Effect of a high fat diet on plasma lipids, lipoprotein lipase, lecithin:cholesterol acyltransferase, and insulin function in adult rabbits

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The influence of a high fat diet (19% wt./wt.) vs. a standard, chow diet (2% fat) on plasma lipids, lipoprotein lipase (LPL), lecithin:cholesterol acyltransferase (LCAT), glucose, and glucose tolerance, was investigated. Both diets had a similar fatty acid pattern and a polyunsaturated:saturated (P:S) fatty acid ratio of 2.7. The high fat diet elevated plasma triglycerides, phospholipids, and cholesterol concentrations and changed the percent distribution of cholesterol and phospholipids among the lipoprotein fractions. Additionally, LCAT and plasma glucose increased, while lipoprotein lipase and its products, free fatty acids and glycerol, were not altered. Glucose tolerance was significantly inhibited in animals on the high fat diet, which also exhibited a diminished insulin secretion. As such, the high fat diet seemed to evoke a diabetogenic situation. The return to the standard chow diet appeared to normalize, to a great extent, the alterations evoked by the high fat diet.

Keywords: fat; diet; lipoprotein; LCAT; acyltransferase; insulin

Introduction

Dietary fats have been shown to assume a primary role in the regulation of carbohydrate and lipid metabolism.[1] Both the effect of dietary fatty acid composition and the influence of the amount of dietary fat are considered important for the process of atherogenesis.[2] High blood and dietary cholesterol levels, in particular, are associated with an increased incidence of atherosclerosis.[3,4]

Studies of non-human primates, such as the marmoset, have shown that a diet of 0.5% cholesterol can increase plasma cholesterol levels from 180 mg% to over 450 mg%, but in combination with a 16% saturated fat diet (lard), levels of >1300 mg% are measured.[5] To investigate the effect of high fat diet alone, we have thus created a high fat diet (lacking cholesterol) having a fatty acid pattern and an energy content per gram-protein, -mineral, and -vitamin resembling that of the standard chow diet. The object of the present study was to investigate the effect of this high fat diet on the alteration of blood lipid metabolism [plasma lipids and lipoproteins], as well as the activity of two key enzymes [lipoprotein lipase (LPL), lecithin:cholesterol acyltransferase (LCAT)], and insulin function [glucose tolerance and immunoreactive insulin].

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Materials and methods

Animals and diet

Although rabbits are herbivorous animals, numerous studies have shown that useful information can be gained from a species so prone to atherosclerosis evoked by dietary manipulation.[6,7] As such, 20 white male New Zealand rabbits (4.1 kg) were housed in single cages in a constant temperature (22° C), well-ventilated room with a controlled light-dark cycle (light, 0700 to 1900). All animals were fed food and water ad libitum. They were maintained on a standard chow diet (2% fat) for 6 weeks (baseline phase),

switched to a fat rich (19% wt./wt.) for 6 weeks (experimental phase), and then returned to the standard diet for an additional 6 weeks (recovery phase). When changing to the high fat diet, no adaption period was necessary as the animals readily accepted the food. Both diets (standard and high fat) were prepared in the laboratory from basic ingredients purchased from Tagger (Graz, Austria) and formed into pellets by use of a sausage-making die.

The components of the two diets are shown in *Table 1*. The standard diet consisted of the following digestible constituents: 12% water, 18% protein, 2% fat, 34% carbohydrates, and 10% fiber. The high fat diet consisted of the following average composition: 13% water, 25% protein, 19% fat, 11% carbohydrates, and 7% fiber. The mean, average energy content of the standard and high fat diets, were 9.62 KJ/g and 13.56 KJ/g, respectively. The two diets were prepared so that the energy content relative to vitamins, minerals, and protein was kept constant. *Table 2* illustrates the fatty acid composition of the two diets; the P/S ratio being about 2.7 in each case. Individual food intake was recorded daily and the body weight weekly.

