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Comparison of a high saturated fat diet with a high carbohydrate diet during pregnancy and lactation: effects on insulin sensitivity in offspring of rats☆

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Abstract

Insulin resistance plays a critical role in metabolic syndrome and is influenced by the amount and saturation of dietary fat. Both prenatal over and under nutrition can program susceptibility to insulin resistance. The aim of the study was to explore the impact of dietary fat given to mothers during gestation on the insulin sensitivity of the offspring. Female Wistar rats were fed with diets rich in carbohydrate (CHO) or saturated fat (SAFA) during pregnancy. The male offspring was split into 5 subgroups: groups 1 (control) and 3 continued on CHO or SAFA after birth, respectively. Group 2 with mothers on CHO continued on the CHO diet during the nursing period and changed to SAFA postweaning. Group 4 with mothers on SAFA continued on SAFA during the weaning period and changed to CHO postweaning. For group 5 the offspring of mothers given SAFA diet was changed to nursing mothers on CHO diet immediately after birth and continued on the same diet postweaning. At the age of 16 weeks, a euglycemic hyperinsulinemic clamp was performed. The glucose infusion rate was lowered in the groups receiving the SAFA diet (group 2, 24.7 ± 2.0 mg/kg per minute; group 3, 22.0 ± 1.9 mg/kg per minute; $P < .05$) compared with group 1 (32.2 ± 2.3 mg/kg per minute). We did not detect any alterations in the rate of glucose disappearance during the clamp for any of the groups compared with group 1. A diet high in SAFA given to mothers during gestation and/or the weaning period does not seem to have deleterious effects on the insulin sensitivity in the offspring.

1. Introduction

Insulin resistance plays a critical role for the etiology of the metabolic syndrome and type 2 diabetes [1]. Although genetic heritability is associated with insulin resistance, the remarkably rapid increase in its prevalence reflects the fact that insulin resistance is also strongly influenced by lifestyle factors, for example, the dietary patterns [1-5] and sedentary lifestyle [6-8].

Fatty acids are known to have both short-term [9] and long-term effects on insulin sensitivity and secretion [10-12]. Changing both the fat content and the fatty acid profile of the diet in adult rodents potently influence insulin action with saturated fats (SAFAs) being particularly detrimental [13].

Although the role of fat amount and type is clear in adult animals, the effects during gestation are less well explored. Hales and Barker [14] put forward the thrifty phenotype hypothesis in an attempt to explain the association between poor fetal and infant growth and increased risk of developing insulin resistance and the metabolic syndrome in adult life. Certainly, early protein restriction has an effect on later impact of high saturated fat diets [15]; however, the effect of level and type of fat feeding on subsequent insulin action has not been explored.

The aim of the present study was to explore the possibility that a high-fat diet during gestation programs the glucose metabolism of the offspring of rats later in life in a diabetogenic direction. Consequently, we focused on the impact of a high carbohydrate (CHO)–rich vs a high fat–rich diet during gestation on whole body insulin sensitivity assessed by the euglycemic hyperinsulinemic clamp procedure in the offspring. A study design was established which enabled us to discriminate between diabetogenic

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effects evolved in uteri during the nursing and in the postweaning period.

2. Materials and methods

2.1. Experimental design

The rat feeding design is shown in Table 1. Nine-week-old Wistar female rats were weight-matched and divided into 2 groups. The rats were fed either a diet rich in SAFA or in CHO for 3 weeks before conception. Before mating, the male Wistar rats were on the same diets as the female rats. The female rats continued on either of the 2 diets through the gestational period (see Table 1). Male offsprings were divided into 5 groups (1-5), continuing on either of the 2 diets. Thus, groups 1 and 3, born by mothers on CHO or SAFA diet, respectively, were nursed by a mother on CHO or SAFA diet, respectively, and continued on the same diet until the end of the study. Animals in groups 2 and 4 were changed to the SAFA-rich and the CHO-rich diet, respectively, after the nursing period. Animals in group 5 were born by a mother on the SAFA-rich diet but were changed to a nursing mother on CHO diet immediately after birth and continued on CHO diet postweaning. Twelve animals from each group entered clamp studies whereas 12 parallel animals were used for sampling of blood for determination of circulating lipids.

