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THE INTERACTIVE EFFECT OF GLYCEROL AND STARCH OR SUCROSE ON RAT TISSUE LIPIDS

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ABSTRACT

This study was undertaken to determine the interactive effect of glycerol and either the polysaccharide, starch or the disaccharide, sucrose on rat tissue lipids and on tissue lipogenesis from labelled glycerol. Male Holtzman rats (71g) were fed fat-free diets consisting of casein, minerals, vitamins and carbohydrates (starch or sucrose with 30% glycerol as well as control carbohydrates without glycerol). The liver weights expressed as % body weights were significantly higher in the glycerol groups than in controls. Even after 28 weeks, the rats fed the starch, fat-free diet had normal levels of liver lipids. The liver total lipids and cholesterol were significantly increased (63 -170%) in the glycerol groups with the greatest values being in the 30% glycerol - 45% sucrose group. The data on serum lipids were less dramatic (18 -52% change) and indicated a trend towards increased values due to the glycerol treatment. [U-¹⁴C]-glycerol incorporation into liver fatty acids was increased (ca. 90%) in the glycerol groups with the difference being significant between the starch-glycerol and starch groups. Long term feeding of glycerol diets did not result in enhanced metabolism of glycerol by epididymal fat tissue. These data have demonstrated important differences between the long term metabolic effects of a starch diet and that supplemented with glycerol.

INTRODUCTION

There is increasing recognition that complex carbohydrates rather than simple sugars should contribute to a substantial part of the total energy intake (1). In previous animal experiments with glycerol (2-5), glucose was invariably used as the additional source of carbohydrate and resulted in fatty livers when glycerol was part of a fat-free diet. Because of the beneficial interactive effect of polyunsaturated fat upon glycerol reported earlier (4), the use of a glycerol diet along with starch, which is known to contain bound unsaturated lipids, was not previously considered as appropriate for a fat-free diet. However, it was considered necessary to establish whether the data obtained with the glycerol-glucose diets were caused by differences in digestion, absorption or metabolism of the monosaccharide, glucose compared with the polysaccharide, starch or the disaccharide, sucrose. This study was, therefore, undertaken to determine (a) the long term effect of the starch, fat-free diet on rat liver and serum lipids; and (b) the

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interactive effect of glycerol with either starch or sucrose on rat liver and serum lipids. Additionally, in an attempt to understand the long term metabolic effects of these carbohydrates, the rates of incorporation of labelled glycerol into lipids of either isolated epididymal fat pads or liver slices were investigated.

MATERIALS AND METHODS

Seventy-two male Holtzman rats (71g) were divided into four groups, A, B, C, and D. The purified diet (2), which was fed to group A consisted of a 1:1 mixture of corn and wheat starches 75%; casein 17%; vitamins and choline chloride in glucose 2.2% with glucose providing 2.0%; and minerals 5.5%. Rats in group B were fed a 30% glycerol diet where the glycerol was added at the expense of the starch mixture. Rats in group C received a diet similar to that for group A except that a sucrose-glucose mixture (1:1.5) was substituted in place of the starch mixture. Rats in group D were fed a 30% glycerol, 45% sucrose diet similar to the diet for group B except for the use of sucrose instead of the starch mixture. With the objective of minimizing the number of rats in animal experimentation, separate control groups with either sucrose or glucose without glycerol was not included because they have been previously reported from this laboratory (5).

The procedures used for chemical analysis of tissues have been described earlier (4). For radioactive studies, 100-200 mg of the epididymal fat tissue or liver slices were incubated in Krebs Ringer bicarbonate buffer containing 10 mM glucose, 10 mM glycerol and insulin (0.3 I.U.) at 37C for four hours along with 1 microcurie of [U-¹⁴C] glycerol. The tissues were thoroughly washed, extracted, fractionated, and counted in a Searle Mark III liquid scintillation counter. The ¹⁴CO₂ expired in 4 hrs was absorbed in hyamine cups and also counted.

RESULTS AND DISCUSSION

At the end of 8 weeks, there were no significant differences between the four groups in body weight gain (Table I) or in feed efficiency which averaged 61 mg gain/kcal/day. The liver weights as % body weight were significantly greater in glycerol groups B and D compared with controls A and C. After 28 weeks on the diets, the body weight gains were lower in the sucrose groups C and D than in the starch groups A and B (Table I), but since the rats in those groups ate less, their feed efficiencies were not significantly decreased. There was no effect of glycerol on body weight gains or on feed efficiency which averaged 24 mg gain/kcal/day for the four groups after 28 weeks.