Sample collection and chemical analysis

Blood samples were withdrawn from the marginal ear artery of animals fasted overnight at the end of the 4th and 6th week in each diet period between the hours of 9 and 10 a.m.[8] An intravenous glucose tolerance test was performed in the 6th week of each diet period. For this purpose, a catheter was introduced into the marginal auricular vein and 0.5 ml blood was removed for the determination of the initial values of glucose and insulin. Subsequently, a solution of 0.6 g of glucose per kilogram body weight was injected into the marginal vein of the opposite ear. After 5, 10, 15, 20, 30, 45, 60, 75, and 120 min a sample of 0.5 ml blood was removed and total cholesterol, triglycerides, free glycerol, phospholipids, and glucose were measured with commercial kits (Boehringer-Mannheim, F.R.G.). Free fatty acids were determined according to the method of Falholt et al.,[9] and the separation of plasma lipoproteins in VLDL, LDL, and HDL was performed by density gradient ultracentrifugation according to Terpstra et al.[10] The recovery of phospholipids and triglycerides in these fractions was 100%, whereas the recovery of cholesterol was 87%. Immunoreactive insulin was determined by the radioimmunoassay method (insulin kit, Radio-Chemical Center, Amersham, UK), while the serum activities of post-heparin lipoprotein lipase and lecithin:cholesterol-acyl-transferase were measured by the method of Schotz et al.[11] and Yao et al.,[12] respectively. Half of the animals were sacrificed after 12 weeks, after being maintained on the high fat diet for 6 weeks, while the remainder were sacrificed at the end of the recovery phase (18 weeks). Significance was determined from the means and standard deviations by Student's *t* test.

Table 1 Composition of diet (percent)

Ingredient	Standard Diet	High Fat Diet
Crushed barley	34.3	1.7
Alfalfa meal	10.0	8.0
Wheat bran	10.0	7.5
Crushed wheat	10.0	2.2
Low fat soybean meal	20.0	45.5
Sunflower hull	6.0	5.0
Cane molasses	4.0	2.0
di-Calcium phosphate	1.7	1.7
Sodium chloride	0.4	0.3
Sodium hydrogen carbonate	0.5	0.4
Mineral mix	0.5	0.7
Vitamin mix	1.0	1.4
Lime stone	1.6	1.6
Shortening ("Becel," Unilever, Vienna, Austria)	—	22.0

Table 2 Fatty acid composition (area%) of dietary lipids

Fatty Acid	Standard Diet	High Fat Diet
C12:0	1.0	2.5
C14:0	0.5	1.0
C16:0	17.0	10.2
C18:0	3.3	7.9
C18:1	17.3	15.3
C18:2	49.5	58.7
C18:3	8.2	1.2

Results

Food consumption and total body weight

Daily food intake of the standard group was about 140 g per day. Although the animals consumed significantly less of the high fat diet (about 100 g vs. 140 g/standard diet/day), the energy intake of all animals on each diet was similar (about 1.33 MJ) due to the higher energy content of the high fat diet. The daily food intake and body weight did not change significantly during all phases of the dietary regimen.

Plasma triglycerides, total cholesterol, and phospholipids

After 4 weeks on the high fat diet, plasma triglyceride (TG) levels were 40% higher than those measured during the baseline phase and remained elevated through the 6th week (*Figure 1*). Upon returning to the standard diet (recovery phase), TG levels fell but still remained about 20% above baseline values. The effect of the high fat diet on plasma cholesterol and phospholipid levels appeared to be biphasic, with elevated values being measured at 4 weeks (72% and 38% above baseline levels, respectively), followed by a slight decline by 6 weeks (*Figure 1*). Upon returning to the standard diet (recovery phase), the levels of both cholesterol and phospholipids declined further to below baseline values. By the end of the feeding regimen (18

