

The Different Effects on the Serum Lipids and Fecal Steroids of High Carbohydrate Diets Given Orally or Intravenously

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ABSTRACT The hypothesis that diets high in carbohydrate produce hyperlipidemia in man was tested in new experiments which provided all calories either by the intravenous route or orally. After a base-line general diet, eight healthy men were fed fat-free diets consisting of 80% of the calories from glucose and 20% from an amino acid hydrolysate. The calories were adequate to maintain body weight. The solutions (1 cal/ml) were infused by constant drip over a 24 h period through either a superior vena cava catheter or a nasogastric tube. Each feeding was for 12 days in sequence but assigned in random order.

The high CHO diet given orally, as expected, increased the mean base-line serum triglyceride level from 176 ± 29 (SE) to 274 ± 47 . The identical diet given intravenously (i.v.) failed to produce hypertriglyceridemia; triglyceride levels were not significantly changed, 154 ± 37 , nor were blood glucose levels. Serum insulin levels were higher during the intravenous feeding.

In contrast, both i.v. and oral feedings greatly lowered mean serum cholesterol concentration from the base-line value of 220 ± 13 mg/100 ml to 135 ± 11 and 151 ± 13 , respectively. However, the serum cholesterol level was significantly lower ($P < 0.01$) with the intravenous feeding than with the oral feeding.

In addition, the fecal excretion of both neutral sterols and bile acids diminished greatly during the period of intravenous feeding. The fecal mass was likewise decreased. The bacterial conversion of cholesterol to coprostanol did not occur with either intravenous or oral feeding, but with both regimens secondary bile acids predominated, as usual, in the bile acid fraction of the stool.

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These results emphasize the key role of the intestinal mucosa in the etiology of carbohydrate-induced hypertriglyceridemia and as a direct or indirect contributor to plasma triglyceride and cholesterol levels in the absence of dietary lipids. When the gut mucosa was bypassed, carbohydrate-induced hypertriglyceridemia did not occur and both serum triglyceride and serum cholesterol levels decreased greatly at a time when the excretion of steroids in the stool was also reduced.

INTRODUCTION

The ingestion of a high carbohydrate diet produces acutely increased serum triglyceride levels in humans (1, 2). This response to increased dietary carbohydrate occurs invariably in normal and frequently in hypertriglyceridemic subjects (3, 4). In typical experiments, the sudden increase in dietary carbohydrate from 45 to 70–80% of the total calories (at the expense of dietary fat) usually doubles or triples serum triglyceride concentrations. The mechanism whereby this effect occurs has been postulated to be decreased removal of triglyceride by the peripheral tissues or increased triglyceride synthesis (5–8). Although the intestinal mucosa synthesizes both very low density lipoproteins (9, 10) and cholesterol (11, 12), most evidence has pointed to the liver as the site of the increased triglyceride synthesis resulting from a high carbohydrate diet (3, 7, 13).

Recently, we observed that patients receiving their total caloric requirements by the intravenous feeding of a solution containing 80% of calories as glucose failed to have the expected elevation of serum triglycerides. This observation stimulated us to design experiments to compare the effects of high carbohydrate feeding on serum lipids and the sterol balance when identical high carbohydrate formulas were given intravenously and orally to the same subjects in sequential fashion.

TABLE I
Description of Subjects

Sub- jects	Age	Weight	Height	Fasting blood sugar	Serum cholesterol	Serum tri- glycerides
				mg/100 ml	mg/100 ml	mg/100 ml
1	40	222	6 ft 1 in	83	166	165
2	42	155	5 ft 9 in	80	214	187
3	40	169*	5 ft 11 in	74	215	121
4	33	227*	6 ft 2 in	83	210	187
5	36	130	5 ft 7 in	75	201	51
6	38	156	5 ft 8 in	93	294	275
7	42	173*	5 ft 8 in	76	253	304
8	32	165*	5 ft 11 in	92	209	124

* Period of nasogastric feeding preceded intravenous period.

