performed using a channels ratio method.[13]

Calculations. Plasma glucose levels did not change significantly during the experimental period, a requirement of the technique used (Fig 1). The curves for plasma glucose specific activity were fitted to two-term exponential equations, the parameters of which were used in the calculations.[9] For the calculation of lipid synthesis in experiments with $^{3}H_2O$, it was assumed that the specific activity of intracellular water was identical to that of plasma water. Rates of tissue and carcass lipid synthesis were calculated assuming that each glycerol and each FA incorporated into TAG contained 3.3 and 13.3 atoms of tritium, respectively.[7,8]

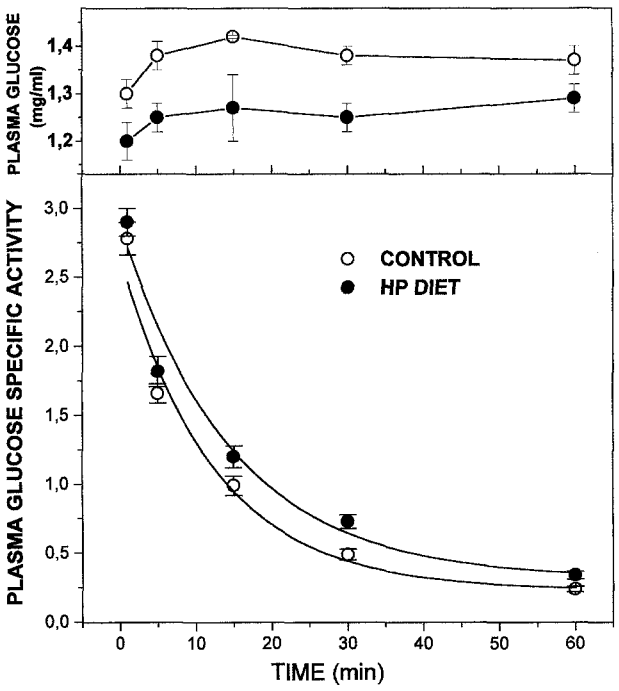

Fig 1. Plasma glucose specific activity (% of injected dose/mg) after intravenous injection of tracer ^{14}C-glucose into rats fed HP or control diet. Each point is the mean ± SEM of 12 to 15 determinations.

Statistical Methods

Differences between groups were analyzed using ANOVA with P less than .05 as the criterion of significance.

RESULTS

Rates of FA Synthesis

The data in Table 1 show a significant reduction in the incorporation rate (per unit tissue weight) of ^{3}H from tritiated water into TAG-FA in the carcass, liver, and epididymal and retroperitoneal adipose tissue of rats adapted to the HP diet. The reduction was more marked in the two adipose tissues, in which rates of FA synthesis were four to six times lower in rats fed the HP diet versus control rats. In the liver, rates in HP rats were reduced to about 50% of control values. The data in Table 1 also show that in the two experimental groups, the rates of incorporation of ^{14}C-glucose into TAG-FA were a small fraction of the rates obtained with $^{3}H_2O$, which estimates synthesis from all carbon sources. Adaptation to the HP diet induced a reduction in FA synthesis from glucose in all tissues examined. From the data in Table 1, it was possible to estimate the contribution of glucose to total FA synthesis in the liver, adipose tissue, and whole body (carcass plus liver) in the two experimental groups. It was assumed that (1) adipose tissue constitutes 9% and 10.6% of the whole carcass weight of HP and control rats, respectively,[1] and (2) the average of the values for the rate of FA synthesis obtained in epididymal and retroperitoneal tissues was representative of synthesis in all body fat. In control animals,

Table 1. In Vivo Incorporation (nmol · g^{-1} · min^{-1}) of ^3H_2O and of U-^{14}C-Glucose Into Glyceride-FA From Carcass, Liver, and Retroperitoneal and Epididymal Adipose Tissue of Rats Adapted to a HP or Control Diet

