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Effect of a High-fat Diet on Pancreatic Insulin Release, Glucose Tolerance and Hepatic Gluconeogenesis in Male Rats *

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Male rats were fed a high-fat diet (B) and compared with age and sex paired controls fed a standard diet (A). After an 18 hr fast, the plasma levels of glucose and insulin were lower in B than in A and the oral glucose tolerance were less in the B group. Insulin release *in vitro* by the pancreas of the rats on the high-fat diet was lower than in the controls. The formation of glucose-C^{14} from alanine-C^{14} (10^{-3} M) by liver slices was higher and that of lactic acid-C^{14}, pyruvic acid-C^{14} and $C^{14}O_2$ was lower in B vs A when the animals were fed. After a 48 hr fast the A group shows higher gluconeogenesis than when fed, while B remains at the fed rate, in such a way that the formation of glucose-C^{14} in A vs B is not different when the animals were fasted. The decreased pancreatic insulin release in B could result in such an activation of hepatic gluconeogenesis, that a further increase with fasting is no longer possible.

The intake of a high-fat diet by rats produces alterations of the carbohydrate and insulin metabolism: plasma insulin and the sensitivity of the tissues to insulin decreases, while plasma glucose is higher (2). This is accompanied by a decreased insulin release by the pancreas (15), and a higher content of adrenal corticosteroids (14). This metabolic setting would be the proper one to induce heightened liver gluconeogenesis, and actually this is emphasized by the lower activity of glycolytic enzymes, such as hexokinase (16) and glucokinase (21) and the increase gluconeogenetic enzymes, such as glucose-6-phosphatase (16).

To complete this metabolic pattern we have evaluated the glucose tolerance and liver gluconeogenetic capacity in rats fed with a high-fat diet.

Materials and Methods

ANIMALS. Wistar male rats from weaning were divided into two groups (A and B). Group A, designated as the control group, was fed on a standard diet: fat, 3.8 %; carbohydrate, 49.5 %; pro-

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tein, 21.4 %. Group B received a high-fat diet (Nutritional Biochemical Corp. U.S.A.): vegetable oil, 45 %; sucrose, 29 %; vitamin-free casein, 18 %. Minerals and vitamins were given to both groups in suitable amounts. The rats were allowed to eat *ad libitum* from the end of the weaning until the two to three months of age (128 to 194 g of body weight in A and B). The animals were sacrificed by decapitation.

ANALYSES OF PLASMA. The blood was obtained from the neck of the animals and protein-free filtrates of plasma were prepared with $Ba(OH)_2$-$ZnSO_4$ (20) and analysed for glucose (12) and total ketones (1). Plasma radioimmunoassayable insulin was evaluated (8) using Novo rat insulin as standard.

TOTAL FATTY ACIDS AND DNA-PHOSPHORUS IN LIVER. Within 18 seconds after sacrifice pieces of liver were frozen in liquid nitrogen, and lipids were them extracted and purified in chloroform-methanol (2:1, Vol./Vol.) (6). Lipid extracts were reduced to dryness under N_2 at 40° C and saponified in 1 N KOH in 95 % ethanol for 2 hours at 100° C. After acidification, total fatty acids were extracted in heptane and measured by the radiochemical method described by Ho and MENG (11). DNA was isolated from the residual pellet after lipid extraction by the method of SCHMIDT and THANNHAUSER (19), and inorganic phosphorus was estimated (5) after digestion with 72 % $HClO_4$.

INSULIN RELEASE BY PANCREAS *in vitro*. Pieces of pancreas tail (35-45 mg) were incubated in 4 ml of KRB, *p*H 7.4, containing glucose (0.6 mg/ml), albumin (Sigma albumin bovine fraction V) (0.5 mg/ml), glutamate (5 mM), fumarate (5 mM) and pyruvate (5 mM) in a Dubnoff metabolic shaker at 37° C (100 cycles/min) under O_2 and CO_2 (95 %:5 %) for 90 min but with a change of medium every 30 min. Insulin was evaluated in a pool of the media from each animal. The determination of insulin was also performed by radioimmunoassay as above.

