Contribution of glycerol and alanine to basal hepatic glucose production in the genetically obese (*fa/fa*) rat

Jacques TERRETTAZ* and Bernard JEANRENAUD

Laboratoires de Recherches Métaboliques, Faculty and Department of Medicine, 64 Avenue de la Roseraie, 1211 Genève 4, Switzerland

Increased hepatic glucose production has been reported to occur in the insulin-resistant genetically obese *fa/fa* rats. The possible existence of an increased basal gluconeogenesis in obese rats was investigated, upon comparing the metabolic fate of glycerol and alanine in liver of fed anaesthetized lean and genetically obese (*fa/fa*) rats. Glycerol turnover rate in obese animals was 3 times that of the lean. This increase in glycerol turnover rate was associated with an increase in blood glycerol levels in obese animals. The contribution of glycerol to glucose production was significantly increased in obese animals. In contrast, the contribution of alanine to the hepatic glucose production was similar in lean and obese animals. A higher incorporation of glucose, glycerol and alanine into hepatic lipids was observed in obese animals than in controls. It is concluded that in fed genetically obese (*fa/fa*) rats the high blood glycerol concentration is a major driving force for the increased basal hepatic conversion of this substrate into glucose.

INTRODUCTION

In the basal state, the liver of the genetically obese (*fa/fa*) rat is characterized by a resistance to the effects of insulin on the glucose production process (i.e. significant amount of glucose produced in the presence of very high basal insulin levels) (Terrettaz *et al.*, 1986a), and by a high production of very-low-density lipoprotein, derived from triacylglycerols, either exogenous or synthesized *de novo* (Schonfeld & Pfeiffer, 1971). This higher lipid and glucose production implicates a higher supply of glucogenic and lipogenic substrates to the liver of these obese animals.

The aim of this study was therefore to measure the contribution of two different gluconeogenic precursors, glycerol and alanine, to the basal hepatic glucose production, and to estimate the distribution of these substrates between the glycogenic and lipogenic pathways in fed anaesthetized lean and genetically obese (*fa/fa*) rats.

MATERIALS AND METHODS

For the studies, 10–12-week-old homozygote male lean (FA/FA) (270 ± 6 g) and genetically obese (*fa/fa*) rats (324 ± 8 g) bred in these laboratories were used. The animals were fed *ad libitum* with standard laboratory chow (by wt.: 51 % carbohydrate, 21 % protein, 2.5 % fat) (Lacta 299; Provimi-Lacta S.A., Cossonay, Switzerland) and were studied between 10:00 and 14:00 h, at 3–4 h after food removal. In these conditions, it has been shown that gut-derived glucose is negligible (Leturque *et al.*, 1981). Rats were anaesthetized (Pentobarbital, 50 mg/kg intraperitoneally) and, once a tracheotomy was performed, two catheters were inserted, one in the right jugular vein for the tracer substrate infusion, one in the left carotid artery for blood sampling. Body temperature was maintained between 36.5 and 37.5 °C by using a heating blanket with a rectal probe.

Two different groups of lean and obese rats were studied. In the first group, a bolus of [U-¹⁴C]glycerol (3.2 µCi) and D-[6-³H]glucose (5.8 µCi; New England Nuclear, Boston, MA, U.S.A.) was administered in 2 min and was immediately followed by a continuous constant infusion of the same tracers ([U-¹⁴C]glycerol at 0.1 µCi/min and D-[6-³H]glucose at 0.18 µCi/

min) during 2 h. Blood was sampled every 15 min to measure the specific radioactivity of [³H]- and [¹⁴C]-glucose and of [¹⁴C]glycerol. In the second group, the same protocol was used, except that [U-¹⁴C]glycerol was replaced by L-[U-¹⁴C]alanine (New England Nuclear). At the end of the experiments, after 2 h of tracer infusion, the main lobe of the liver was cut and quickly frozen in liquid N₂.