	Incorporation Into Glyceride-FA			
	^3H_2O		U-^{14}C-Glucose	
Site	Control	HP	Control	HP
Carcass†	9.68 ± 0.52 (n = 12)	4.83 ± 0.49 (n = 14)*	2.47 ± 0.43 (n = 14)	0.83 ± 0.09 (n = 15)*
Liver	24.37 ± 2.34 (n = 10)	13.54 ± 1.31 (n = 14)*	0.74 ± 0.07 (n = 10)	0.20 ± 0.02 (n = 12)*
Retroperitoneal	41.58 ± 8.69 (n = 7)	7.22 ± 0.89 (n = 10)*	3.67 ± 0.91 (n = 6)	0.50 ± 0.08 (n = 8)*
Epididymal	36.90 ± 9.23 (n = 8)	8.10 ± 1.80 (n = 8)*	4.25 ± 0.81 (n = 6)	0.69 ± 0.11 (n = 5)*

NOTE. Data are the mean ± SEM, with the number of observations in parentheses.
*$P < .01$ *v* control.
†Including label incorporated into glyceride-FA of retroperitoneal and epididymal fat depots.

the estimated contribution of glucose to FA synthesis was relatively small (liver, 2.9% ± 0.6%; adipose tissue, 10.1% ± 0.3%; whole body, 22.8% ± 1.0%) and was even smaller in rats adapted to the HP diet (1.5% ± 0.2%, 7.8% ± 1.2%, and 14.0% ± 0.8%, respectively).

Glyceride-Glycerol Synthesis

The data in Table 2 show that in contrast to the reduction of FA synthesis, the rate of incorporation (per unit tissue weight) of ^{3}H from tritiated water into TAG-glycerol in rats fed the HP diet either did not differ significantly from that in controls (in carcass, liver, and retroperitoneal tissue) or were even higher (by about 50% in epididymal adipose tissue).

In both experimental groups, the rate of incorporation of ^{14}C from glucose into TAG-glycerol (Table 2) was always much higher than the rate of hexose carbon incorporation into TAG-FA (Table 1) in all tissues examined. Adaptation to the HP diet did not significantly affect the rate of in vivo incorporation of ^{14}C from glucose into TAG-glycerol in the two adipose tissues, but induced a reduction in TAG-glycerol synthesis in the carcass and especially the liver, where the rates were less than half of those in control rats (Table 2).

Rates of TAG-glycerol synthesis from nonglucose sources (via dicarboxylic acid shuttle: glyceroneogenesis) can be estimated by subtracting TAG-glycerol synthesis from glucose from the rate obtained with ^3H_2O, which estimates synthesis from all carbon sources. The results of these calculations indicate that glyceroneogenic activity in the liver was not affected by the diet, but was significantly higher in both retroperitoneal and epididymal tissues of HP rats (Fig 2).

DISCUSSION

The finding in the present study that FA synthesis from glucose represents only a small fraction of total FA synthesis (estimated with ^3H_2O), even in animals fed a balanced carbohydrate-rich diet, is in agreement with previous studies in vivo showing low rates of incorporation of ^{14}C from glucose into the carcass, liver, and/or adipose tissue FAs of normally fed rats[14,15] or mice.[9,16] Also, although glucose has a stimulating effect on in vitro lipogenesis from several precursors,[17-19] it has been shown in experiments with hepatocytes,[20] adipose tissue,[21] and adipocytes[22] that hexose is a relatively poor substrate for FA synthesis. The finding that the contribution of glucose to total FA synthesis was even lower in rats adapted to the HP diet is consistent with the low insulin levels in these animals.[23]

On the other hand, in all tissues of both control and HP rats, the rate of incorporation of ^{14}C from glucose into glyceride-glycerol was always much higher than that into FAs. Evidence that glucose does not constitute a preferential substrate for FA formation but is a major contributor to glyceride-glycerol synthesis has also been obtained with adipose tissue in vitro[21] and in experiments in which substrates were directly infused into periuterine adipose tissue in situ.[24] Of special interest is the finding that glyceroneogenesis, estimated by subtracting TAG-glycerol synthesis from glucose from the rate obtained with ^3H_2O, was increased in rats adapted to the HP diet (Fig 2). In a previous study,[5] we showed that adaptation to the HP diet induces a twofold increase in the activity of adipose tissue phosphoenolpyruvate carboxykinase and an increase in the tissue capacity to convert ^{14}C-pyruvate into glyceride-glycerol