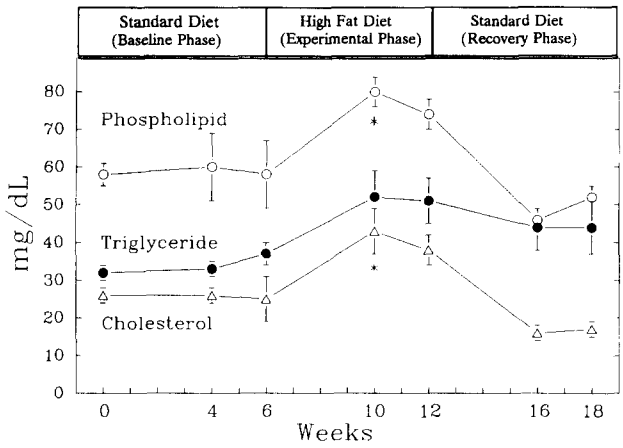

Figure 1 Concentrations of plasma phospholipids, triglycerides, and cholesterol (mean ± SD) in the blood of six rabbits drawn after an overnight fast from the marginal ear vein at the time points indicated. Data presented in *Figures 1–4* are from animals that received a standard diet (baseline and recovery phase) interrupted by a high fat diet (experimental phase). * = significant difference ($P < 0.05$) from baseline and recovery phase values.

weeks), phospholipids returned toward normal while cholesterol values were still lower than baseline.

Cholesterol distribution among the lipoproteins

Similar to our previous results,[7] during the first feeding period (baseline phase) about 81% of the total cholesterol was found in the HDL, 11% in the LDL, and 8% in the VLDL lipoprotein fraction (*Table 3*). The high fat diet increased cholesterol to different extents in all lipoprotein fractions so that the percent distribution was altered. At the end of the experimental period (6 weeks on the high fat diet), the HDL-cholesterol dropped to 73%; LDL- and VLDL-cholesterol increased to 16% and 11%, respectively. The switch back to the standard diet (recovery phase) resulted in a fall in the absolute amount of cholesterol in all three lipoprotein fractions, as was noted for plasma cholesterol (*Figure 1*). However, the percent distribution did not revert back to baseline values. Only the percentage of LDL-cholesterol returned to the range of the starting levels.

Phospholipid distribution among the lipoproteins

The distribution of phospholipids in the lipoprotein fraction resembled that of cholesterol, with an elevation in the percent of VLDL- and LDL-phospholipid at the expense of the percentage of the HDL-phospholipid during the experimental phase (*Table 4*). After the switch back to the standard diet (recovery phase), the distribution of phospholipids among the lipoprotein classes was similar to that of cholesterol; the percentage of the LDL-phospholipids returning to the range of the starting values, while the phospholipids of the VLDL fraction remained high and HDL fraction remained low.

TG distribution among the lipoproteins

The increase of plasma TG levels seen in *Figure 1* was mirrored equally in all three lipoprotein fractions so that the percentage distribution was not altered significantly by the high fat diet (*Table 5*). The levels of TG in the VLDL, LDL, and HDL lipoproteins re-

Table 3 Distribution of plasma cholesterol in the lipoprotein classes: mg/dl (percent)

	Standard Diet (Baseline Phase)		High Fat Diet (Experimental Phase)		Standard Diet (Recovery Phase)	
	4 weeks	*6 weeks*	*4 weeks*	*6 weeks*	*4 weeks*	*6 weeks*
VLDL	1.9 (8.4%) ±0.3	1.9 (8.2%) ±0.3	4.1 (9.8%) ±0.9	3.8 (11.1%) ±1.1	2.4 (15.6%) ±0.8	2.8 (17.1%) ±0.6
LDL	2.1 (9.3%) ±0.8	2.5 (10.7%) ±0.6	6.6 (15.8%) ±1.0	5.4 (15.8%) ±1.6	2.0 (13.0%) ±0.4	2.0 (12.2%) ±0.4
HDL	18.5 (82.2%) ±2.9	18.9 (81.1%) ±2.7	31.1 (74.4%) ±3.5	24.9 (73.0%) ±1.8	11.0 (71.4%) ±1.6	11.6 (70.7%) ±2.1

Table 4 Distribution of plasma phospholipids in the lipoprotein classes: mg/dl (percent)