As early as the eighth week of feeding, dietary glycerol dramatically elevated liver total lipids and cholesterol (Table II). Since the liver phospholipids are unaffected by dietary manipulations and usually average (25 mg/g liver), it can be concluded that liver triglycerides were substantially increased by glycerol treatment. This is the first time it has been shown that a

TABLE I. EFFECT OF FAT-FREE CARBOHYDRATE DIETS ON RAT BODY AND LIVER WEIGHTS

Group No. Diet	Weight gain (g)		Feed efficiency mg gain/kcal/Day		% Liver Weight	
	Week 8	Week 28	Week 8	Week 28	Week 8	Week 28
A						
75% Starch	254 ± 27 a	431 ± 37 a	58.4 ± 7.0 a	26.3 ± 3.7 a	3.32 ± 0.26 a	2.85 ± 0.22 a
B						
30% Glycerol 45% Starch	255 ± 27 a	415 ± 65 a	59.0 ± 8.5 a	25.8 ± 5.8 a	3.96 ± 0.10 b	3.56 ± 0.38 b
C						
30% Sucrose 45% Glucose	269 ± 18 a	359 ± 34 b	67.9 ± 6.1 a	22.6 ± 2.0 a	3.73 ± 0.19 b	3.68 ± 0.26 b
D						
30% Glycerol 45% Sucrose	237 ± 32 a	333 ± 38 b	58.6 ± 8.0 a	20.9 ± 2.9 a	4.83 ± 0.41 c	4.49 ± 0.34 c

Values are mean ± of 6 rats. Means not sharing a common letter in a column are significantly different (analysis of variance, P < 0.05). Where required, data were normalized by log transformation.

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TABLE II. EFFECT OF FAT-FREE CARBOHYDRATE DIETS ON RAT LIVER LIPIDS

Group No. Diet	Total lipid (mg/g liver)			Cholesterol (mg/g liver)		
	Week 8	Week 18	Week 28	Week 8	Week 18	Week 28
A 75% Starch	44.2 ± 12 a	32.6 ± 2.4 a	35.2 ± 6.9 a	2.19 ± 0.29 a	1.54 ± 0.14 a	1.79 ± 0.3 a
B 30% Glycerol 45% Starch	75.8 ± 7.5 b	87.6 ± 35 b	88.8 ± 19 b	4.26 ± 0.92 b	4.33 ± 2.5 b	4.0 ± 1.0 b
C 30% Sucrose 45% Glucose	72.6 ± 27 b	68 ± 15 b	74.9 ± 22 b	2.83 ± 0.8 a	3.47 ± 0.75 b	3.78 ± 0.71 b
D 30% Glycerol 45% Sucrose	119 ± 33 c	135 ± 21 c	121 ± 29 c	5.27 ± 1.2 b	7.9 ± 0.84 c	8.52 ± 2.7 c

See footnote to Table I.

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fat-free diet containing glycerol and starch caused fatty livers in rats. Additionally, it was seen that the fat-free diet containing a mixture of sucrose and glucose was equally effective in 8 weeks as the 30% glycerol, 45% glucose used earlier (5) in causing liver fat deposition. Thus, at week 8, the sucrose-glucose, fat-free diet was almost as bad as the glycerol-starch fat-free diet. The additive effect of both these dietary undesirables (sucrose and glycerol) is seen in Table II, group D. The extent of fat deposition seen in the liver of group D was certainly the worst seen at the early time period of 8 weeks. In comparison with the previous data with the sucrose diet (ref 5, Table II), the present liver lipid values at 8 weeks are almost twice as large.

After 18 weeks, the liver lipids were further increased in the glycerol groups compared with controls (Table II). The liver cholesterol was considerably elevated in the glycerol-sucrose group. Data obtained at 28 weeks on liver lipids was essentially the same as that for the 18 week and further confirmed the deleterious effect of glycerol in a fat-free or an extremely low fat diet.

At 8 weeks, the serum total lipids were significantly higher in groups B and D compared with groups A and C, respectively (Table III). The serum cholesterol values also tended to be higher in the glycerol groups B and D compared with controls. The elevation in serum total lipids in groups B and D occurs with a concurrent increase in liver lipids at this time period. Feeding the diets for 28 weeks, the serum cholesterol was significantly higher in group B compared with group A (Table III). Between groups D and C, no significant differences in serum lipids were seen. However, these values in both these groups at week 28 were considerably lower than in groups A or B, suggesting that trace amounts of polyunsaturates in starch were providing the necessary additional "lipotropic" factor lacking in groups C and D.

To account for the fat deposition in the livers of rats fed fat-free diets containing glycerol, it is worthwhile to consider the possibility that dietary glycerol may stimulate fatty acid synthesis to the extent that fat accumulation becomes inevitable. Furthermore, the lipid transport out of the liver by lipoproteins is coordinatively aggravated by essential fatty acid deficiency. With the isolated fat tissue at week 18, neither the catabolism nor the anabolism of glycerol was influenced by dietary glycerol as judged by the rate of [U-¹⁴C] glycerol incorporation into CO₂ or into lipids (Table IV). Although the fat tissue is a major, if not the main, site of fatty acid synthesis in the rat, this tissue is generally deficient in glycerokinase. The low rate of ¹⁴C incorporation into CO₂ and glyceride-glycerol by fat tissue from all groups supports this conclusion. More important, these data demonstrate that glycerokinase activity is not enhanced in the fat tissue as a result of prolonged feeding of dietary glycerol.