Analytical methods

Blood (100 µl) was collected in heparinized tubes and immediately deproteinized in 500 µl of ZnSO₄ (0.15 M) and 500 µl of Ba(OH)₂ (0.15 M). Then 400 µl of the supernatant was passed through three stacked ion-exchange resin columns (Poly-Prep columns; Bio-Rad Laboratories A.G., Glattburg, Switzerland) to allow for the separation of the various labelled compounds. The top column contained a cation-exchange resin (AG 50W-X8; 100–200 mesh, H⁺ form; Bio-Rad) and was eluted with 4 ml of 2 M-NH₃ to recover amino acids. This fraction was further treated with glutaminase and then passed through an AG 1-X8 (100–200 mesh, acetate form) column to recover alanine, as described by Golden *et al.* (1981). The second column, containing an anion-exchange resin (AG 1-X8; 100–200 mesh, Cl⁻ form; Bio-Rad), was eluted with 5 ml of 0.1 M-HCl to recover lactate. The third column, containing another anion-exchange resin (AG 1-X8; 100–200 mesh, borate form; Bio-Rad), was first eluted with 5 ml of 20 mM-sodium tetraborate to recover glycerol and then with 4 ml of 0.5 M-acetic acid to recover glucose. Each eluted fraction was evaporated, redissolved in 300 µl of water, and a 200 µl sample was counted for radioactivity in a liquid-scintillation counter (RackBeta, LKB Wallac) by using a dual-label program with correction for quenching and spill-over. With each batch of columns, labelled standards of glucose, glycerol and alanine were processed to measure the recovery of the separation, which averaged 75 % for glycerol, 63 % for alanine and 97 % for glucose. Glucose concentration was measured with a glucose oxidase kit (Boehringer, Mannheim, Germany). Glycerol and alanine were measured by fluorimetry (Bergmeyer, 1974). The incorporation of ³H and ¹⁴C into liver glycogen was measured as described previously (Postle & Bloxham, 1980). Total hepatic lipids were extracted (Folch *et al.*,

* To whom correspondence should be addressed.

1957), saponified in ethanolic KOH, and fatty acids were then extracted with light petroleum (b.p. 30–45 °C), dried, and counted for radioactivity. Glycogen synthase and phosphorylase activities were assayed as described previously (Hue *et al.*, 1975; Stalmans & Hers, 1975). Hepatic glycerol 3-phosphate was determined in a neutralized trichloroacetic acid extract (Bergmeyer, 1974) by fluorimetry. Insulin was measured by radioimmunoassay, as described previously (Terrettaz *et al.*, 1986a).

Calculations

Calculations were made with the valuations obtained during the last 30 min of the study, when steady-state levels of tracers were achieved. Glucose turnover rate was calculated by dividing the rate of [^3H]glucose infusion by the blood [^3H]glucose specific radioactivity. Glycerol and alanine turnover rates were calculated by dividing the rate of [^{14}C]-glycerol or -alanine infusion by the [^{14}C]-glycerol or -alanine blood specific radioactivity. An index of the rate of conversion of glycerol into glucose (R_{gly}) was calculated as follows:

$$R_{\text{gly}} = \frac{[^{14}\text{C}]\text{glucose SA} \times R_{\text{g}}}{[^{14}\text{C}]\text{glycerol SA}}$$

and for conversion of alanine into glucose (R_{Ala}):

$$R_{\text{Ala}} = \frac{[^{14}\text{C}]\text{glucose SA} \times R_{\text{g}}}{[^{14}\text{C}]\text{alanine SA}}$$

where R_{g} = glucose turnover rate and SA = arterial specific radioactivity.

These calculations provide an estimation of the actual rate of conversion of precursors into glucose, since the arterial specific radioactivity of the precursors is used instead of the mixed portal–arterial one.

The index of incorporation of the labelled substrates into glycogen and lipids was calculated by dividing the amount of ^3H or ^{14}C incorporated by the blood specific radio-activity of the labelled substrate.

Statistical analysis

Comparison between experimental groups was made by Student's *t* test for unpaired data.

RESULTS

The time courses of the arterial specific radioactivity of [^3H]glucose, [^{14}C]glycerol, [^{14}C]alanine and [^{14}C]glucose measured during this study in lean and obese animals are shown in Figs. 1 and 2. These specific radioactivities were normalized to an infusion rate of tracer of 100 000 d.p.m./min. The blood concentrations, the turnover rates of glucose, glycerol and alanine and their rate of conversion into glucose, measured in the lean and obese animals, are shown in Table 1. Glucose turnover rate was slightly but significantly increased in obese animals compared with lean controls (Table 1). This slight increase in glucose turnover in obese animals was associated with an increased blood glucose concentration (Table 1). Glycerol turnover rate was much increased in obese animals, and this increase was associated with a higher blood glycerol concentration (Table 1). Blood alanine concentration and alanine turnover were not different in lean and obese animals (Table 1).