	Standard Diet (Baseline Phase)		High Fat Diet (Experimental Phase)		Standard Diet (Recovery Phase)	
	4 weeks	*6 weeks*	*4 weeks*	*6 weeks*	*4 weeks*	*6 weeks*
VLDL	2.0 (3.5%) ±0.3	2.3 (3.9%) ±0.4	4.2 (5.4%) ±0.7	4.6 (6.2%) ±1.0	4.9 (10.2%) ±1.3	4.6 (8.8%) ±1.6
LDL	2.0 (3.5%) ±0.5	2.2 (3.8%) ±0.6	3.7 (4.7%) ±0.8	3.9 (5.3%) ±1.1	1.8 (3.7%) ±0.9	2.0 (3.8%) ±0.5
HDL	52.5 (92.9%) ±5.4	53.2 (92.2%) ±6.8	70.3 (89.9%) ±6.8	65.3 (88.5%) ±4.1	41.4 (86.1%) ±3.8	45.7 (87.4%) ±7.1

Table 5 Distribution of plasma triglycerides in the lipoprotein classes: mg/dl (percent)

	Standard Diet (Baseline Phase)		High Fat Diet (Experimental Phase)		Standard Diet (Recovery Phase)	
	4 weeks	*6 weeks*	*4 weeks*	*6 weeks*	*4 weeks*	*6 weeks*
VLDL	12.2 (38.6%) ±0.5	13.4 (39.6%) ±1.6	19.7 (41.0%) ±3.9	20.2 (41.5%) ±3.7	17.3 (42.3%) ±5.1	18.0 (43.1%) ±6.5
LDL	6.2 (19.6%) ±0.6	7.2 (21.3%) ±0.9	9.2 (19.2%) ±1.9	9.2 (18.9%) ±1.4	9.3 (22.7%) ±2.1	9.2 (22.0%) ±1.1
HDL	13.2 (41.7%) ±1.3	13.2 (39.1%) ±0.3	19.1 (39.8%) ±1.7	19.3 (39.6%) ±0.9	14.3 (35.0%) ±2.0	14.6 (34.9%) ±1.0

mained at about 40%, 20%, and 40% throughout the entire feeding regimen (18 weeks).

Plasma-free fatty acids (FFA) and glycerol

During the high fat dietary regime, the levels of free fatty acids and glycerol decreased slightly, but not significantly (from 0.5 to 0.42 mM/L and 0.17 to 0.12 mM/L, respectively). The switch back to the standard diet (recovery phase) brought the levels back into the range of the starting values.

Blood glucose

The high fat diet resulted in an elevated blood glucose content from 123 mg/dl to 139 mg/dl (16% increase) after 4 weeks, and further to 152 mg/dl (24% increase) after 6 weeks (*Figure 2*). The switch back to the standard diet (recovery phase) lowered the blood glucose level to starting values.

Plasma lipoprotein lipase (LPL) and lecithin:cholesterol acyltransferase (LCAT) activity

These enzymes represent the two major enzymes involved in the metabolism of plasma lipoproteins. The high fat diet resulted in a slight decline in LPL activity (not significant), which remained about the same in the recovery phase (*Figure 3*). On the other hand, the activity of LCAT was elevated by 47% in animals on the high fat diet after 4 weeks but returned to baseline values during the recovery phase.

Glucose tolerance

After a bolus injection of glucose in the marginal ear vein, the animals on the standard diet (baseline phase) achieved 5-min peak blood glucose values of about 345 mg/dl, while those consuming the high fat diet (experimental phase) reached levels of 400 mg/dl (*Figure 4*). These animals not only attained a higher peak level but also sustained elevated glucose values 40% to 50% above baseline throughout the period of glucose tolerance measurement (120 minutes; *Figure 4*). When the animals were switched back to a standard diet (recovery phase), the peak glucose levels following a bolus injection were as high as those seen in animals on the high fat diet (400 mg/dl); however, there was a distinct improvement during the subsequent 2 hr. During this period, blood glucose levels approached those