2.2. Diet

Before entering the experiment the mother rats were fed a standard chow (Altromin, Lage, Germany). The composition of the CHO- and SAFA-rich diets is given in Table 2 (made by Altromin). The SAFA diet is based on 30% coconut oil. The total digestible energy in the CHO diet was 15 and 19 MJ/kg for the SAFA diet. The SAFA diet was in powder form whereas the CHO-rich diet was in pellet form. The content of fibers, vitamins, and minerals was slightly different in the 2 diets. Controlled feeding was carried out with ad libitum feeding on the CHO diet (pellet), and because the animal weights were similar throughout the study period, there was no need for intervention (eg, energy restriction) in the SAFA-fed groups.

Table 1
Experimental design

Group	−4 to 0 wk (mothers' diet)	0-4 wk (offspring nursed by mothers on diet)	4-16 wk (weanlings diet)
1	CHO	CHO	CHO
2	CHO	CHO	SAFA
3	SAFA	SAFA	SAFA
4	SAFA	SAFA	CHO
5	SAFA	CHO	CHO

The offspring of female rats fed with either CHO-rich diet (CHO, groups 1 and 2) or at diet rich in SAFA (groups 3-5) were nursed by mothers and later fed with CHO and SAFA diets as indicated in the table.

Table 2
Composition of the diets rich in CHO and SAFA

	CHO		SAFA	
	E%	g/kg	E%	g /kg
Fat	7.53	0	58.5	300
SAFA	0.7		51.0	
C10:0		–		19
C12:0		–		134
C14:0		–		57
C16:0		2		33
C18.0		1		22
Monounsaturated fat	1.7		3.3	
C18:1		7		17
Polyunsaturated fat	5.1		4.2	
C18:2		21		16
C18:3		–		6
CHOs	79.6	741	27.7	324
Monosaccharides	5.3	49	4.2	49
Disaccharides	71.9	670	12.1	141
Polysaccharides	2.4	22	11.4	134
Protein	13.7	123	14.6	171
Fiber		20		39
Mineral mix		27		40
Vitamin mix		17		17

Percentage of total energy (E%) and actual content (g/kg) are shown.

2.3. Bioimpedance

A multifrequent bioelectrical bioimpedance meter (SEAC, UniQuest Ltd, Brisbane, Australia) was used to estimate the percentage of body fat in the animals [16].

2.4. Euglycemic hyperinsulinemic clamp

At the age of 15 weeks, 12 animals from each group were equipped with intravascular catheters for blood sampling and infusion. The animals were anesthetized and the catheters were inserted into the right carotid artery and right external jugular vein and exteriorized at the neck. Animals were caged individually thereafter and allowed free access to food and water. After 5 days of recovery, the animals underwent the euglycemic hyperinsulinemic clamp.

The animals were fasted for 14 hours (water ad libitum) before the clamp experiments. The animals were conscious and freely moving in separate plastic cylinders. Human insulin (Actrapid, Novo Nordisk, Bagsværd, Denmark) was dissolved to a final concentration of 0.2 U/mL in 0.9% NaCl supplemented with 0.5% human serum albumin (essentially fatty acid–free, Sigma, St Louis, Mo). Glucose was prepared as a 30% D-glucose solution in 0.9% NaCl.