The basal rate of conversion of glycerol into glucose was markedly increased in obese animals as compared with lean ones (Table 1), and corresponded to $73.6 \pm 7.8\%$ of the total rate of glycerol utilization for the obese and to $89.9 \pm 7.1\%$ for the lean animals. In contrast, no difference was observed in the basal rate of conversion of alanine into glucose between lean and obese

Fig. 1. Blood specific radioactivity of [^3H]- and [^{14}C]-glucose in lean and genetically obese *fa/fa* rats

Blood specific activities of (a) [^3H]glucose and of (b) [^{14}C]glucose were measured every 15 min as described in the Materials and methods section, in lean (○) and genetically obese (*fa/fa*) rats (●) given a constant infusion of [^3H]glucose and [^{14}C]glycerol. Results are the means $\pm$ S.E.M. for 7 lean and 9 obese animals.

animals (Table 1). In lean rats $19.2 \pm 1.1\%$ and in obese rats $11.5 \pm 1.0\%$ of the total amount of alanine utilized was converted into glucose.

The incorporation of [^3H]glucose and [^{14}C]glycerol into liver glycogen was not different between lean and obese animals (Table 2), but less [^{14}C]alanine was incorporated into glycogen in liver of obese animals. Hepatic glycogen levels were similar in lean and obese rats (17.2 ± 1.7 mg/g in lean and 20.5 ± 1.1 mg/g in obese), as well as the activities of glycogen synthase *a* (25.3 ± 3.7 m-units/g in lean and 42.1 ± 14.5 m-units/g in obese) and of glycogen phosphorylase *a* (7.6 ± 0.8 units/g in lean and 8.5 ± 1.2 units/g in obese). Hepatic glycerol 3-phosphate levels were significantly ($P < 0.05$) higher in the obese animals (184 ± 24 nmol/g of liver) than in controls (126 ± 13 nmol/g of liver).

The incorporation of [^3H]glucose, [^{14}C]glycerol and [^{14}C]alanine into fatty acyl moieties of triacylglycerols was markedly increased in liver of obese animals compared with lean

Fig. 2. Blood specific radioactivity of (a) [^{14}C]glycerol and (b) [^{14}C]alanine in lean and genetically obese *fa/fa* rats

Blood specific radioactivities of (a) [^{14}C]glycerol and of (b) [^{14}C]alanine were measured every 15 min as described in the Materials and methods section, in lean (○) and genetically obese (*fa/fa*) rats (●) given (a) a constant infusion of [^3H]glucose and [^{14}C]glycerol and (b) a constant infusion of [^3H]glucose and [^{14}C]alanine. Results are means $\pm$ S.E.M. for (a) 7 lean and 9 obese animals, and (b) 6 lean and 5 obese animals.

animals (Table 2), suggesting that the flux of both the glycolytic and the lipogenic pathways was increased in the liver of obese animals. The incorporation of label into the glycerol moiety of triacylglycerols from both glucose and glycerol was markedly increased in liver of obese animals (Table 2). In contrast, no

Table 2. Incorporation of [^3H]glucose, [^{14}C]glycerol and [^{14}C]alanine into glycogen and lipids in liver of lean and genetically obese (*fa/fa*) rats

Lean and obese rats were infused with [^3H]glucose and [^{14}C]glycerol or [^{14}C]alanine; see the Materials and methods section. Values are means $\pm$ S.E.M. of 7–9 experiments: NS, not significantly different.

		Incorporation into		
		Glycogen ($\mu\text{mol/g}$ of liver)	Triacylglycerols	
			Fatty acids (nmol/g of liver)	Glycerol ($\mu\text{mol/g}$ of liver)
[^3H]Glucose	Lean	2.7 ± 0.8	30 ± 4	0.2 ± 0.01
	Obese	2.2 ± 0.8 NS	370 ± 80 $P < 0.001$	1.2 ± 0.2 $P < 0.005$
[^{14}C]Glycerol	Lean	0.23 ± 0.08	6.8 ± 2.2	0.1 ± 0.02
	Obese	0.5 ± 0.12 NS	428 ± 126 $P < 0.005$	0.8 ± 0.14 $P < 0.001$
[^{14}C]Alanine	Lean	2.2 ± 0.6	30 ± 2	0.27 ± 0.04
	Obese	0.5 ± 0.1 $P < 0.02$	2360 ± 470 $P < 0.001$	0.32 ± 0.03 NS

change was observed in the incorporation of alanine into the glycerol of triacylglycerols between lean and obese animals. Plasma insulin levels were much higher in obese animals (13.2 ± 1.4 ng/ml) than in the lean ones (2.3 ± 0.5 ng/ml).