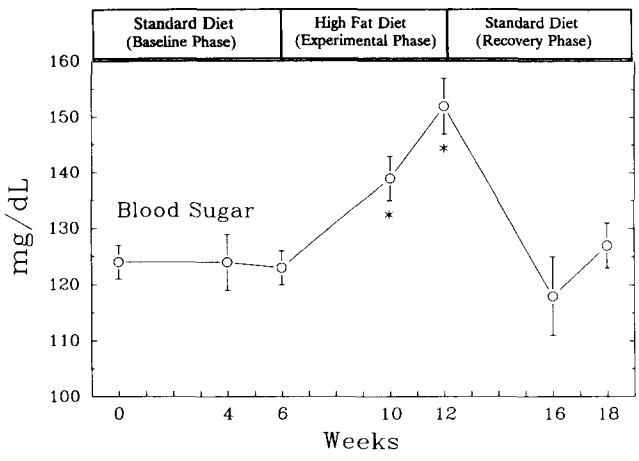

Figure 2 Baseline blood glucose levels (mean ± SD) measured after an overnight fast in six rabbits. * = significant difference ($P < 0.05$) from baseline and recovery phase values.

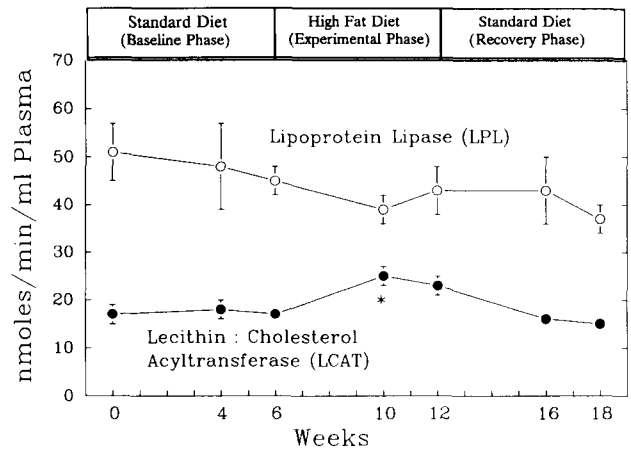

Figure 3 Lipoprotein lipase (LPL) and Lecithin:Cholesterol Acyltransferase (LCAT) activity (mean ± SD) measured after an overnight fast at the time points indicated. Activity measured during the experimental phase for LCAT, but not LPL, was statistically different from those measured during the baseline and recovery phases. * = significant difference ($P < 0.05$) from baseline and recovery phase.

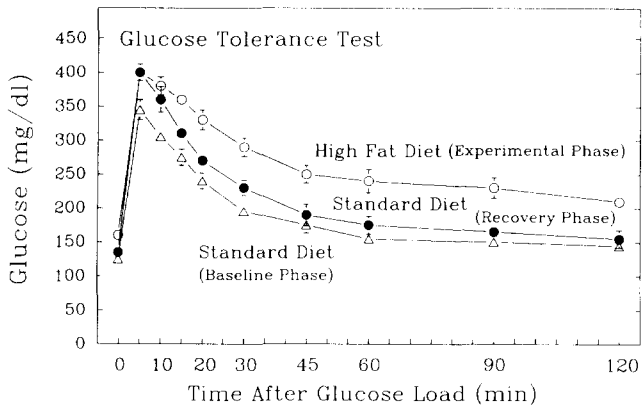

Figure 4 Plasma glucose levels (mean ± SD) measured at the times indicated following a bolus injection of glucose (0.6g glucose/kg body weight) into the marginal ear vein of six rabbits. Measurements were made from blood collected from the opposite ear.

found in animals maintained on the standard diet during the baseline phase (*Figure 4*).

Following the glucose load, plasma immunoreactive insulin of both groups of animals on the standard diet (baseline and recovery phase) peaked after 5 min, at 80 and 74 μU/ml, respectively, and then sharply declined to baseline values after about 30 min. However, animals on the high fat diet (experimental phase) exhibited a plasma immunoreactive insulin peak at 5 min of only 50 μU/ml and remained elevated for an additional 30 min prior to a gradual decline to values approximately 30 to 40% above baseline (*Figure 5*).

