The Review of DIABETIC STUDIES

ORIGINAL DATA

High-Fat Feeding During Gestation and Nursing Period have Differential Effects on the Insulin Secretory Capacity in Offspring from Normal Wistar Rats

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■ Abstract

Restriction of protein or energy intake during gestation or early life is linked to developmental defects in the endocrine pancreas and insulin resistance. AIMS: To study whether a saturated fatty acid-rich diet during gestation and/or after the weaning period may be detrimental to the insulin secretory capacity later in life. STUDY DESIGN: Female Wistar rats were fed diets rich in carbohydrate (CHO) or saturated fat (SAFA) during pregnancy. The male offspring were split into five subgroups: after birth group 1 (control) continued on CHO and group 3 on SAFA. Group 2 continued on the CHO diet during the nursing period but changed to SAFA post weaning. Group 4 continued on SAFA, but changed to CHO post weaning. For group 5 the offspring of mothers given a SAFA diet were changed to nursing mothers on a CHO diet immediately after birth, and continued on the same diet post weaning. After 14 wk, the islets of Langerhans were isolated for determination of insulin secretory capacity in static incubation and dynamic perifusion experiments. RESULTS: We found a negative correlation (Coef: -3.1, 95% CI: -6.1 to -0.0, $p < 0.05$) between a diet rich in saturated fat fed to mothers during gestation and a positive correlation (Coef: 4.4, 95% CI: 0.9 to 7.8, $p = 0.01$) between nursing mothers' diet and the capability to secrete insulin in the offspring. CONCLUSION: Our results indicate the importance of applying a nutrient-balanced diet during pregnancy and the nursing period on the later insulin secretory capacity in the offspring.

Keywords: dietary fats · gestation · insulin · islet of Langerhans · rats

Introduction

The rapid increase in type 2 diabetes indicates that environmental factors e.g. the diet and physical inactivity, in addition to genetic disposition, are of major importance for the development of the disease. Both the amount and the individual subtypes of dietary fats seem to influence insulin sensitivity [1]. Insulin resistance is a major player in the etiology of type 2 diabetes, which requires enhanced insulin secretion to maintain normal intermediary metabolism. If enhanced insulin secretion is not able to compensate the insulin resistance, type 2 diabetes develops [2, 3].

Hales and Barker [4] have hypothesized that type 2 diabetes may be programmed *in utero* i.e. that intrauterine malnutrition would lead to a low birth weight and increased the risk of type 2 diabetes later in life.

Experimental investigation on organogenesis shows the pivotal role of adequate protein availability as well as total caloric intake [5, 6]. Amino acid metabolism in

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the feto-maternal unit appears to play a key role for the development of organs involved in chronic disease e.g. diabetes in offspring [7, 8]. Experimental studies have also highlighted the influence of carbohydrate metabolism on the fetus, i.e. diabetes in pregnant rat, has developmental effects on the endocrine pancreas [9].

Insulin secretion can be differentially influenced by individual fatty acids [10-12]. *In vitro* studies have shown that long-term exposure of pancreatic β-cells to high levels of fatty acids has a detrimental effect on insulin secretion [13] corresponding to changes in a large number of genes involved in fatty acid and glucose metabolism as well as insulin signal transduction [14]. Changing both the fat content and the fatty acid profile of the diet in rodents [12, 15] and humans [16] potently influence insulin action and secretion, with saturated fats being particularly detrimental. Siemelink *et al.* [12] studied the impact of fatty acid composition of the maternal diet (unsaturated vs. saturated fat-rich diets) on fetal and postnatal growth, morphology of the pancreas and glucose metabolism in adult offspring. Interestingly, they found that the nutritional fatty acid balance during development programs pancreatic responsiveness, observable as early as at 12 wk of age [12]. The maternal diet rich in saturated fat (SAFA), compared to a maternal diet rich in unsaturated fat, caused an increased insulin response at week 12 in the offspring of the SAFA group [12].

This raises the question whether an abundant amount of saturated fat during gestation may directly or indirectly be detrimental to the secretory responses of the pancreatic β-cells in the offspring. Consequently, we carried out the present study to elucidate whether the amount of saturated fat in the diet of mothers during the gestational or nursing period may induce a subsequent alteration in the β-cell function in the offspring.