METHODS

Eight healthy male volunteers from the Iowa State Penitentiary were admitted to the Clinical Research Center (Table I).¹ All men had normal fasting and 2 h post-prandial blood sugars. None were grossly obese. Base-line serum lipid analyses showed six men to have normal fasting serum cholesterol (<210 mg/100 ml) and triglyceride (<190 mg/100 ml) levels. Two men (subjects 6 and 7) were hyperlipidemic (cholesterol 294 and 253 mg/100 ml; triglycerides 275 and 304 mg/100 ml, respectively). Their lipoprotein electrophoretic strips indicated typical type IV (pre-beta) patterns.

A diet was selected which could be fed during both the intravenous and oral periods of the study (Table II). Of the total calories, 20% were derived from a casein amino acid hydrolysate (Amigen, Baxter Laboratories, Inc., Morton Grove, Ill.) and 80% from glucose. Appropriate electrolytes and vitamins were added to meet the daily nutritional requirements. Sterile solutions were prepared daily at a caloric equivalent of 1 cal/ml. The total nitrogen content was 5.2 g/liter and the amino acid nitrogen was 3.12 g/liter. To minimize the effect of weight change on serum lipids, a careful dietary history was taken on admission to the Clinical Research Center and caloric needs were estimated from this information and from the height and weight. The men were ambulatory throughout the study and carried out usual ward recreational activities.

The experimental design of the study is shown in Table III. During the initial 7 day period, all subjects were fed a eucaloric (calories adequate to maintain body weight) general diet. During the next two 12 day sequential periods each subject was fed the eucaloric, fat- and cholesterol-free diet by either the nasogastric or intravenous routes. The solutions (1 cal/ml) were given as constant rate 24-h infusions. Alternate pairs of subjects received their initial feeding either by the intravenous route or oral routes. During the final 7 day period a general diet was again fed. Two of the subjects were studied for a third 12 day period during which the experimental diet was fed by nasogastric catheter as four equal feedings instead of by continuous 24 h drip.

¹ The proposed study had been fully explained to each man and informed, written consent was obtained. The protocol of the study had been approved by the College of Medicine Research Committee in charge of all prison volunteer studies and by the Human Use Committee. The study was in accord with the principles of the Declaration of Helsinki.

TABLE II
Composition of the Nasogastric/Intravenous Diet (3,000 Cal)

20% Casein hydrolysate (% of calories)		
80% Dextrose		
Electrolytes		
Sodium	90	meq
Potassium	45	meq
Calcium	37	meq
Chloride	59	meq
Magnesium	12.6	meq
Phosphate	74	meq
Vitamins		
Thiamine hydrochloride	25	mg
Riboflavin	10	mg
Sodium pantothenate	20	mg
Niacinamide	100	mg
Pyridoxine hydrochloride	20	mg
Ascorbic acid	500	mg
Phytonadione	10	mg
Calorie content: 1 cal/ml		

The diet was given intravenously through a 17 gauge plastic catheter which had been threaded through the subclavian vein into the superior vena cava under fluoroscopic observation. All infusing solutions were passed through a 44 µm filter placed immediately proximal to the subclavian catheter. Every 3rd day all dressings were removed and skin around the catheter was defatted with acetone. The catheter tip was cultured at the termination of the study.

Each day at 8:00 a.m., the subjects were weighed and blood was drawn for cholesterol, triglyceride, lipoprotein electrophoresis, glucose, and serum insulin determinations. During the base line periods (initial and final) and the study periods in which the experimental diet was fed as meals, the subjects were fasted from 10:00 p.m. to 8:00 a.m. before blood specimens were obtained. All stools were collected and stored in plastic containers at -20°C until analyzed.

The cholesterol content of serum was measured by the methods of Abell, Levy, Brodie, and Kendall (14). Serum triglycerides were determined fluorometrically using the Technicon Auto Analyzer (Technicon Instruments Corp., Tarrytown, N. Y.) following the method of Kessler and Lederer (15). Insulin levels in serum were quantified by the radioimmuno assay method of Hales and Randle (16). Bile acid and neutral steroid excretion in feces were determined using previously described methods (17-19). All data

TABLE III
The Experimental Design of the Study
Sequence of Dietary Periods

General diet	Eucaloric, fat and cholesterol-free, high carbohydrate diet		General diet
Base line	Period I (nasogastric)*	Period II (intravenous)*	Base line
7 days	12 days	12 days	7 days

* Order randomly assigned.