The first blood sample was drawn 20 minutes before the insulin and glucose infusion started and at subsequent 10-minute intervals for measurement of glucose on 20 μL whole blood on an YSI 2300 STAT Plus Glucose Analyzer (YSI Inc, Yellow Springs, Ohio). At time point 0 minutes, the insulin infusion started at a continuous rate of 0.25 U/h per kg body weight) and the glucose infusion was started 2 minutes after followed by variable infusion according to the prevailing blood glucose measurements. After stable euglycemia (3.5-4.0 mmol/L) was reached and maintained for 30 minutes,

a 300-μL mixture of 50 μCi ^{14}C-glucose and 50 μCi ^{3}H-deoxy-glucose was given as a bolus infusion. At time points 2, 5, 10, 15, 20, 25, and 30 minutes, hereafter, 200-μL blood samples were collected for measurement of plasma glucose and plasma-specific activity. A final 1000-μL sample was taken for measurement of plasma human insulin. After the final sample, the animal was killed by infusion of pentobarbital intravenously, and specific tissues immediately excised (see below) and frozen in liquid nitrogen (see below).

The Animal Experiments Inspectorate under the Danish Ministry of Justice has approved the study.

2.5. Tissue analysis

Tissue samples were collected from the liver, brown adipose tissue (BAT; interscapular), white adipose tissue (WAT; intra-abdominal), and from the following muscles: red quadriceps (RQ), white quadriceps (WQ), soleus (SOL), and heart (COR). The samples were frozen immediately at −80°C for later analysis.

The tissue samples were loaded into tubes containing 2.2 mL of 1 mol/L KOH and heated at 70°C for 40 minutes with continuous shaking (hereafter referred to as digests). Eight hundred microliters of each digest was used for the glucose uptake assay and lipogenesis, and two 100-μL aliquots of the digest were loaded onto filter paper for glycogen assays.

2.6. Lipid assay

Tissue digests were analyzed for the rate of lipogenesis by measuring the ^{14}C-labeled fatty acids. To each digest, 1 mL (100%) ethanol was added followed by 20-minute incubation at 70°C. Tubes were cooled in ice water whereafter 200-μL 9 mol/L H_2SO_4 was added and tubes rapidly shaken. Five milliliters of hexane were added, shaken, briefly centrifuged, and the top (organic) layer transferred to fresh centrifuge tubes. Five milliliters of H_2O was added and tubes were shaken and centrifuged. The organic (top layer) was transferred to scintillation vials.

Rate of lipogenesis was calculated using the equation: rate = ^{14}C counts (dpm)/tissue weight (g) × A, where A denotes the area under the ^{14}C-glucose disappearance curve until the time of killing of the animal.

2.7. Glucose uptake assay

The glucose uptake in individual tissues was determined by measurement of the accumulation of 2-[^{3}H]-deoxy glucose (2-[^{3}H]-DG) which is phosphorylated and thus trapped within the cells without being further metabolized [17].

The digests were neutralized and centrifuged (10 000g, 3 minutes, room temperature). To separate 2-[^{3}H]-DG and 2-[^{3}H]-deoxyglucose phosphate (2-[^{3}H]-DGP), the supernatant (700 μL) was loaded onto pre-prepared Dowex-2-formate columns (Poly-Prep; Bio-Rad, Hercules, Calif). 2-[^{3}H]-DG was removed by washing the columns with water (5 times, 1 mL) and Dowex-bound 2-[^{3}H]-DGP was finally removed by washing with 1 mol/L formate−0.3 mol/L ammonium formate (6 times, 1 mL). One milliliter of the pooled formate wash was then counted in 5-mL scintillation fluid (EcoScint A, National Diagnostics, Georgia, USA). Glucose uptake was assessed using the same equation as described for determination of lipogenesis.

2.8. Glycogen synthesis

The digest was dried on filter paper and transferred to scintillation vials. Precipitation of glycogen on the squares was achieved by addition of 3 mL 70% ethanol to each vial. An ethanol precipitation was carried out twice followed by addition of 1 mL acetone to each vial, which was then allowed to dry overnight. Toluene/2,5-diphenyloxazole (PPO) (6 g/L, 5 mL) was added to each vial and then counted for ^{14}C.