GLUCOSE TOLERANCE CURVES. 2 g of glucose/kg body weigth were administered *per os* to 18 hr fasted rats, 30 and 60 min before sacrifice. Basal samples were obtained in animals under the same fasting period but with no glucose administration. Plasma glucose, insulin and total ketones were evaluated as above.

ALANINE-U-C^{14} UTILIZATION BY LIVER SLICES. Liver slices, 0.5 mm in thickness, were cut freehand and floated in KRB. Slices were briefly bloatted, weighed on a torsion balance, and 90-120 mg portions were put into vessels filled with 1 ml of KRB containing 10^{-3}M alanine-U-C^{14} (1 μCi/ml) (Radiochemical Centre, Amersham, England). The vessels were covered with rubber caps from which a small polyethylene cup was suspended. They were gassed with O_2 and CO_2 (95 %:5 %) through out needles for 5 min in a metabolic shaker at 37° C and the incubation was processed for 90 min at 100 cycles/min. $C^{14}O_2$ was evolved at the end of incubation by the introduction of 0.1 ml N H_2SO_4 into the medium and the $C^{14}O_2$ was «trapped» in hydroxide of hyamine (250 μl) (Packard, U.S.A.) placed in the polyethylene cup by gently shaking for 90 min at room temperature.

PROCESSING OF MEDIA. The media were centrifuged to remove insoluble debris. 50 μl of each supernatant were chromatographied in two systems: *a*) pyridine, acetic acid, water, isopropyl alcohol (8:1:4:8, by volume) (PAWI) and *b*) the upper phase of n-butanol, water, methanol and formic acid (320:320:80:1, by volume) (BWMF). Ascending chromatography was done in both systems and one-inch wide strips of Whatman paper N.° 3, with a carrier of cold glucose, lactic, pyruvic, aspartic and glutamic acid and alanine (5 μg of each in 10 μl) and