TABLE IV
The Effect of the High Carbohydrate Diet and the Route of Administration upon the Serum Triglyceride Levels*

Subjects	Dietary period and route of feeding			
	Initial base line	High carbohydrate diet		Final base line
		Nasogastric	Intravenous	
	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml
1	165	210	144	178
2	187	271	151	165
3‡	121	268	102	132
4‡	187	324	101	160
5	51	56	50	47
6	275	468	383	290
7‡	304	422	212	280
8‡	124	173	95	130
Mean ±SE	176 ±29	274 ±47	154 ±37	172 ±28
Mean changes		<div> <div> <div>— +98§ —</div> <div>— -120 —</div> </div> <div> <div>— -22¶ —</div> <div>— -4¶ —</div> </div> </div>		

* Mean of last three determinations each period.

‡ Period of nasogastric feeding preceded intravenous period.

§ $P < 0.01$ (Student's t test).

|| $P < 0.005$.

¶ Not significantly different.

were expressed as the mean \pm standard error. Statistical comparisons of paired data were made by means of Student's t test.

RESULTS

The mean serum triglyceride level increased significantly from the initial base-line value of 176 mg/100 ml to 247 mg/100 ml when the high carbohydrate diet was fed by constant nasogastric drip (Table IV). With the administration of the high carbohydrate diet intravenously, the serum triglyceride concentration was 154 mg/100 ml,

vastly different (-120 mg/100 ml, $P < 0.005$) from that of the nasogastric feeding.

To determine the possible effect of the order in which the two different routes of administration were given, four subjects were fed the experimental diet intravenously first, followed by the nasogastric feeding (subjects 1, 2, 5, 6). The order of entry into the study was exactly opposite in the remaining four subjects; that is, the nasogastric feeding preceded the intravenous feeding. One subject from each of these subgroups was hyperlipemic (subjects 6 and 7 with base-line serum triglycerides of 275 and 304 mg/100 ml, respectively). After the nasogastric feeding first, the serum triglyceride level increased from the mean base-line value of 184 mg/100 ml in all four subjects to 297 mg/100 ml and decreased in all subjects to 128 mg/100 ml after the succeeding intravenous feeding (Table V). The four subjects who received the diet by the intravenous route during the first period of the study, followed by a period of constant drip feeding through a nasogastric tube, had an increase of serum triglycerides from a mean of 182 mg/100 ml to 251 mg/100 ml (Table V).

Any changes in serum triglyceride concentration which followed the various diets began at 24 h and reached its maximum at 72 h where it plateaued until the end of the dietary period. With the nasogastric feeding, the serum triglyceride levels plateaued by 72 h and continued to be elevated for the remainder of the period. In contrast, during the intravenous feeding, the serum triglyceride levels remained essentially the same as during the base-line diet or else fell drastically (Table IV) from 274 mg/100 ml to 154 mg/100 ml in the switch from the nasogastric to the intravenous feeding.

TABLE V
The Effect of the Order in the Feeding Periods upon the Serum Lipid Concentrations and Body Weight

	Nasogastric feeding period first (subjects 3, 4, 7, 8)			
	Initial base line*	Nasogastric	Intravenous	Final base line
Triglyceride, mg/100 ml	184 ±42.8	297 ±52	128 ±28	176 ±36
Cholesterol, mg/100 ml	222 ±11	164 ±14	144 ±14	208 ±8.3
Weight, lb	184 ±15	184 ±14	183 ±14	184 ±14
	Intravenous feeding period first (subjects 1, 2, 5, 6)			
	Initial base line	Intravenous	Nasogastric	Final base line
Triglyceride, mg/100 ml	169 ±46	182 ±71	251 ±85	170 ±50
Cholesterol, mg/100 ml	218 ±27	127 ±19.7	140 ±21	202 ±23.2
Weight, lb	166 ±20	166 ±19	165 ±20	168 ±21

* Data expressed as mean values \pm SE.