Table 2. In Vivo Incorporation (nmol · g^{-1} · min^{-1}) of ^3H_2O and of U-^{14}-Glucose Into Glyceride-Glycerol From Carcass, Liver, and Epididymal and Retroperitoneal Adipose Tissue of Rats Adapted to a HP or Control Diet

	Incorporation Into Glyceride-Glycerol			
	^3H_2O		U-^{14}C-Glucose	
Site	Control	HP	Control	HP
Carcass†	31.18 ± 2.97 (n = 9)	23.05 ± 2.42 (n = 10)	12.17 ± 1.26 (n = 8)	8.14 ± 0.91 (n = 7)*
Liver	136.64 ± 10.41 (n = 8)	123.94 ± 13.50 (n = 6)	36.09 ± 3.06 (n = 6)	15.16 ± 2.47 (n = 16)†
Retroperitoneal	36.04 ± 4.22 (n = 7)	42.63 ± 2.09 (n = 6)	15.70 ± 2.90 (n = 12)	12.31 ± 2.25 (n = 9)
Epididymal	33.99 ± 3.48 (n = 10)	47.24 ± 3.98 (n = 11)*	13.62 ± 1.70 (n = 11)	10.05 ± 2.00 (n = 9)

NOTE. Data are the mean ± SEM, with the number of observations in parentheses.
*$P < .05$ *v* control.
†$P < .01$ *v* control.
‡Including label incorporated into glyceride-glycerol of retroperitoneal and epididymal fat depots.

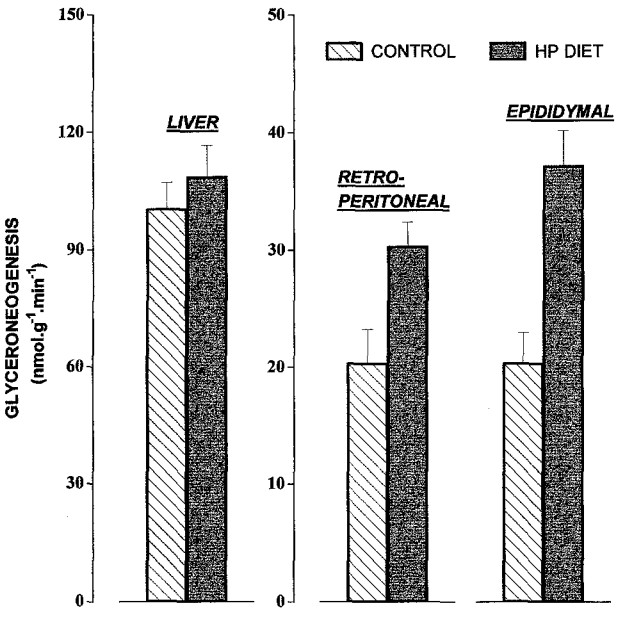

Fig 2. Glyceroneogenesis in liver and epididymal and retroperitoneal adipose tissue of rats fed a HP or control diet. The rate of de novo glyceride-glycerol synthesis was calculated from the data in Table 2 by subtracting the rate obtained with ^{14}C-glucose from that obtained with $^{3}H_2O$.