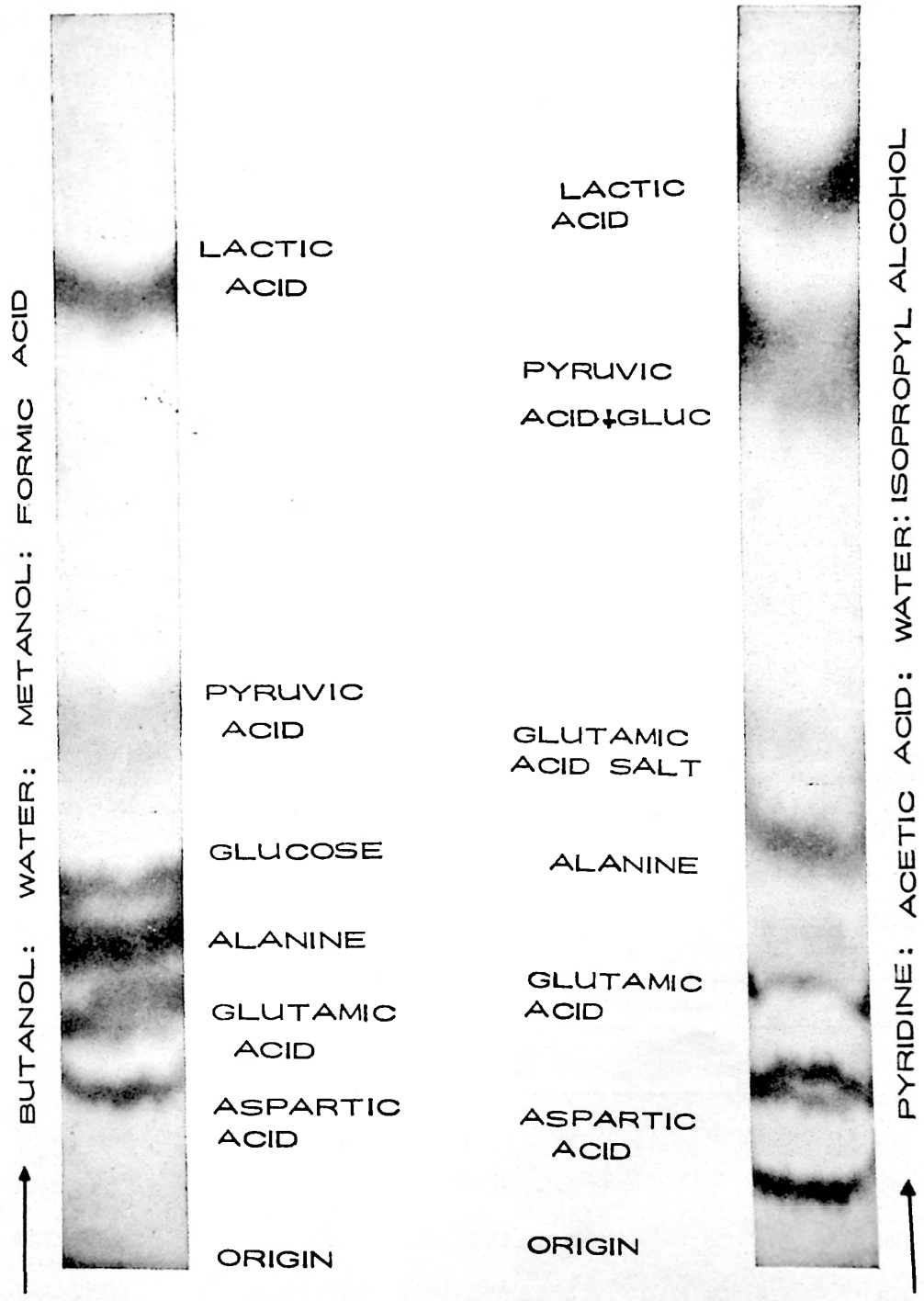

Fig. 1. *Radioautograph of l-dimensional chromatograms of radioactive standards suspended in non-radioactive KRB and developed in two different systems: pyridine, acetic acid, water, isopropyl alcohol (8 : 1 : 4 : 8, by volume) and the upper phase of n-butanol, water, methanol, formic acid (320 : 320 : 80 : 1, by volume).*

the spots were identified through radioactive guide standards by autoradiography. Figure 1 illustrates typical radioautograps of chromatograms of radioactive standards suspended in non-radioactive incubation medium (KRB) and developed in both chromatography systems. As may be seen PAWI system does not separate pyruvic acid from glucose whereas the BWMF system gives a good separation of pyruvic acid from the other metabolites. However, with the latter system separation of the other metabolites tested (lactic acid excepted) is less efficient than with the PAWI system. The difference between the percentage of radioactivity in the pyruvic acid + glucose spot after chromatography in PAWI was substracted from that of the pyruvic acid spot after chromatography on BWMF to calculate the percentage of total radioactivity converted to glucose.

RADIOACTIVE ASSAY. Hyamine cups ($C^{14}O_2$), appropriate spots of chromatograms and aliquots of the media were counted in liquid scintillation media containing 15 g PPO, 1150 mg of POPOP and 240 g naphthalene in 3000 ml of xylene: dioxane: 95 % ethanol (5:5:3, by volume). Radioactive measurements were expressed as a function of the appropriate counting standard (i.e., as percentages of the total alanine-U-C^{14} that had been added to each vessel) and were related to the initial wet weight of the slices.

Results

RELEASE OF INSULIN BY PANCREAS. 18 hours fasted rats were used to determine, in pancreas pieces incubated *in vitro*, the basal release of insulin into the media. After 90 min of incubation the amount of insulin secreted was 40.16 ± 10.90 μU/mg for the A group and 19.72 ±5.35 μU/mg for the B ($P < 0.01$), indicating a decreased capacity to release insulin in the animals under high-fat diet.

RESPONSE TO ORAL GLUCOSE ADMINISTRATION. To check whether or not the decreased insulin release by the pancreas in the B group is accompanied by a different tolerance to a high ingestion of glucose, it was administered, *per os*, to

Table I. *Effect of high-fat diet on plasma components after oral glucose administration in the 18 hours fasted rat.*

Results are expressed as means ± S.E.M. of 5-8 animals/group. Glucose was administered at 0 time *per os* (2 g/kg of body weight) and the animals sacrificed at the appropriate time by decapitation. Rats sacrificed at 0 time did not receive any glucose. P values denote the statistical significance between the groups.