TABLE VI
The Effect of the High Carbohydrate Diet and the Route of Administration upon the Serum Cholesterol Levels*

Subjects	Dietary period and route of feeding			
	Initial base line	High carbohydrate diet		Final base line
		Nasogastric	Intravenous	
		mg/100 ml	mg/100 ml	
1	166	119	102	173
2	214	157	147	222
3†	215	196	161	215
4‡	210	131	105	190
5	201	93	87	155
6	294	189	172	257
7‡	253	178	165	227
8‡	209	150	144	198
Mean ± SE	220 ± 13.4	151 ± 12.7	135 ± 11.5	204 ± 11.5
Mean changes		-69§	-16§	
		-85	-16¶	

* Mean of last three determinations each period.

† Period of nasogastric feeding preceded intravenous period.

§ $P < 0.01$ (Student's t test).

|| $P < 0.001$.

¶ Not significant.

The serum cholesterol level dropped significantly from the base-line value of 220 mg/100 ml during both the periods of intravenous and nasogastric feeding (Table VI). When the gut was completely bypassed by the intravenous route of nutrient administration, the serum cholesterol was lower than with oral feeding (151 to 135 mg/100 ml). Serum cholesterol levels were lower with the intravenous feeding irrespective of order of entry into the study protocol (Table V). The mean serum cholesterol level of the four subjects fed intravenously first was 127 mg/100 ml and rose to 140 mg/100 ml during the nasogastric period. Those fed by the nasogastric route for the first period had a mean cholesterol concentration of 164 mg/100 ml which decreased to 144 mg/100 ml after intravenous feeding.

During the final base-line dietary period (general diet), it was of interest that the serum triglyceride and cholesterol levels returned to the values of the initial base-line period (Tables IV and VI). For those subjects whose immediate preceding diet was the intravenous feeding, both serum cholesterol and triglyceride levels increased greatly (Table V). The subjects with an immediate preceding nasogastric feeding had a decline in the serum triglyceride concentration and a distinct rise in the serum cholesterol level as fat and cholesterol were again added to the diet.

Serum insulin and blood glucose determinations were carried out in all subjects at 8:00 a.m. on the 12th day of each intravenous constant feeding nasogastric period (Table VII). The mean serum insulin levels in all subjects during intravenous feeding were 101.5 ± 43.0 and

TABLE VII
Serum Insulin and Glucose Concentrations on the 12th Day of Constant Nasogastric and Intravenous Feeding

Subject	Nasogastric period		Intravenous period	
	Insulin	Glucose	Insulin	Glucose
1	38	116	132	108
2	32	120	146	126
3	21	95	70	105
4	39	105	130	95
5	76	98	152	100
6	32	130	76	140
7	28	120	66	125
8	32	105	40	90
$\bar{X} \pm SE$	37.3 ± 16.6	111.1 ± 4.3	$101.5 \pm 43.0^*$	$111.1 \pm 6.2^\dagger$

* Nasogastric vs. intravenous; $P < 0.02$.

† NS.

37.3 ± 16.6 μ U/ml after nasogastric feeding, a greatly lower level ($P < 0.02$). Mean daily blood glucose concentrations for all subjects were 111.1 ± 4.3 mg/100 ml during constant nasogastric feeding and 111.1 ± 6.2 mg/100 ml during intravenous feeding. Serum insulin and glucose measurements were made in the last two subjects in the study on the 3rd, 8th 11th, and 12th day of each period (Table VIII). Again serum insulin levels were consistently lower when the diet was administered by the nasogastric route. At no time did glucosuria occur in any of the subjects. The weights of the individual subjects throughout the 5½ wk were constant.

Because we were concerned that the effect of the nasogastric feeding on serum triglycerides might only occur with constant nasogastric drip feeding over a 24 h period (i.e. analogous to "nibbling"), the final two subjects received the same formula diet in four equal feedings during a third 12 day period. One of these subjects was initially hypertriglyceridemic and the other had a normal serum triglyceride concentration. Neither serum triglyceride nor serum cholesterol level was different

TABLE VIII
Serum Insulin and Glucose Concentration in Two Subjects (Subjects 7 and 8) during Nasogastric and Intravenous Feeding

Study day	Nasogastric period		Intravenous period	
	Insulin	Glucose	Insulin	Glucose
	μ U/ml	mg/100 ml	μ U/ml	mg/100 ml
3	78* 38	55 93	200 146	110 95
8	32 34	145 95	130 66	168 115
11	30 20	110 130	150 132	150 145
12	28 32	120 105	66 40	125 90

* Data from subject 7 appears first in each column.