With liver tissue (Table IV), the catabolism as well as the anabolism was substantially higher (ca. 10 fold) in all groups compared with the fat tissue. The rate of ¹⁴C-glycerol incorporation into

TABLE III. EFFECT OF FAT-FREE CARBOHYDRATE DIETS ON SERUM LIPIDS

Group No. Diet	Total lipid (mg/100 ml serum)		Cholesterol (mg/100 ml serum)	
	Week 8	Week 28	Week 8	Week 28
A 75% Starch	243 ± 50 a	336 ± 46 a	52.6 ± 8.4 a	68 ± 9.5 a
B 30% Glycerol 45% Starch	332 ± 53 b	439 ± 110 a	60.8 ± 4.7 a	87.4 ± 18 b
C 30% Sucrose 45% Glucose	233 ± 27 a	221 ± 31 b	52.1 ± 7.5 a	47.1 ± 6.6 c
D 30% Glycerol 45% Sucrose	354 ± 41 c	260 ± 71 b	75.1 ± 20 b	57.8 ± 15 ac

See footnote to Table I.

TABLE IV. EFFECT OF FAT FREE CARBOHYDRATE DIETS ON GLYCEROL METABOLISM BY RAT TISSUE
Incorporation (nmoles/g tissue/hr of [U - ¹⁴C] Glycerol into

Group No. Diet	¹⁴ CO ₂		Fatty Acids		Glyceride-glycerol	
	Fat tissue	Liver	Fat tissue	Liver	Fat tissue	Liver
A 75% Starch	462 ± 50 a	6010 ± 1200 ab	190 ± 28 a	1600 ± 610 a	325 ± 31 a	2870 ± 400 a
B 30% Glycerol 45% Starch	528 ± 120 a	6670 ± 1100 b	169 ± 52 a	3130 ± 480 b	314 ± 32 a	4470 ± 1000 b
C 30% Sucrose 45% Glucose	649 ± 92 b	4570 ± 1100 c	224 ± 73 a	668 ± 240 c	423 ± 110 b	2980 ± 200 a
D 30% Glycerol 45% Sucrose	545 ± 38 a	4680 ± 1400 ac	211 ± 41 a	1270 ± 640 ac	292 ± 38 a	3150 ± 730 a

Rats on diets for 18 weeks. See also footnote to Table I.

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CO₂ was not influenced by the glycerol treatment but the sucrose groups apparently catabolized glycerol at a slower rate (26%) than the starch groups. With the starch-glycerol group compared with starch group, the rates of incorporation of ¹⁴C into fatty acids, total lipids (not shown) and glyceride-glycerol were greatly increased and correlated with the increase in liver lipids in these groups. The metabolism of glycerol in the liver was obviously stimulated by dietary glycerol possibly through induction of glycerokinase (6,7). With the sucrose-glycerol group D, the rates of ¹⁴C incorporation into fatty acids and total lipids (not shown) were higher than those in group C, but the differences were not significant. The lack of increased incorporation into glyceride-glycerol in group D suggests that there is no additional induction of glycerokinase by dietary glycerol in the presence of fructose derived from dietary sucrose. Additionally, the dilution by the existing large pool of alpha-glycerophosphate arising from fructose metabolism may result in diminished rate of incorporation and, therefore not reflect the true rate of synthesis.

Both glycerol and fructose are more rapidly metabolized by the liver than is glucose to alpha-glycerophosphate (5, 6, 7). This intermediate plays a key role in the synthesis of triacylglycerol and phospholipids. The data obtained here with [U-¹⁴C] glycerol incorporation suggests, but does not prove, that fatty acid synthesis is enhanced by feeding glycerol along with the starch diet. Increased pool size of alpha-glycerophosphate arising from fructose may have contributed to a lack of statistical significance in incorporation data between groups D and C. The lower rates of incorporation into fatty acids in these two groups compared with groups B and A is in agreement with this suggestion.

With respect to the fat-free starch diet, the original study of Burr and Burr (8) has shown that this diet does not produce the classical symptoms of essential fatty acid (EFA) deficiency. Trace amounts, presumably less than 0.1%, of linoleic acid are bound to starch and are sufficient to prevent the rat from developing EFA deficiency. This observation has been confirmed in the present investigation over a long time period (28 weeks). Furthermore, other parameters, namely liver total lipids and cholesterol were shown to be normal. Trace amounts of linoleic acid may also be sufficient to prevent fat deposition in rats fed a glucose, fat-free diet. Both glycerol and sucrose apparently require a higher level of linoleic acid to prevent liver fat accumulation. Highly purified diets containing linoleic acid levels ranging from 0.02 to 0.5% may help to provide a better comparison of the various carbohydrates with respect to their specific minimum requirement of linoleic acid to prevent essential fatty acid deficiency.

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