Discussion

It is now generally recognized that the type of dietary fat can influence blood cholesterol and lipid levels in animals and man. Dietary fat rich in saturated fatty acids has been shown to induce a higher cholesterol level and degree of atherosclerosis than fats rich in polyunsaturated and poor in saturated fatty acids.[3,13] Whereas the effect of the different dietary fatty acids has been studied intensively, the influence of the amount of fat has attracted less attention. It is now known that numerous metabolic interactions are affected by the proportion of fat and carbohydrates in the diet.[14]

The present study was designed to elucidate the effects of the ingestion of a high quantity of fat with a high unsaturated fatty acid pattern similar to a standard "chow" diet, on serum lipid parameters of adult rabbits. Since it is known that refined diets are hyperlipemic by themselves, in contrast to chow diets which seem to have an unidentified protective factor,[10] we prepared an experimental diet by adding fat to the natural chow compounds while keeping the protein, mineral, and vitamin content per KJ the same as the chow diet. The fat diet pellets resembled the chow diet pellets in appearance, size, and smell and were readily accepted by the animals. As the energy intake was the same for both groups, the often-seen obesity by energy-rich diets was not observed.

Consumption of the high fat diet resulted in an elevation of all blood lipids after four weeks (*Figure 1*), but the levels of cholesterol and phospholipid declined during the remaining two weeks on this diet. This phenomenon of a decrease in blood lipids in spite of a continuing high fat diet, indicates a better management of the fat load and is supported by the observations of others.[15,16] The three lipoprotein fractions contributed to various degrees to the increase in total plasma cholesterol, thus altering the percentage distribution (*Table 3*): HDL-cholesterol dropped from 81% to 73%, while VLDL- and LDL-cholesterol increased from 8% to 11% and 11% to 16%, respectively. Similar shifts in the distribution of cholesterol between the lipoprotein fractions have also been observed by others.[7,17] The switch back to the standard diet (recovery phase) decreased total cholesterol to below the starting values (*Figure 1*), without restoring the altered cholesterol distribution. Since the diet was completely cholesterol-free, the increase in plasma cholesterol can be attributed to an endogenous origin. The majority of published reports support an increase in hepatic cholesterolgenesis evoked by a high fat diet[18-21] although contrasting results have been described.[22] It has been postulated that the enhanced cholesterol synthesis may represent an alternative pathway for acetyl-CoA when lipogenesis is suppressed.[19]

The enzyme responsible for the formation of esterified cholesterol in blood is lecithin-cholesterol: acyltransferase (LCAT). We found a significant increase of the LCAT activity during the high fat feeding period, which correlated with the increase in plasma cholesterol (*Figure 1*). A positive correlation of LCAT activity with blood lipid concentrations, particularly cholesterol, has also been seen by others in man[23,24] and animals.[25] LCAT was also found to be increased in diabetes[26]; a metabolic situation that is similar in many aspects to high fat feeding.[26] As these conditions

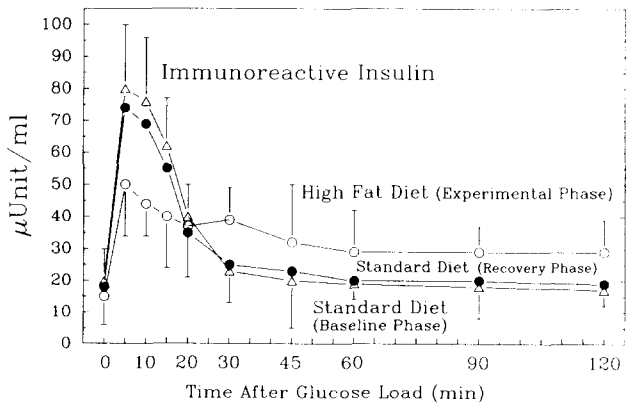

Figure 5 Plasma immunoreactive insulin levels (mean ± SD) measured at the times indicated following a bolus injection of glucose (0.6 g glucose/kg body weight) into the marginal ear vein of six rabbits. Measurements were made from blood collected from the opposite ear.