Group	Time min.	Glucose mg/100 ml	Insulin μU/ml	Ketone bodies μM/l
Control (A)	0	99.2±14.8	25.2±3.8	1891±338
P		N.S.	< 0.05	< 0.05
High-fat (B)	0	82.4±10.3	17.0±1.7	3390±564
Control (A)	30	205.1±10.3	105.8±19.4	260±25
P		N.S.	N.S.	< 0.001
High-fat (B)	30	233.9±15.5	83.8±9.6	1872±144
Control (A)	60	165.3±26.3	31.2±4.6	836±178
P		N.S.	< 0.02	N.S.
High-fat (B)	60	253.9±34.6	58.8±7.6	1128±376

18 hours-fasted rats, 2 g/kg BW of glucose. The results are summarized in Table I. Basal plasma insulin and glucose were lower in the B than in the A group although the differences in the glucose values were not significant. Plasma total ketones prior glucose administration were higher in B than in A (Table I). The A animals respond normally to the ingestion of glucose: an increase in plasma glucose and insulin 30 min after the glucose load, followed by a decrease in both parameters after 60 min, although without attaining the basal level. Ketone bodies response is practically the opposite the high fasted levels of plasma ketones fall to nadir levels at 30 min of glucose treatment to increase later (60 min). The response of the B group is quite different, there is a continuous increase in plasma glucose up to 60 min of the glucose load. Plasma insulin, which started at a lower basal level, increases after 30 min of treatment but remains high after 60 min. The picture presented by the plasma ketone bodies is very interesting in the B group; they decrease after 30 min of the glucose administration, but much less than in the A group (86.5 % in A vs 44.8 % in B). With longer time, while the plasma ketones of the A group begin to recover, the basal levels in the B rats continues to fall.

In short, these results indicate that a high-fat diet produces a decreased tolerance to oral glucose administration.

In vitro LIVER GLUCONEOGENESIS. The above results show an altered insulin metabolism in the rats fed on a high-fat diet. To check whether or not this is accompanied by alterations in the *in vitro* liver gluconeogenetic capacity, we studied the disposition of alanine-U-C^{14} by liver slices

Table II. *Effect of high-fat diet on disposal of L-alanine-U-C^{14} (10^{-3} M)* in vitro *by liver slices in fed and 48 hours fasted rats.*

Details of the sacrifice of animals and *in vitro* incubation of liver slices are given in text. The disposal of labelled alanine to the different metabolites during the 90 minutes of incubation has been expressed as a function of the total counts initially present within each flask. P denotes the significance of the differences between mean ± S.E.M. values of 6 animals/group for control and high-fat fed rats. The significance of the differences between mean values for 48 hours fasted animals and fed animals is denoted by asterisks: x = $P < 0.05$; xx = $P < 0.02$; xxx = $P < 0.01$; xxxx = $P < 0.001$.

Dietary Status	% total initial counts/100 mg					
	FED			48 Hours FAST		
Group	Control (A)	High-Fat (B)	P	Control (A)	High-Fat (B)	P
Alanine-C^{14} * uptake	74.18±1.91	63.85±2.67	< 0.02	45.50±8.58 xxx	41.34±6.80 xx	N.S.
Glucose-C^{14}	1.53±0.64	7.94±1.66	< 0.01	3.19±1.06	5.16±1.47	N.S.
$C^{14}O_2$	17.47±1.32	13.87±0.86	< 0.05	10.40±2.23 x	9.04±1.96 x	N.S.
Lactic acid-C^{14}	19.86±1.82	10.29±2.09	< 0.01	10.65±2.29 x	4.54±0.71 x	< 0.05
Pyruvic acid-C^{14}	6.88±0.33	3.72±0.29	< 0.001	3.59±0.72 xxx	2.48±0.50	N.S.
Aspartic acid-C^{14}	2.36±0.26	2.78±0.25	N.S.	2.35±0.44	2.77±0.34	N.S.
Glutamic acid-C^{14}	5.81±0.62	5.57±0.51	N.S.	3.90±0.49 x	4.49±0.69	N.S.