TABLE IX
*The Effects of the Nasogastric Feeding Given by
a Constant 24 h Drip or as Four Meals*

	24 h drip	Four meals*
Serum triglyceride, mg/100 ml		
Subject 7	296	297
Subject 8	126	120
Serum cholesterol, mg/100 ml		
Subject 7	182	186
Subject 8	173	172

* Values are fasting levels.

when the entire formula was fed in four meals in contrast to the 24 h drip (Table IX).

The quantity of stool diminished greatly during the period of eucaloric intravenous feeding as compared with the same feeding given by the nasogastric route: the mean stool weight being 21.4 g/day during the intravenous period and 74.4 g/day for the nasogastric period (Table X). These data compare with usual mean stool weights of 50–94 g of patients receiving a formula diet containing 40% of the total calories as fat.

Correspondingly, the total fecal steroid excretion likewise decreased from 461 mg/day to 205 mg/day during the period of intravenous alimentation (Table X). Neutral steroid excretion declined from 283 to 160 mg/day but the decrease in bile acid excretion was even more pronounced a change, from 178 to 45 mg/day. In five of the eight subjects bile acid excretion was below 50 mg/day and in three subjects it was under 20 mg/day.

The composition of the fecal neutral sterols for both routes of feeding was almost entirely cholesterol (Table XI). The usual bacterially altered products of cholesterol in the feces, coprostanol and coprostanone, were virtually absent during the nasogastric feeding and greatly reduced or absent for the intravenous period. Bile acid excretion, especially low during the intravenous feeding, continued to be composed largely of the secondary bile acids, lithocholic and deoxycholic acids; 76% of the total bile acids after the intravenous feeding and 83% after the nasogastric route (Table XII).

DISCUSSION

Heretofore, the liver has been presumed to be the primary source of the hypertriglyceridemia in response to high dietary carbohydrate feeding by virtue of enhanced triglyceride synthesis (3, 7, 13). The failure to produce hypertriglyceridemia in our experiments when the high carbohydrate diet was infused intravenously, thus bypassing the gut, suggests that the intestinal mucosa may have a prime role in the synthesis of triglyceride and lipoprotein resulting from a high carbohydrate diet. When the 80% carbohydrate diet was given orally, the mucosal absorption of glucose apparently stimulated triglyceride synthesis which then made a major contribution to the carbohydrate-induced hypertriglyceridemia.

The ingestion of a high carbohydrate diet is believed to elevate serum triglyceride levels by virtue of increased synthesis which results in an enlarged serum triglyceride pool (20). The increased rate of synthesis induced by carbohydrate feeding is apparently the same in normal subjects and in patients with known hypertriglyceri-

TABLE X
*The Effect of the Route of Administration of the High Carbohydrate Diet upon the Fecal
Mass and upon the Fecal Excretion of Neutral Sterols and Bile Acids*

Subject	Nasogastric period				Intravenous period			
	Fecal mass	Fecal steroid excretion			Fecal mass	Fecal steroid excretion		
		Neutral sterols	Bile acids	Total		Neutral sterols	Bile acids	Total
	g/day		mg/day		g/day		mg/day	
1	75.6	195	183	378	6.1	59	19	78
2	75.7	190	150	340	37.5	216	61	277
3	101.3	222	336	558	31.5	219	98	317
4	68.0	235	179	414	8.6	170	14	184
5	49.4	305	91	396	13.4	12	1	13
6	89.2	215	92	307	31.5	227	126	353
7	77.4	512	296	808	22.9	267	31	298
8	58.7	393	96	489	19.4	108	10	118
$\bar{X} \pm \text{SE}$	74.4 \pm 5.8*	283.4 \pm 40.5†	177.9 \pm 33.1§	461.3 \pm 57.1†	21.4 \pm 4.1	159.8 \pm 32.0	45.0 \pm 16.2	204.8 \pm 44.2

* $P < 0.001$, Nasogastric vs. intravenous.