Materials and methods

Experiment design

The present study on islet cell function is a separate part of a previously reported study [17].

Nine-week-old, female Wistar rats were weight-matched and divided into two groups. The animals were fed a diet rich in either saturated fatty acids (SAFA) or carbohydrates (CHO) for 3 wk until the mating. The animals continued on the same diet during pregnancy.

After birth, the male offspring were divided into 5 groups, and fed the different diets according to Table 1. Thus, groups 1 and 3 were born and nursed by mothers on a CHO or SAFA diet, respectively, and continued on the same diet until the end of the study. Groups 2 and 4 were born and nursed by mothers on a CHO or SAFA-rich diet, respectively, but changed to the "opposite" diet at the time of weaning. The offspring in group 5 were born by mothers on a fat-rich diet, but we removed the newborn pups from their biological mother and placed them with nursing mothers receiving a carbohydrate-rich diet. These animals continued on the CHO diet after weaning. Thus, we divided the offspring of mothers on a CHO diet into two groups, whereas those on the SAFA diet were divided into three groups. Each subgroup of offspring consisted of 12 rats. We transferred the offspring from the breading facilities (Taconic M&B, Denmark) to our own housing facilities when they were thirteen wk old. The animals acclimatized for one week, and continued on the diets described in Table 1 for this period. The animal facility was temperature-controlled (22-24°C) with a 12:12-h light-dark cycle.

The animals were handled in accordance with Danish law, with the approval of the Animal Experiments Inspectorate under the Ministry of Justice.

Table 1. Experimental design of studies using diets rich in carbohydrate (CHO) or saturated fat (SAFA)

Group	A: Mothers' diet	B: Offspring nursed by mothers on diet	C: Offspring on diet
1	CHO	CHO	CHO
2	CHO	CHO	SAFA
3	SAFA	SAFA	SAFA
4	SAFA	SAFA	CHO
5	SAFA	CHO	CHO

Legend: the study period can be divided into three sections: **Period A** including preconception, conception and gestation, **Period B** representing the nursing period, and **Period C** from wk 4-14 which represent the period from weaning until the end of the study. Each group of offspring consisted of 12 animals.

Diet

The two diets used in this experiment were close to iso-caloric. The diets differed in their content of saturated fatty acids and carbohydrates, but had the same protein content. The diet rich in saturated fatty acids (SAFA) was composed of 60 E% fat (which were mostly saturated fatty acids), 25 E% carbohydrates, and 15 E% protein. The carbohydrate rich diet (CHO) contained 10 E% fat, 75 E% carbohydrates, and 15 E% protein. Consequently, we switched 50 energy percent from fat in the SAFA diet to carbohydrate in the

CHO-rich diet. We have previously published the specific diet compositions [17].

Table 2. Characteristics of the offspring of mothers fed on diets rich in carbohydrate (CHO) or saturated fat (SAFA)

Parameter	CHO	SAFA	p-value
Litter weight (total g)	71.2 ± 2.4	69.2 ± 2.4	ns
Male pups (n)	5.2 ± 0.3	5.5 ± 0.3	ns
Female pups (n)	5.8 ± 0.3	5.1 ± 0.3	ns
Offspring (total n)	10.9 ± 0.4	10.6 ± 0.4	ns
Offspring weight (g)	6.6 ± 0.1	6.7 ± 0.1	ns

Legend: data were collected at time of birth, before division into subgroups. ns: not significant. Data given as mean ± SEM (n = 46-63).

Body weight and food consumption

The animals were weighed on a regular basis. At the age of 4 wk the offspring were marked individually, and after this time the groups only contained the animals that were later part of the experiment. We would like to stress that we separated the rat offspring into five groups immediately after birth. The food consumption was monitored throughout the study period. Controlled feeding was carried out with *ad libitum* feeding on the CHO diet (pellet), and since the animal weights were similar throughout the study period, there was no need for intervention (e.g. energy restriction) in the SAFA-fed groups.