* The alanine-C^{14} represents the % of alanine captured by 100 mg of tissue (uptake).

in the A and B group, both fed and fasted for 48 hours. When the animals were fed, the uptake of alanine-C^{14} after 90 min of incubation was higher in A than in B ($p < 0.02$) (Table II). Glucose-C^{14} formation was 518.9 % higher in B than in A ($p < 0.01$) and this difference would be even greater when corrected by the decreased uptake of the precursor by the tissue. In agreement with the above, the percentage of alanine-U-C^{14} converted to $C^{14}O_2$, lactic acid and pyruvic acid-C^{14} was lower in B than in A (Table II), suggesting a shift to gluconeogenesis. The percentage of radioactivity converted into aspartic and glutamic acid was the same in both groups. As the livers of the B group are bigger than in A (Table III), this increased gluconeogenetic capacity would be even more when calculated by the total liver. After a fasting period of 48 hours, the gluconeogenitic pattern was quite different. The uptake of alanine-U-C^{14} was lower in both groups when related to their fed values and there was no difference between the A and B animals. Despite this decreased utilization of alanine in the fasted A group vs fed, the amount converted to glucose-C^{14} was higher than when the rats were fed, indicating an increased gluconeogenesis with fasting, which is corroborated by a lower conversion of the substrate to $C^{14}O_2$, lactic acid, pyruvic acid and glutamic acid-C^{14} in the fasted than in the fed rats. However, the B group synthesize the same amount of glucose-C^{14} when fasted as when fed (Table II) so that the glucose-C^{14} formed in the A and B groups was not different when the animals were fasted, although when the values are calculated per total liver, the B group formed approximately twice more glucose-C^{14} than the A and this is due to the fact that with fasting the B group remains with larger liver than the A (Table III). The percentage of radioactivity converted to $C^{14}O_2$, pyruvic acid, aspartic acid and glutamic acid-C^{14} was the same and lactic acid-C^{14} was lower in B than in A, when the animals were fasted (Table II).

DNA-phosphorus (and presumably the number of liver cells) per g of liver is the same in A and B groups, when fed (Table III). The response of this parameter to fasting is the same for both groups, namely an increase in DNA-phosphorus per g of liver (Table III). This is such that its concentration per whole liver did not change with fasting in either group, confirming previous findings in other experimental situations (9, 10). As the livers of the B group rats are larger, the total liver content of DNA-

Table III. *Effect of high-fat diet on adrenal weight and liver weight and composition in fed and 48 hours fasted rats.*
Details of statistical analysis as in table II. In parenthesis number of animals.

Group	Liver weight g	Liver total fatty acids μmoles/g	Liver DNA-phosphorus μg/g	Adrenal weight mg
Fed control (A)	6.38±0.35 (6)	98±5 (5)	207±8 (5)	27.3±1.1 (6)
	< 0.05	< 0.05	N.S.	< 0.01
Fed high-fat (B)	7.84±0.42 (6)	149±9 (5)	200±29 (5)	40.8±3.0 (6)
Fasted control (A)	4.42±0.26 (6)	137±13 (5)	267±16 (5)	34.0±2.7 (6)
	xxxx	x	x	x
	< 0.05	N.S.	N.S.	N.S.
Fasted high-fat (B)	5.31±0.26 (6)	138±1 (5)	281±14 (5)	32.4±1.9 (5)
	xxxx		x	x

phosphorus is higher for these animals, suggesting hyperplasia.

The concentration of total fatty acids for g of liver is higher for B than for A groups, when fed (Table III). After fasting the difference between the groups disappears which is due to an increase in the total fatty acids per g of liver in the A group, without any change observed for B group animals (Table III).

The adrenal weights show changes similar to those described above for total fatty acid/g liver and *in vitro* liver gluconeogenesis (Table III): B group rats have heavier adrenals than the A animals. Fasting increases the weight of the glands in the A group, whereas the adrenals of the B group are even smaller than those of fed B group animals and thus difference between fasted A and B rats disappears.