† $P < 0.005$, Nasogastric vs. intravenous.

§ $P < 0.05$, Nasogastric vs. intravenous.

TABLE XI
*Fecal Excretion of Cholesterol and Its Bacterial Degradation Derivatives during
Nasogastric and Intravenous Feeding*

Subject	Nasogastric period			Intravenous period		
	Cholesterol	Coprostanol +coprostanone	Total	Cholesterol	Coprostanol +coprostanone	Total
	<i>mg/day</i>			<i>mg/day</i>		
1	195	—	195	59	—	59
2	190	—	190	198	18	216
3	222	—	222	218	1	219
4	235	—	235	144	26	170
5	305	—	305	10	2	12
6	210	5	215	179	48	227
7	509	3	512	255	12	267
8	393	—	393	108	—	108
$\bar{X} \pm \text{SE}$	281.1 \pm 39.9*		283.4 \pm 40.5	146.4 \pm 29.4		159.8 \pm 32.0

* Nasogastric vs. intravenous, $P < 0.02$.

demia (usually type IV hyperlipidemia). The greater pool size induced in the latter by a high carbohydrate feeding may result in part from a decreased rate of triglyceride removal by peripheral tissues. The significant finding in our experiments concerns the organ apparently responsible for increased triglyceride synthesis induced by carbohydrate feeding; i.e., the intestinal tract. All subjects, including two subjects who were initially hypertriglyceridemic, had decreased triglyceride levels when the intestine was bypassed by the intravenous feeding.

One alternative hypothesis might be that dietary carbohydrate, given orally, elevates serum triglycerides through a "gut" hormone or other factor which in turn acts to stimulate hepatic very low density lipopro-

tein (VLDL) synthesis. Another hypothesis might be that oral dietary carbohydrate elevates serum triglyceride through a "gut" factor which acts to inhibit the removal of VLDL triglyceride.

Our hypothesis that the intestine may itself be responsible for increased triglyceride synthesis induced by carbohydrate conforms to the increasing recognition of the role of the intestinal mucosa in lipid synthesis. It has been found that significant triglyceride synthesis in the gut continues in triton-treated, hepatectomized rats (21) and in hepatectomized dogs (22). Ockner and Laster (11) and Baxter (10) found that 40% of the plasma triglyceride in the rat fed a fat-free diet was derived from intestinal lymph, a figure considerably higher than the 10% found by Windmueller and Levy

TABLE XII
The Fecal Excretion of Individual Bile Acids during Nasogastric and Intravenous Feeding

Subject	Nasogastric period				Intravenous period			
	Lithocholic	Deoxycholic	Others	Total	Lithocholic	Deoxycholic	Others	Total
	<i>mg/day</i>				<i>mg/day</i>			
1	93	71	19	183	6	8	5	19
2	44	51	55	150	28	10	23	61
3	118	171	47	336	33	56	9	98
4	57	97	25	179	7	1	6	14
5	38	40	13	91	0.3	—	0.7	1
6	52	33	7	92	58	49	19	126
7	127	118	51	296	10	3	18	31
8	29	45	22	96	1	2	7	10
$\bar{X} \pm \text{SE}$	69.8 \pm 13.3*	78.2 \pm 16.9†	29.8 \pm 6.5§	177.9 \pm 33.1	17.9 \pm 7.1	16.1 \pm 8.0	11.0 \pm 2.8	45.0 \pm 16.1

* Nasogastric vs. intravenous, $P < 0.005$.

† Nasogastric vs intravenous, $P < 0.01$.

§ Nasogastric vs. intravenous, $P < 0.02$.

(23). This difference may be accounted for by the much lower hourly lymph triglyceride flow of 2.7 mg observed by Windmueller as compared to a rate of 6 mg/h observed independently by Ockner and Baxter. Furthermore, very low density lipoprotein production has been observed in the intestinal mucosa of man and rat (24) and found in intestinal lymph under fasting conditions (9, 25). Low density (beta) lipoprotein synthesis also occurs in the rat intestinal mucosa (23, 26).