Blood samples

Blood samples were collected using the retro-orbital method [18], with the animals slightly sedated with pentobarbital (30 mg/kg BW), after overnight fasting at the age of 8 and 14 wk. Blood samples were collected on ice in tubes containing heparin/aprotinine (10 µl/ml blood) (Løvens Kemiske Fabrik, Ballerup, Denmark), rapidly centrifuged (4000 g, 60 sec, 4°C) and frozen for subsequent analysis of insulin, leptin and glucose at the age of 8 wk and insulin at age 14 wk. At the termination of the study, we collected a sample of whole blood for analysis of glycated hemoglobin (Table 4).

Islet isolation

Prior to experiments, the animals were fasted for 12h. Islets were isolated by the collagenase digestion technique [19]. In brief, the animals were anesthetized by injection of pentobarbital (50 mg/kg BW intraperitoneally), and a midline laparotomy was performed. The pancreas was retrogradely filled with 10 ml ice-cold Hanks balanced salt solution (HBSS, Sigma Chemical, St Louis, MO, USA) supplemented with 0.3 mg/ml collagenase P (Boehringer Mannheim GmbH, Mannheim, Germany) through the pancreatic duct. The pancreas was subsequently removed and placed on ice in a glass tube, until digestion at 37°C for 16.5 min. After incubation, HBSS was added and the tubes were shaken rigorously. After sedimentation, the suspension was washed three times with HBSS. The islets were then collected under a microscope and incubated overnight at 37°C and 95% normal atmosphere/5% CO_2 in 10 ml RPMI 1640 containing 11.1 mM glucose supplemented with 10% fetal calf serum, 2.06 mM L-glutamine, 100 IU/ml penicillin G and 100 µg/ml streptomycin (all GIBCO BRL, Paisley, UK) to recover. The islets originating from each animal were kept separate during the whole experiment.

Table 3. Animal weight (g) measured after weaning and at the end of the study in the five experimental groups

Week	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
4	78.0 ± 2.0	83.0 ± 2.0	83.0 ± 2.0	81.0 ± 1.0	77.0 ± 1.0	ns
14	227.0 ± 3.0	226.0 ± 4.0	227.0 ± 5.0	233.0 ± 3.0	228.0 ± 4.0	ns

Legend: Values are mean ± SEM (n = 23-26). ns: not significant compared to group 1 (control). The experiment design for the five groups is provided in Table 1.

Incubation studies

After overnight culture, the islets were rinsed twice with a modified Krebs-Ringer buffer (KRB) supplemented with 2.0 mM glucose and 0.1 % human serum albumin (Sigma). The KRB contained 125 mM NaCl, 5.9 mM KCL, 1.2 mM $MgCl_2$, 1.28 mM $CaCl_2$ and 25 mM HEPES (pH 7.4; all Sigma), and the islets were then pre-incubated for 1 h at 37°C in normal atmosphere. Islets from each animal were used in 8 single islet incubations with 100 µl of the modified KRB-buffer supplemented with 2.0 mM glucose, 11.1 mM

glucose, 16.7 mM glucose, 2.0 mM glucose with 5 mM leucine, or 11.1 mM glucose with 0.04, 0.4 mM palmitate, or 0.1 μM glucagon-like peptide 1 (GLP-1), respectively. After 1 h incubation at 37°C, 50 μl from each well were collected and frozen for later insulin analysis.

Pharmacia Mono S HR 5/5 cation-exchange column, and standardized according to recommendations set by the International Federation for Clinical Chemistry.

Insulin sampled in the perifusion and incubation experiments was determined by RIA with a guinea-pig anti-porcine insulin antibody (PNILGP4, Novo Nord-

Table 4. Metabolic parameters

	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
Week 8						
Glucose (mM)	6.59 ± 0.48	5.68 ± 0.22	5.61 ± 0.36	6.42 ± 0.37	5.46 ± 0.48	ns
Insulin (ng/ml)	0.18 ± 0.06	0.13 ± 0.13	0.09 ± 0.02	0.14 ± 0.02	0.14 ± 0.02	ns
Leptin (ng/ml)	2.14 ± 0.41	2.27 ± 0.33	2.36 ± 0.37	2.60 ± 0.22	1.89 ± 0.21	ns
Week 14						
Insulin (ng/ml)	0.59 ± 0.22	1.08 ± 0.19	1.19 ± 0.23	0.74 ± 0.20	0.91 ± 0.21	ns
HbA1c (%)	1.63 ± 0.04	1.64 ± 0.04	1.69 ± 0.04	1.66 ± 0.03	1.70 ± 0.02	ns

Legend: Metabolic parameters collected during the study period. Blood samples collected at the age of 8 and 14 wk (n = 12 animals per group). Glycated hemoglobin (HbA1c) was analyzed on whole blood samples (n = 6 animals per group). Values are mean ± SEM. ns: not significant compared to group 1 (control). The experimental design for the five groups is provided in Table 1.