In short, the rats under a hig-fat diet have larger livers (presumably due to hyperplasia) and adrenals, higher liver total fatty acids concentration and an increased *in vitro* gluconeogenesis, as compared to those of fed controls. Although fasting mantains the larger liver of the fat-fed rats, the adrenals shrink to the control's weight, the liver total fatty acids concentration is no longer different as compared to that of the control animals and a further increase of their *in vitro* liver gluconeogenesis is not possible.

Discussion

The present results demonstrate that administration of a diet containing 45 % fat to rats produces higher plasma ketones and lower insulin levels and decreased release of the hormone by the pancreas *in vitro*, when the animals were fasted for 18 hours. In these animals there is a slower response to the oral glucose load as far as changes of plasma glucose, insulin and ketone bodies are concerned. This *in vivo* glucose tolerance is followed by a lower sensitivity of the tissues to insulin, as we have previously shown (2). The findings are in agreement with CHRJSTOPHE (3) who found a decreased rate of glucose utilization *in vivo* in rats given a high-fat diet (54 %) for three weeks, and with MALAISSE *et al.* (15) who describe a reduction in the insulin secretory response of the beta cells to glucose and a smaller sensitivity of muscle and adipose tissue to insulin in rats fed on a high-fat diet (40 % fat for three weeks).

We have found here that decreased sensitivity to insulin is accompanied by high circulating ketone bodies and higher *in vitro* liver capacity to synthesize glucose from physiological concentrations of alanine, when the animals were fed. This enhanced fed-liver gluconeogenesis may be due to one or all of these three possibilities: i) the high lipaemia in these animals, which would presumably produce high levels of intracellular liver FFA. In the present study we have found a higher content of total fatty acids in the liver of the rats fed with the fat diet as compared to their controls, thus confirming the fatty liver described by LEMONNIER (13) in animals under a similar fat diet. It is known that there is heightened liver gluconeogenesis wherever the flow of lipids to the liver is increased, as is the case with fasting and diabetes (7), cold acclimation (17), pregnancy and fasting (10) and obesity (18). ii) The lower insulin release by the pancreas, plasma insulin levels, and the sensitivity of the tissues to it would also raise liver gluconeogenesis because the well-known antigluconeogenetic effect of insulin. iii) A higher release of steroids by the adrenal cortex would act synergetically with the above factors. Here we have found that the adrenals are larger in the fat-fed rats compared with their controls. This confirms the findings of others (4). This overall observation is accompanied by more specific findings: high fat diet have been described as producing hypertrophy of the fasciculatis and reticularis zona of the adrenal cortex (13) and higher

content of steroids in the adrenals (14).

Our studies in the 48 fasted animals complete the above picture. We observed here that fasting produces a normal response in the control animals, i.e. an increased *in vitro* liver gluconeogenesis. However, when the animals were under the high-fat diet before fasting, they could not further increase their fed-liver gluconeogenesis when food was witheld for 48 hours. This lack of response to fasting may be due to a maximal enzymatic activity of their livers when fed, driven by the above-cited inductors (lipaemia, low insulin and high steroids), which makes a further increase impossible. In fact, some of these inductors can be modified by starvation: i) The fat intake is withheld and so the lipaemia is maintained by endogenus resources which would have difficulty in maintaining such high levels of circulating fat as the fat intake. In agreement with this interpretation we have found here that the liver fatty acid concentration in the rats fed with high-fat diet does not change with fasting. ii) In the normal fasted animals the adrenals increased in size with fasting as was to be expected, while in the rats that were under fat diet before fasting the adrenal weight is smaller than when the animals were fed and equal or even smaller than those of the fasted controls. There are no data in the literature concerning adrenal steroid content in this situation but these findings would suggest a lower adrenal activity.

This whole situation is consistent with current studies in obese hyperglycemic mice (18) in which, together with high lipaemia, there is a lack of response to fasting as far as liver gluconeogenesis is concerned, concomitantly with smaller lipaemia.

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