It is of interest that serum insulin levels in our subjects were generally higher during the continuous intravenous than during the oral feeding. Blood glucose values were similar. The higher insulin levels during the intravenous diet thus correlated inversely with the lower triglyceride concentrations occurring at the same time. These findings are in harmony with the overall hypothesis that insulin promotes the removal of triglyceride from the blood as recently reiterated by Barter, Carroll, and Nestel (27) who have extended the previous observations (28, 29) that serum insulin levels in human subjects fed a high carbohydrate diet show a highly significant inverse correlation with the serum triglyceride levels.

The mechanism by which insulin secretion was enhanced when the diet bypassed the gastrointestinal tract is unclear. Ordinarily, glucose alone stimulates more insulin release by the oral route vs. the intravenous route because of the release of various humoral factors (30). Plasma free amino acid levels are known to influence secretion of hormones by the endocrine glands, and may act as regulators for the secretion of insulin, glucagon, growth hormone, and the corticosteroids (31, 32). Studies using isolated pancreatic preparations *in vitro* (33, 34) and those using the intravenous administration of various amino acids indicate that elevated levels of certain amino acids in the plasma (particularly arginine, leucine, lysine, and possibly histidine) cause increased insulin secretion (32, 35-39). However, with the exception of an effect upon plasma cystine levels (40), few differences were noted in plasma amino acid levels in our subjects with the differing routes of alimentation. Plasma levels of the insulin-stimulating amino acids arginine, leucine, lysine, and histidine were nearly identical during the intravenous and oral feeding periods.

The absence of nutrients in the small intestine during the period of intravenous feeding undoubtedly decreased bile flow into the intestine. This hypothesis is suggested by, and would help to explain, the extremely low output of fecal bile acids when the gut was bypassed. Decreased enterohepatic circulation of bile would also result in decreased biliary lipid available in the intestinal lumen for triglyceride synthesis. The very small fecal mass during intravenous feeding may provide an index to

the low metabolic activity of the intestinal mucosa when the gut was bypassed. Our experimental diet was obviously residue-free, so that the fecal mass would be entirely composed of the products of desquamated mucosal cells and bacteria.

If fatty acid for triglyceride synthesis by the intestinal mucosa is derived from bile phospholipid and the fatty acids of sloughed mucosal cells as reported by previous investigators (9, 10), then both decreased metabolic activity of the gut mucosa and the decreased bile flow would limit substrate for triglyceride synthesis. Fatty acid available for triglyceride synthesis may be further depleted by its use for the metabolic needs of the intestinal mucosa. Conversely, oral feeding of carbohydrate may provide a substitute source for fatty acid otherwise needed for intestinal metabolic needs; the fatty acid spared could then be used for triglyceride synthesis. Triglyceride synthesized in the intestinal wall is not thought to be derived from glucose carbons (5, 6, 10). Fatty acids from biliary phospholipid accounted for approximately 50% of the fatty acids in lymph triglyceride in fasting rats (10). Bile diversion with constant bile acid infusion resulted in a 60% fall from base line in intestinal lymph triglycerides (22). With total bile diversion, lymph lipid was further decreased (9, 41-43).

The use of a fat and cholesterol-free feeding during both of the experimental high carbohydrate dietary periods produced a sharp decline in serum cholesterol levels. These decreases are explainable on a twofold basis. Cholesterol was absent in the diet (44). Furthermore, even that cholesterol derived from the bile and the intestinal mucosa would have lessened absorbability because of the absence of dietary fat (45). These changes occurred with the oral feeding of the high carbohydrate diet at the same time that serum triglyceride concentrations were increasing some 50%. Our assumption is that the decrease of cholesterol-rich beta lipoproteins more than compensated for the increase of very low density lipoproteins whose predominant lipid is triglyceride and which contain a much lower content of cholesterol.

The further lowering of the serum cholesterol level with the intravenous feeding probably occurred because the more metabolically quiescent intestinal mucosa was no longer contributing its customary amount of synthesized cholesterol to the plasma. It is of interest that this further 10% reduction of serum cholesterol matched the data of Dietschy and Wilson (46). They calculated that the intestinal mucosa contributes about 11% of the synthesized cholesterol entering the serum pool of cholesterol.