Perifusion studies

We performed six perifusion experiments simultaneously using a Brandel Suprafusion 2500 apparatus (Brandel Inc., Gaithersburg, MD USA). Each perifusion chamber (300 μl) contained a gel (Biogel P4, Pharmacia, Uppsala, Sweden), and we perfused 10 islets with a modified KRB-buffer (see above) supplemented with stimulatory agents as described below.

Ten min basal perifusion at 2.0 mM glucose initiated each perifusion experiment. The perifusion experiment consisted of three stimulatory periods with 11.0 mM glucose, 25.0 mM glucose and 11.1 mM glucose +0.1 μM GLP-1 each for 20 min, respectively. Each stimulation period was separated by 30 min basal perifusion with 2.0 mM glucose.

All perifusion buffers were temperated at 37°C. The labile GLP-1 was added just before use to protect it from destruction. Fractions of the perifusion medium were collected for 2-min periods and frozen for later analysis. Total duration of the experiments was 200 min.

Analysis

Blood glucose was determined using the Glucose Oxidase method (GOD-PAP, Boehringer Mannheim, Germany). Blood samples were analyzed for leptin and rat insulin using sensitive radioimmunoassay kits (both Linco Res. Inc., Mo., USA). Glycated hemoglobin was measured by HPLC (Hitachi, Tokyo, Japan) with the

isk, Bagsvaerd, Denmark) and mono-^{125}I-(Tyr A14) labeled human insulin (Novo Nordisk) as tracer and rat insulin (Novo Nordisk) as standard. Free and bound radioactivity was separated using ethanol [20]. Inter- and intra assay variation was below 10%.

Insulin biosynthesis

The islets were washed three times in modified MEM (Minimal Essential Medium (Eagle) from GIBCO purchased without glucose or leucine) supplemented with 8.3 mM glucose and 10% (v/v) Nuserum (Invitrogen, Taastrup, Denmark) whereafter the islets were pre-incubated for 30 min in the same medium (37ºC, 95% O_2, 5% CO_2). The islets were divided into groups of 10 and incubated for 90 min at 37ºC (95% O_2, 5% CO_2) in 250 μl of the modified MEM supplemented with 10 μCi 4,5-^{3}H(N)-Leucine (specific activity 52 Ci/mmol; Amersham Pharmacia Biosciences, Hillerød, Denmark) and supplemented with different concentrations of glucose and palmitate (see results). After incubation, the medium was removed for insulin measurements and the islets were washed seven times with modified ice-cold MEM supplemented with 2.8 mM glucose. Then, the islets were transferred to microfuge vials containing 1 ml of ice-cold glycine-BSA (750.7 mg glycine, 250 mg bovine serum albumin (Sigma, MO, USA), 100 ml of distilled water, pH 8.8 adjusted with NaOH) and sonicated in an ice-bath (2 x 15 s, setting 4, (Branson, Danbury, CT, USA)). The

suspension was hereafter placed in an ultra-centrifuge (30000 g, 30 min, 4ºC), and the supernatant removed for determination of specific insulin binding and total islet insulin.

Table 5. Insulin secretion responses during static incubation of isolated islets of Langerhans