are often accompanied by atherosclerosis, it has been speculated that lysolecithin, the co-product of the LCAT activity, may be a causal agent for arterial damage.[27]

The time course of total phospholipids resulting from the dietary regimens was similar to that exhibited by total plasma cholesterol, with a peak after four weeks on the high fat diet. Since the changes in the lipoprotein phospholipid fractions were similar to those seen with cholesterol, this pattern of distribution may reflect a compensatory mechanism necessary to maintain the integrity of the lipoprotein particles.

Triglyceride levels were also found to be increased during the high fat feed phase. However, in contrast to cholesterol and phospholipids, the switch back to the standard diet (recovery phase) did not return the TG levels to the starting values. Thus, the increase may have resulted from a saturation of the catabolic system by the high level of dietary fat. A strong inhibition of lipogenic activity[19,28,29] and the associated enzymes[30-32] has been demonstrated previously by fat rich diets, especially those containing unsaturated fatty acids. The elevated levels of triglycerides shown in *Figure 1* may have resulted from the decline in extrahepatic lipoprotein lipase, the inhibition of which could hinder the breakdown of triglycerides and reduce levels of free fatty acids and glycerol (see section on *Plasma-free fatty acids (FFA) and glycerol*, above).

During the high fat feeding period, blood glucose levels increased by 25% (*Figure 2*) and returned to normal during the recovery phase. However, the expected elevation in free fatty acids after a diet of high fat content[33,34] was not observed. The high fat (experimental phase) was also associated with an impaired recovery in the glucose tolerance test, with glucose levels remaining about 30% above baseline after 120 min. However, upon a return to a standard diet (recovery phase), the glucose tolerance test was somewhat normalized (*Figure 4*). The normalization occurred about 30 min after the administration of glucose, since the peak value of plasma glucose during the recovery phase remained high (400 mg/dl), similar to the value recorded during the high fat diet regimen (*Figure 4*). Similar impairments of carbohydrate metabolism by the excess intake of fat have been observed by others.[35,36]

High levels of dietary fat have been reported to increase,[37] decrease,[38] or have no effect on plasma insulin concentrations in rats.[39] In our study, the insulin values at time zero in the glucose tolerance test were not significantly different in animals on the high fat diet compared to those on the standard diets (baseline and recovery phases; *Figure 5*). However, after glucose challenge, there was a decreased maximum output and higher values of insulin from 30 min throughout the observation period (120 min).

The results of this study indicate that a high fat diet can induce elevations in plasma lipids, blood glucose, and a diminished glucose utilization combined with a blunted insulin responsiveness during glucose challenge. This is consistent with other reports demonstrating that insulin binding to liver plasma membranes and the number of insulin receptors decreased by over 50% in normal rats fed a high fat diet.[40] High fat diets have also been shown to increase insulin resistance in skeletal muscle and adipose tissue,[41,42] which may be related to a depletion of glucose transporters at the post-receptor level.[43]

Further, it has also been speculated that plasma cholesterol level could be a determinant of glucose tolerance by changing the cholesterol-phospholipid ratio of membranes, which in turn could result in a diminished sensitivity of the membrane-bound insulin receptor.[44] However, the small rise in plasma cholesterol we observed may not have been sufficient to have brought about such alterations. Further, diet-induced differences in the fatty acid composition and the resultant fluidity changes also have been shown to lead to impaired insulin receptor function.[45] Although these parameters were not measured directly in the present study, the high fat diet used had a fatty acid composition similar to the standard diet, and as such, changes in membrane lipids and/or fluidity may not have occurred. We conclude that the high fat diet seems to have induced a diabetogenic condition and that greater attention should be paid to the quantity of fat consumption in relation to the diabetic condition.

Acknowledgments

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