The much lower excretion of cholesterol in the stool during the intravenous feeding further corroborated the

presumed low mucosa metabolic activity. We assume when no nutrients are entering the gastrointestinal tract that bile flow (and cholesterol excretion by this route) is at a minimum. Under this state of affairs most of the fecal cholesterol is probably derived from desquamated mucosal cells. The ratio of fecal bile acids to neutral sterols decreased during the intravenous feeding period. What cholesterol was present in the serum must have then been derived from hepatic synthesis.

The fecal neutral steroids are derived from three sources: unabsorbed dietary cholesterol, unreabsorbed bile cholesterol, and cholesterol secreted through the intestinal mucosa into the lumen and that contained in desquamated mucosal cells (47). In the present study, since cholesterol was absent in the diet, the fecal neutral steroids came from the latter two sources. An earlier study indicated that the bile flow decreased during fasting (48). In view of the fact that there was no food going through the gastrointestinal tract to stimulate normal digestive secretions and the low fecal bile acids excretion during the intravenous feeding period, it is probable that bile secretion was low during that period. Since the molar ratio of bile acid to cholesterol in the human bile is usually 12:1 (49) and since the fecal bile acid content was very low, it is likely that the contribution of bile cholesterol to the fecal neutral sterols was small during the intravenous feeding period. The fecal neutral steroids thus represented mainly that cholesterol derived from the desquamation of mucosal epithelium, or about 160 mg/day. This value is slightly lower than the reported value measured in three patients with complete obstruction of biliary and pancreatic ducts (250–400 mg/day) (50). This may be due to a lower turnover rate of the mucosal cells in our normal subjects in the absence of the usual stimulation from the passage of nutrients through the intestinal tract. The excretion of both bile acids and neutral steroids was higher during the nasogastric than during the intravenous feeding period. Since the serum cholesterol in this period was also higher (151 mg/100 ml vs. 135 mg/100 ml), the larger secretion of fecal steroids can therefore be interpreted as the result of increased cholesterol synthesis in the mucosa and increased mucosal cell turnover.

Interestingly, the fecal steroids excretion during nasogastric feeding period was still lower than that of six normal subjects fed formula diets, cholesterol-free in our previous study (neutral steroid 388–419 mg/day, bile acids 244–426 mg/day) (19). The serum cholesterol of those subjects ranged from 177 to 226 mg/100 ml. The difference between nasogastric feeding and the previous formula feeding was that the fat content of the latter was 40% of the total calories. It is known that dietary fat stimulates the flow of bile (20, 51). The nasogastric feeding may have had the same effect as the intravenous

feeding because of the low fat content. The movement of lipids from the liver may in turn stimulate the synthesis of cholesterol. It was reported that in the rat fed a cholesterol-free diet, dietary fat stimulated the removal of cholesterol from liver and increased cholesterol biosynthesis (52, 53).

A major fraction of fecal neutral steroids is usually excreted in the form of the saturated sterol, coprostanol (54, 55). In the feces of the eight subjects during both nasogastric and intravenous feeding periods, coprostanol was either completely absent or very low, from only 5 to 48 mg/day. The virtual absence of bacterial activity for this sterol transformation may result from the character of the diet which was residue-free, fat-free, and cellulose-free. These constituents influence the bacteria flora and its metabolic activity. Phrenosin, which historically has been thought to influence the conversion of cholesterol to coprostanol, was also absent in the diet.

On the other hand, the percentage of the deoxycholic acid and lithocholic, secondary bile acids produced in the gut by bacterial action (56, 57), was 83% of total bile acid excretion for nasogastric feeding and 76% of total for intravenous feeding. These figures are comparable with a percentage of 87% usually found in human beings fed formula diets. This indicates that the activity of the bacteria responsible for the formation of secondary bile acids was not deterred by the experimental conditions of this study. It is not known what species of bacteria are responsible for these two different biochemical reactions in the colon (cholesterol and bile acid transformations).

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