in vitro. Although these results were indicative of a HP diet–induced increase in adipose tissue glyceroneogenesis, it was found in these experiments[5] that in the presence of a physiological concentration (5 mmol/L) of glucose, the contribution of pyruvate to glyceride-glycerol synthesis was insignificant, with no difference observed between HP and control rats. As previously discussed,[5] this in vitro effect of glucose raised doubts about the importance of glyceroneogenesis in in vivo conditions, as rats fed the HP diet have a markedly increased gluconeogenic activity and normal levels of blood glucose.[6] In fact, the same doubts might be raised in relation to the increased glyceroneogenesis previously observed in fasted rats under a balanced regimen,[25,26] whose adipose tissue has high levels of phosphoenolpyruvate carboxykinase activity with high rates of incorporation of ^{14}C-pyruvate into glyceride-glycerol in vitro, which are also markedly inhibited in the presence of glucose.[26] The data of the present study constitute the first in vivo evidence of the physiological adaptative role played by adipose tissue glyceroneogenesis in situations of reduced carbohydrate availability in the diet. A possible explanation for the apparent contradiction of the in vivo and in vitro (in the presence of glucose) findings could be a reduced hexose utilization by the adipose tissue of HP rats induced by the metabolic and hormonal environment prevailing in vivo, not reflected in vitro.

It can also be inferred from the data in Tables 1 and 2 that most of the glyceride-glycerol synthesized by adipose tissue of both HP and control rats is used for esterification of preformed FAs, which include, in addition to FAs recycled after hydrolysis of stored TAG, FAs taken up by the tissue from the circulation, mainly in the fed state, and FAs produced by breakdown of chylomicron-TAG. Indeed, the portion of glycerol used for esterification of preformed FA can be obtained by discounting the glycerol used to esterify FA synthesized de novo, which corresponds to one third of the TAG-FA synthesized from $^{3}H_2O$ (Table 1), if it is assumed that 3 mol FA is esterified by 1 mol glycerol. Thus, it can be estimated [(^{3}H-glycerol − ⅓ ^{3}H-FA) × 100/^{3}H-glycerol] that about 63% of total glycerol synthesized in both the retroperitoneal and epididymal adipose tissue of rats fed the balanced diet was used to esterify preformed FA. In the same tissues of rats adapted to the HP diet, in which FA synthesis is markedly reduced, almost all (94%) of the glycerol formed was used for preformed FA esterification.

Using the same type of calculation for glyceride-glycerol formed via glyceroneogenesis (subtracting ^{14}C-glycerol from ^{3}H-glycerol in the formula), it is possible to estimate from the data in Tables 1 and 2 that even if glyceroneogenesis provided all of the glycerol needed to esterify FA synthesized de novo, with no contribution of glycerol formed from glucose, about 92% of the glycerol derived from glyceroneogenesis in both the retroperitoneal and epididymal adipose tissue of rats adapted to the HP diet would be used for esterification of preformed FA. Therefore, there can be little doubt that the supply of α-glycerophosphate to esterify these FAs is the primary reason for the increased glyceroneogenic activity in HP rats. It is not possible with the present data to estimate the relative contribution of stored TAG hydrolysis and of tissue FA uptake from the circulation to preformed FA generation. It has been found that the adipose tissue of fasted rats maintains a high rate of reesterification of endogenously produced FAs, which may be even higher than the rate in the fed state,[27] and it has been proposed[28] that the TAG-FA cycle may play an important physiological role in controlling the sensitivity of lipolysis and/or esterification to hormones or regulators. On this basis, it seems reasonable to suppose that high reesterification rates are also maintained in adipose tissue from rats adapted to the HP diet, thus permitting a rapid adaptative response of the tissue to changing conditions. However, this process probably accounts for utilization of only part of the greatly increased production of glyceride-glycerol. It would thus appear that a sizable fraction of glycerol formed via glyceroneogenesis in adipose tissue of HP rats is used to esterify FA taken up from the circulation, especially FA produced by hydrolysis of chylomicron-TAG.

We have previously hypothesized[3] that in addition to an increased metabolic efficiency consequent to a reduced thermogenic activity of brown adipose tissue, a more efficient utilization of diet-derived FAs could contribute to preserve body fat stores in rats adapted to the HP diet, despite the reduced lipogenic activity. The results of the present study seem to support this hypothesis.

ACKNOWLEDGMENT

The technical assistance of M.A.R. Garófalo, N.M.Z. Resano, and J.R. Oliveira is gratefully acknowledged.

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