2.9. Total glycogen content

The digest was handled as mentioned above with precipitation with ethanol and acetone drying. Thereafter, the filter papers were incubated at room temperature for 45 minutes with glucose assay mixture (2 mL, Boehringer Mannheim, Mannheim, Germany) and 200 μg amylase (Boehringer Mannheim). Type 2 glycogen (Sigma) was used to plot a standard curve. The amount of glycogen was determined colorimetrically. The total glycogen content is expressed as microgram of glycogen per gram of tissue.

2.10. Analysis of blood parameters

Human insulin was determined using enzyme-linked immunosorbent assay (DAKO Insulin, K6219, DAKO Diagnostics Ltd, Cambridgeshire, UK). Blood glucose was determined using the Glucose Oxidase method (GOD-PAP, Boehringer Mannheim). Triglyceride, free fatty acids, and total cholesterol were determined using colorimetric kits (Boehringer Mannheim).

2.11. Statistics

Group 1 (receiving the CHO-rich diet during the whole experiment) is referred to as the control group. Analysis of variance (ANOVA) was used to assess overall differences between groups, and if significant, Newman-Keul test for group differences compared with group 1 (control) was performed. When data were not normally distributed, even after transformation, Kruskal-Wallis test was used to assess overall differences between groups. If significant, Mann-Whitney test was used to compare differences between experimental groups and the control group. Bonferroni correction was applied to adjust for multiple comparisons. Results are presented as mean ± SEM.

3. Results

No differences in the average weights of the offspring were found between the groups at weeks 4 and 16, respectively (Table 3). Thus, the mean weights of animals

Table 3
Animal weights (g) for the 5 experimental groups (1-5) at the start and at the end of the study (weeks 4 and 16, respectively; n = 24 in each group)

	Group					
	1	2	3	4	5	*P*
Weight at week 4	78 ± 2	83 ± 2	83 ± 2	81 ± 1	77 ± 1	NS
Weight at week 16	222 ± 2	224 ± 7	225 ± 7	225 ± 5	212 ± 3	NS
Fat mass (%)	32 ± 2	37 ± 2	42 ± 1*	35 ± 1	34 ± 2	<.01

Percentage of body fat was determined by bioimpedance at week 16 (see Materials and methods; n = 12 in each group). Data given as mean ± SEM. NS indicates not significant.

* $P < .01$ compared to group 1.

entering the clamp study did not differ, but the percentage of body fat was higher in group 3 compared with the control group.

3.1. Triglycerides, free fatty acids, and cholesterol

Plasma triglycerides were different among groups (ANOVA: $P < .001$, n = 10-12 in each group) with significantly higher levels in groups 2 (1.03 ± 0.09 mmol/L) and 3 (1.12 ± 0.16 mmol/L) compared with the control group 1 (0.63 ± 0.09 mmol/L). The circulating levels of free fatty acids and cholesterol did not differ among groups. The average of all groups for free fatty acids was 0.66 ± 0.03 mmol/L and for cholesterol 1.97 ± 0.06 mmol/L.

3.2. Glucose metabolism

Stable euglycemia was assured by measurements on whole blood before tracer infusion and no significant changes in blood glucose during the 30-minute sampling period were found (data not shown). Circulating human insulin levels were measured at the end of the clamp. As has been noted in many other studies, there was a tendency toward a higher clamp insulin level in groups with evidence of impaired insulin action (Table 4). The glucose infusion rates (GIRs) during the stable phase of the euglycemic clamp are shown in Fig. 1. Analysis of variance analysis showed differences between groups ($P = .0079$) and test for group differences showed lower GIR in groups 2 and 3 compared with the control group.

When attempting to correct for the tendency toward higher circulating insulin levels in groups 2 and 3, the *P* value was .0019 (dividing GIR by human insulin level).