DISCUSSION

In genetically obese *fa/fa* rats, several defects of the hepatic glucose production process have been reported. When tested under high insulin levels (during euglycaemic-hyperinsulinaemic clamps), hepatic glucose production was much less responsive to insulin in obese than in lean animals (Terrettaz *et al.*, 1986a). After oral glucose ingestion, obese animals were characterized by a lack of suppression, and even by a stimulation, of the hepatic production of glucose (Rohner-Jeanrenaud *et al.*, 1986). In the basal state, a slight increase in hepatic glucose production has usually been measured (Rohner-Jeanrenaud *et al.*, 1986; Terrettaz *et al.*, 1986a). Since the hepatic glycogen content in obese animals has been reported to be equal in the fed state and higher in the fasted state compared with control rats (Hue *et al.*, 1983), a higher supply of glucogenic precursors other than glycogen may possibly contribute to the increased hepatic glucose production in genetically obese *fa/fa* rats. It was therefore decided to investigate the fate of two gluconeogenic precursors,

Table 1. Blood levels and turnover rates of glucose, glycerol and alanine measured in lean and genetically obese (*fa/fa*) rats

Lean and obese rats were infused with [^{14}C]glycerol or [^{14}C]alanine; see the Materials and methods section. Values are means $\pm$ S.E.M. of 7–11 experiments: ND, not determined; NS, not significantly different.

Groups	Blood glucose (mmol/l)	Glucose turnover ($\mu\text{mol/min}$)	Blood glycerol ($\mu\text{mol/l}$)	Glycerol turnover ($\mu\text{mol/min}$)	Glycerol conversion into glucose ($\mu\text{mol/min}$)	Blood alanine ($\mu\text{mol/l}$)	Alanine turnover ($\mu\text{mol/min}$)	Alanine conversion into glucose ($\mu\text{mol/min}$)
Lean + [^{14}C]glycerol	4.9 ± 0.4	12.9 ± 0.6	43.4 ± 5.9	0.9 ± 0.1	0.8 ± 0.1	ND	ND	ND
Obese + [^{14}C]glycerol	5.8 ± 0.3 ($P < 0.05$)	15.8 ± 1.3 ($P < 0.05$)	107.9 ± 5.6 ($P < 0.001$)	2.8 ± 0.4 ($P < 0.005$)	2.0 ± 0.4 ($P < 0.005$)	ND	ND	ND
Lean + [^{14}C]alanine	4.3 ± 0.2	12.8 ± 0.5	ND	ND	ND	128 ± 20	7.3 ± 0.6	1.4 ± 0.2
Obese + [^{14}C]alanine	5.1 ± 0.3 ($P < 0.05$)	17.7 ± 1.1 ($P < 0.001$)	ND	ND	ND	153 ± 25 NS	8.7 ± 0.6 NS	1.0 ± 0.1 NS

glycerol and alanine, in liver of lean and genetically obese *fa/fa* rats.

A first observation in this study was that the availability of some substrates to the liver was different in lean and obese animals. Thus a large increase in blood glycerol levels was observed in obese rats, which may be explained by an increased lipolysis in the enlarged adipose cells of the obese animals (Gruen & Greenwood, 1981). In contrast, similar alanine blood levels were measured in both groups of animals.

The difference in the rate of glycerol utilization observed between lean and obese animals was correlated with the differences in blood glycerol concentrations, indicating that the metabolic clearance of glycerol was not changed in obese rats compared with lean ones. The elevated hepatic glycerol utilization observed in obese animals led to an increase in the rate of conversion of glycerol into glucose, as well as an increase in the index of incorporation of glycerol into glycogen. Since glycerol is entering the gluconeogenesis at a relatively late step of the pathway, a very large proportion of this substrate was directly converted into glucose (89% in lean and 74% in obese animals). This suggests, as previously observed in perfused rat liver (Brunengraber *et al.*, 1973), that liver does not glycolyse at high rates in the basal state, especially in lean animals. Although the amount of glycerol converted into glucose represents only a small proportion of the total hepatic glucose production (3.3% in lean and 6.4% in obese animals), the higher conversion of glycerol into glucose observed in obese animals is quantitatively of importance, since it corresponds, over a 24 h period, to an excess of about 20 mg of glucose/g of liver. This excess of glucose produced from glycerol in obese animals may therefore completely explain the hepatic glycogen retention observed in obese animals (Hue *et al.*, 1983).