The calculated rate of disappearance of glucose is shown in Fig. 2. No significant differences were detected for any of

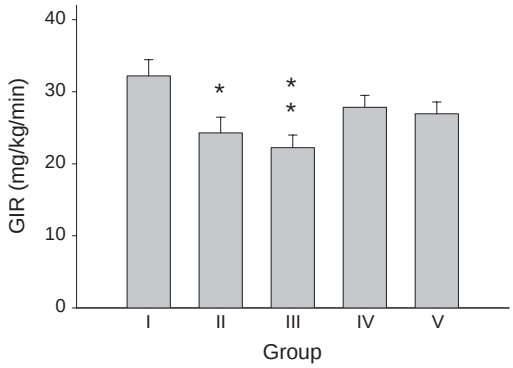

Fig. 1. Steady-state GIRs in the 5 experimental groups during the euglycemic hyperinsulinemic clamp. Results are presented as mean ± SEM; n = 9-12 in each group. *$P < .05$; **$P < .01$ compared with group 1.

the groups compared with the control group. Hepatic glucose output for individual animals was calculated as GIR-Rd. No differences between groups were detected (ANOVA: $P = .17$) (Table 4).

3.3. Glucose uptake

The glucose uptake in individual insulin-sensitive tissues is shown in Table 5. The only significant difference was found in WAT in group 5 compared with the control. Taken as a whole, the order of glucose uptake rates was BAT COR > SOL > RQ > WQ > WAT.

3.4. Lipid synthesis

The incorporation of ^{14}C-glucose into lipid in the individual insulin-sensitive tissues is shown in Table 5. Lipid synthesis rates in BAT were lower in groups 2, 3, and 5 compared with the control group. Taken as a whole, the order of glucose incorporation into lipid was BAT > WAT > liver > COR > SOL > RQ > WQ.

3.5. Glycogen synthesis and glycogen content

The glycogen synthesis was measured as the incorporation of ^{14}C-glucose into glycogen. The rates are shown in Table 5 for the individual insulin-sensitive tissues. No significant differences were detected for groups 2 to 5 compared with group 1 in any of the tissues.

The rate of glycogen synthesis in BAT was greater than the remaining tissues, which have comparable rates also in liver tissue.

We also measured the glycogen content in the same tissues. We observed a lower glycogen content in BAT for

Table 4
Data from the euglycemic hyperinsulinemic clamps

	Group					
	1	2	3	4	5	ANOVA
HGO (mg/kg per minute)	2.7 ± 2.2	1.1 ± 1.7	−1.7 ± 0.9	−1.1 ± 1.5	2.4 ± 1.5	NS
Human insulin (pmol/L)	419 ± 21	457 ± 61	529 ± 37	400 ± 50	392 ± 18	NS

Hepatic glucose output (HGO) was calculated as GIR-Rd. Levels of circulating human insulin after the clamp (t = 30 minutes) are shown. Results are presented as mean ± SEM; n = 9-12 in each group.

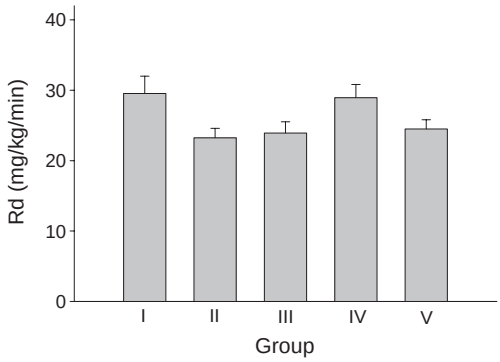

Fig. 2. Steady-state rate of glucose disappearance (R_d) of ^{14}C-glucose in the 5 experimental groups. ^{14}C-glucose was infused as a bolus during euglycemia. Results are presented as mean ± SEM; n = 9-12 in each group.

group 3, whereas in the liver the glycogen content was significantly increased in groups 2 and 3 compared with group 1.

4. Discussion

In the present study, we investigated if a diet rich in SAFA ingested during pregnancy influences glucose metabolism later in life. The effect was compared with that of a CHO-rich diet. The study design enabled us to differentiate the impact of SAFA during gestation, in the nursing period, and postweaning. The euglycemic hyperinsulinemic clamp technique was applied together with analysis of glucose uptake, lipogenesis and glycogen synthesis, and total glycogen content in key metabolic tissues. A reduced insulin sensitivíty was only observed in the 2 groups of animals still on a high-fat diet (Fig. 1). This was corroborated by our finding of a slightly higher percentage of body fat in group 3 compared with the control group.

Taken as a whole, the well-known reduction in the insulin sensitivity induced by high-fat feeding is confirmed. Our results, however, do not support the hypothesis that a SAFA-rich diet during pregnancy is deleterious

Table 5
Metabolic parameters in insulin-sensitive tissues for the 5 experimental groups

	Group					
	1	2	3	4	5	*P*
Glucose-uptake (nmol 2-DGP/min per g tissue)						
WAT	14 ± 3	9 ± 1	9 ± 1	10 ± 2	7 ± 1*	.02
BAT	868 ± 82	661 ± 92	889 ± 84	962 ± 201	861 ± 128	NS
RQ	59 ± 9	54 ± 7	43 ± 4	62 ± 9	59 ± 8	NS
SOL	82 ± 6	73 ± 8	71 ± 6	98 ± 7	65 ± 9	NS
WQ	28 ± 2	31 ± 5	23 ± 3	35 ± 6	29 ± 3	NS
COR	192 ± 36	116 ± 13	138 ± 17	178 ± 14	160 ± 18	NS
Rate of lipid synthesis (nmol/min per g tissue)						
WAT	55.5 ± 12.5	44.1 ± 8.6	36.6 ± 8.2	43.9 ± 11.4	26.7 ± 9.7	NS
BAT	10319 ± 705	5877 ± 558*	6561 ± 932*	10298 ± 1915	7952 ± 478*	.05
RQ	3.2 ± 0.3	2.3 ± 0.4	3.4 ± 0.9	3.3 ± 0.5	2.5 ± 0.5	NS
SOL	6.1 ± 1.0	3.4 ± 0.4	3.8 ± 0.4	5.3 ± 0.5	4.2 ± 0.8	NS
WQ	1.8 ± 0.2	1.2 ± 0.1	1.4 ± 0.1	2.0 ± 0.3	1.6 ± 0.2	NS
COR	8.6 ± 1.1	6.0 ± 2.0	4.8 ± 0.8	7.2 ± 1.2	10.1 ± 4.1	NS
Liver	29.0 ± 10.1	16.2 ± 1.8	18.3 ± 2.6	22.0 ± 3.4	26.7 ± 11.0	NS
Rate of glycogen synthesis (nmol/min per g tissue)						
WAT	17.9 ± 8.9	9.2 ± 1.9	10.7 ± 3.0	10.4 ± 2.4	12.9 ± 4.6	NS
BAT	850 ± 124	506 ± 64	492 ± 92	671 ± 140	608 ± 71	NS
RQ	32.6 ± 7.7	33.6 ± 3.0	26.6 ± 5.5	39.5 ± 8.9	27.6 ± 3.9	NS
SOL	39.5 ± 9.2	42.8 ± 9.8	23.9 ± 3.9	49.6 ± 8.3	30.2 ± 4.2	NS
WQ	11.7 ± 3.0	16.8 ± 3.2	13.2 ± 2.7	16.5 ± 2.5	12.9 ± 1.4	NS
COR	15.2 ± 1.8	13.6 ± 1.0	15.2 ± 1.6	13.2 ± 1.7	13.8 ± 2.1	NS
Liver	34.3 ± 1.8	31.5 ± 2.6	43.0 ± 4.3	36.7 ± 1.5	38.7 ± 3.8	NS
Glycogen content (μg/g tissue)						
WAT	1.4 ± 0.1	1.1 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	NS
BAT	105 ± 13	70 ± 7	30 ± 7*	62 ± 20	94 ± 12	.01
RQ	47 ± 7	42 ± 4	36 ± 3	32 ± 5	38 ± 4	NS
SOL	29 ± 6	26 ± 3	16 ± 3	22 ± 3	21 ± 2	NS
WQ	38 ± 2	37 ± 5	32 ± 3	31 ± 3	34 ± 5	NS
COR	20 ± 2	16 ± 3	17 ± 3	16 ± 2	17 ± 3	NS
Liver	6 ± 3	30 ± 12*	19 ± 4*	5 ± 2	8 ± 3	.05