When alanine was studied, a different pattern of results was observed in lean and obese animals. First, both blood alanine concentration and alanine utilization rates were similar in the two groups of rats. Secondly, the absolute rate of conversion of alanine into glucose was not different in liver of lean and obese animals, and the index of incorporation of alanine into liver glycogen was diminished in obese rats. Since glucose turnover was higher in obese animals, the contribution of alanine to the total glucose production was significantly lower in obese animals when expressed as a percentage of the total glucose produced (2.9% in obese versus 5% in lean animals). This is in contrast with a previous study (Triscari *et al.*, 1979), which showed an increased incorporation of [¹⁴C]alanine into hepatic glycogen in obese rats compared with controls. In this study, a bolus of both labelled and unlabelled alanine was administered, and therefore the blood concentration and/or the blood specific radioactivity of alanine (which were apparently not measured) may have been different between lean and obese animals.

In this study the animals were in the post-absorptive state, and therefore under these conditions glycogenolysis is a major contributor to the hepatic glucose production. A minimal estimation of the contribution of gluconeogenesis to glucose production in lean and obese animals can be calculated by adding the contributions of both glycerol and alanine, which is equal to 8.3% in lean and 9.3% in obese of total glucose production. This indicates that the contribution of gluconeogenesis (as estimated by the added contributions of glycerol and alanine) to total glucose production is similar in lean and obese animals. Since the hepatic glucose production is significantly higher in obese animals, it indicates that the absolute rate of gluconeogenesis is increased in the obese group. Since the hepatic glycogen concentration and the activities of both glycogen synthase *a* and phosphorylase *a* were similar in lean and in obese animals, it may be hypothesized that the contribution of

glycogenolysis to hepatic glucose production is similar in lean and obese rats. In the fed obese rats, the large increase observed in the incorporation of alanine into fatty acyl moieties of liver triacylglycerols indicates that the hepatic lipogenic pathway *de novo* is stimulated. The fact that the incorporation of both glucose and glycerol into lipids was also increased in the obese animals further suggests that both the hepatic glycolytic pathway and the '*de novo*' lipogenic one are independently stimulated in the genetically obese rat. This is in keeping with a previous study showing that the levels of fructose 2,6-bisphosphate were higher in liver of obese rats, that the activities of both phosphofructokinase 1 and pyruvate kinase were increased in the liver of the obese animals (Hue *et al.*, 1983) and that hepatic lipogenesis was augmented in the obese group (Godbole & York, 1978). This suggests that, in the liver of obese animals, the glycolytic and lipogenic pathways are not insulin-resistant, and are stimulated by the basal hyperinsulinaemia present in obese animals.

Previous studies have shown, *in vitro*, an inhibitory action of glycerol 3-phosphate on the activity of both hepatic 6-phosphofructo-1-kinase and 6-phosphofructo-2-kinase (Claus *et al.*, 1981; Stewart *et al.*, 1985). In this study, in obese animals, as well as in normal rats during euglycaemic-hyperinsulinaemic clamp (Terretaz *et al.*, 1986b), we measured an increase in hepatic glycerol 3-phosphate levels together with an increase in the rate of glycolysis. This may indicate that this inhibitory action of glycerol 3-phosphate on 6-phosphofructo-1-kinase and 6-phosphofructo-2-kinase is not occurring *in vivo*, or that the stimulation of 6-phosphofructo-2-kinase by insulin (Assimacopoulos-Jeannet & Jeanrenaud, 1990) over-rides this inhibitory effect.

In summary, the elevated glycerol concentration observed in the blood of genetically obese (*fa/fa*) rats leads to an increased total glycerol utilization. The resulting higher rate of conversion of glycerol into glucose observed in the obese rats contributes to an increased absolute rate of hepatic gluconeogenesis in the obese group. The much higher incorporation of glucose, glycerol and alanine measured in liver lipids of obese, compared with that of lean animals, suggests that the flux of these substrates through the hepatic glycolytic and lipogenic pathways is increased in the obese group, and suggests that these pathways are not resistant to insulin in these obese animals.

In fed genetically obese (*fa/fa*) rats and in the basal state, the high blood glycerol concentration is therefore a major driving force for the increased hepatic conversion of this substrate into glucose. Since an activation of the glycolytic pathway and/or an inhibition of the gluconeogenesis are involved in the suppression of hepatic glucose production by insulin in normal rats (Terretaz *et al.*, 1986a), it remains to be determined to what extent the increased glycerol turnover and/or a lack of inhibition of gluconeogenesis contributes to the resistance to insulin of the inhibition of the hepatic glucose production in genetically obese (*fa/fa*) rats.

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