Glucose ([^{3}H]2-deoxy-D-glucose) uptake in individual tissues in rats during euglycemic hyperinsulinemic clamp. Rate of lipid synthesis was determined as uptake of ^{14}C-glucose into lipid. Rate of glycogen synthesis was determined as uptake of ^{14}C-glucose into glycogen. Total glycogen content was expressed as milligram of glycogen per gram of tissue. See Materials and methods for statistical and biochemical analysis. Results are presented as mean ± SEM. Significant differences compared with group 1 (control) are marked with asterisks; n = 9-12 animals in each group.

on the insulin sensitivity later in life. Storlien et al [18] have previously shown that the type of dietary fat may affect insulin sensitivity in rats by changing the fatty acid composition of the membrane lipids. A higher proportion of SAFA may impair insulin signaling by decreasing membrane fluidity. Consistent with this, the proportion of SAFA in skeletal muscle membrane lipids was negatively associated with insulin sensitivity in humans [19]. A change in the proportion of fatty acids, reducing SAFA and increasing monounsaturated fat induced a significant improvement in insulin sensitivity in humans on a diet with a fat content below 37% E% [5].

In the present study, we found that dietary SAFA during gestation alone or gestation and nursing period does not lower the insulin sensitivity of the offspring. On the other hand, as expected, the diet after the weaning period (ie, from week 4 to week 16) critically influences the insulin sensitivity. In addition to inducing lower whole body glucose uptake the SAFA diet also elevated the triglyceride levels corroborating a diabetogenic tendency.

The high-fat diet before and during pregnancy did not, in our rats, induce increased body weight in the offspring in the postweaning period. In our experiments, we used a controlled feeding design with ad libitum feeding on the starch-rich, pellet food. The waste of food of the fat-rich, powder food appeared higher than on the pellet diet, probably because of its powder texture. It was not possible exactly to differentiate between the powder food and feces; consequently, we were unable to exactly determine the food intake.

Most commercial laboratory rat diets contain 4% to 6% dietary fat and this amount is adequate for growth and maintenance of rats. The National Research Council [20] sets a requirement of 5% dietary fat. In addition, no definite CHO requirement has been established for rats [21]. We consider the CHO-rich diet a suitable control diet when comparing it with a diet rich in saturated fatty acids. Our study contrasts with one other study focusing on the impact of high-fat feeding during pregnancy on the offspring metabolism. Guo [22] found that a high-fat diet (40 E%) caused the weanlings to weigh more and have higher blood glucose and triglyceride levels than the low-fat (4.5 E%) weanlings. The reason for this difference is unclear. However, the present results are consistent with those of Elton et al [23] in terms of weight gain. The latter study has reported results that are somewhat in contrast to the present in relation to offspring insulin sensitivity. This study of Elton et al [23], however, is very difficult to interpret. Apparently, profound reduction in basal and insulin-stimulated glucose uptake in skeletal muscle was found in offspring of high fat–fed mothers. However, this contrasts with actually lower basal serum glucose and insulin levels, which would indicate, if anything, improved insulin sensitivity. In our study, despite an apparent reduction of insulin sensitivity in individual muscles of some 75%, whole body glucose disposal was only changed by some 13%. We have no explanation for these discrepancies.

Our results may be more comparable to the human condition where somewhat higher fat in the diet has been shown to have positive impact upon glucose metabolism during pregnancy in human mothers and certainly, a low polyunsatured-to-saturated fat ratio consistently seems to impact negatively on maternal glucose metabolism [24,25]. Dietary recommendations during pregnancy are a controversial topic, with little evidence for the currently recommended high-CHO diet.

One other study that has examined another aspect of metabolic syndrome, namely, hypertension, after a high-fat diet during pregnancy found a higher blood pressure in female offspring fed with a high-fat diet than those on low-fat diet [26]. Had we studied female rats, we may have found more pronounced effects on glucose and lipid metabolism.

We introduced the potentially diabetogenic diet 3 weeks before mating to ensure that the animals were metabolically equilibrated before mating. It cannot be ruled out that feeding the mother with the SAFA-rich diet for even longer periods than 3 weeks would have had a negative effect on the insulin sensitivity of the offspring.

However, duration of 3 to 4 weeks on a SAFA-rich diet should be sufficient to lower insulin sensitivity. In this context, it is noteworthy that it has previously been shown that vascular dysfunction develops in offspring of normal and diabetic rats after high-fat feeding [27].

The observed changes in whole body insulin sensitivity would be expected to correlate with the glucose uptake in insulin-sensitive tissues. However, by measuring the DGP uptake we were not able to detect consistent patterns of decreases in the tissue-specific glucose uptake between individual groups. We cannot exclude the possibility that the change in whole body insulin resistance is too small to detect on individual tissues and what we detect as the decreased whole body glucose uptake during the clamp is the sum of minor changes that only become significant when accumulated. The same may be valid for the lipid and glycogen synthesis where no consistency in the patterns was detected. In this regard, we have no good explanation for the observation that levels of glycogen synthesis and content apparently were not higher than that found in other insulin-sensitive tissues, as would have been expected. In this context, we must emphasize, however, that our aim was not to compare different tissues, rather to detect changes between groups. In general, the high metabolic rate of BAT is confirmed for both the glucose uptake and lipid synthesis.

It should be noted that gestation is very different in the rat vs human. Rats are relatively immature at birth and their growth rate is substantially greater than that of humans. The equivalent day conversion is about 1 rat-day equals 30 human-days.

The content of fibers, vitamins, and minerals was nearly similar in the commercially produced diets and both diets conform with current knowledge on sufficient amounts of these substances to prevent deficiencies [20].

From epidemiologic studies in humans [28], total fat and saturated fatty acid intakes are not associated with the risk of type 2 diabetes in women, whereas high *trans* fat intake is associated with increased and high polyunsaturated fat intake associated with reduced risk. Also in men, the intake of total fat and SAFA was not associated with a higher risk of type 2 diabetes after adjustment for BMI [29].

As mentioned earlier we did not detect differences in the fasting blood glucose levels between groups and, therefore, it may be argued that the intrauterine environment may not have been disturbed to a sufficient degree to induce detectable metabolic changes later in life. In this respect, it is worth noting that induction of even mild hyperglycemia solely during the late part of gestation can induce impaired insulin secretion and glucose intolerance in offspring [30], but that may have more to do with impairment of neural regulation of insulin secretion than to impairment in insulin action.

In contrast to what has been observed during famine and selective malnutrition (eg, protein deprivation), it seems that high-fat diet during pregnancy and the weaning period in normal rats does not induce detrimental effects on insulin sensitivity at least at the age of 16 weeks. We do not know if the present data can be extrapolated to human beings.

In conclusion, taking into account the extreme dietary manipulation we instituted and the rather negative results, it does not seem probable that it should be more deleterious during gestation to ingest a high-fat diet as compared with a high-CHO diet.

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