Medium	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
2.0 mM glucose	1.8 ± 0.2	1.9 ± 0.2	1.9 ± 0.1	1.8 ± 0.2	1.7 ± 0.2	ns
2.0 mM glucose +5 mM leucine	3.8 ± 0.5	4.2 ± 0.7	4.5 ± 0.6	5.0 ± 0.7	2.7 ± 0.3	ns
11.1 mM glucose	11.5 ± 0.9	10.0 ± 0.6	12.3 ± 0.8	13.1 ± 1.0	9.6 ± 0.9	ns
11.1 mM glucose +0.04 mM palmitate	9.8 ± 0.8	10.6 ± 0.8	10.7 ± 0.8	11.3 ± 0.9	7.5 ± 0.7	ns
11.1 mM glucose +0.4 mM palmitate	17.1 ± 1.2	19.0 ± 1.0	19.8 ± 1.6	21.8 ± 2.0	13.4 ± 1.4*	0.01
11.1 mM glucose +0.1 µM GLP-1	27.5 ± 2.2	29.0 ± 2.0	24.8 ± 1.7	25.4 ± 1.9	16.5 ± 1.7*	0.01
16.7 mM glucose	13.3 ± 1.2	13.3 ± 1.0	16.3 ± 1.1	13.8 ± 1.1	11.2 ± 1.0	ns
25.0 mM glucose	12.8 ± 1.1	13.6 ± 0.8	14.8 ± 1.0	15.4 ± 1.1	10.8 ± 0.9	ns

Legend: Incubation experiments were performed as described in the methods section. The insulin concentration (ng/ml) is shown as mean ± SEM. Islets from each animal were used in 8 single islet incubations in 8 well racks with 100 µl medium in each well, and each well counts as one experiment. (n = 52-87 single experiments for each condition per group n = 9-12 animals per group). Asterisk (*) denotes $p < 0.05$ compared to group 1 (control). ns: not significant compared to group 1 (control). The experiment design for the five groups is provided in Table 1.

Immunoprecipitation

300 µl of a glycine-BSA-NP40-protein-A Sepharose solution (3 ml of glycine-BSA-NP40 was supplemented with 150 mg protein-A-Sepharose (CL4B, Pharmacia)) mixed to homogeneity, and washed 2 times with 1 ml glycine-BSA-NP40 and centrifuged (8000 g, 30 s) between each wash. After the last wash, 1 ml glycine-BSA was added to each tube. The tubes were supplemented with 10 µl guinea pig anti-insulin serum (AIS, donated by Novo Nordisk) or 10 µl guinea pig normal serum (NS, Sigma) and mixed for 60 min at room temperature. Hereafter, the tubes were centrifuged (8000 g, 30 s) and washed with 1 ml glycine-BSA-NP40 three times. We added 1.5 ml of glycine-BSA-NP40 to each vial, and thereafter 10 µl of the islet sonicate was mixed for 60 min at room temperature with 100 µl of either AIS or NS and 100 µl glycine-BSA-NP40. The tubes were centrifuged (8000 g, 30 s) and the supernatant discarded. The precipitate was washed 4 times with 250 µl glycine-BSA-NP40, and we discarded the supernatant after each wash. Hereafter, the precipitate was resuspended in 250 µl acetic acid-BSA (1 M acetic acid, 2.5 mg/ml BSA). Finally, the precipitate suspension was transferred to the scintillation fluid (Eco Scint, National Diagnostics, Georgia, USA) and counted in a liquid scintillation counter.

Statistics

Group 1 received a CHO-rich diet during the whole experiment and is referred to as the control group. ANOVA was used to assess overall differences between groups. If significant, Newman-Keul's test for multiple differences was used to compare differences between the different experimental groups and the control group. When data were not normally distributed, even after transformation, the Kruskal-Wallis test was used to asses overall differences between groups. If significant, Mann-Whitney's test to compare differences between the different experimental groups and the control group was applied. The Bonferroni method was used to adjust for multiple-comparisons. Only significant differences compared to group 1 (control) are shown. Data are shown as means ± SEM. Linear regression analysis was applied using the data from incubation and perifusion studies. The regression model contained the insulin responses to the different experimental conditions, grouping variable and the interactions between diet during gestation, nursing mothers' diet or the diet applied to the offspring post weaning, according to the experimental setup (Table 1). Intercooled STATA 8 (StataCorp LP, College Station, TX, USA) was used for all statistical analyses.

Results

The offspring were separated into five subgroups immediately after birth, as described in Table 1. Table 2 summarizes birth weight and litter size. There was no

difference in litter size, average weight, or sex distribution between the two diets at the time of birth.

group for all the applied stimuli, but this only reached significance for high palmitate and GLP-1.

Table 6. Insulin responses from isolated islets of Langerhans during dynamic perifusion experiments

Medium	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
11.1 mM glucose	27.8 ± 5.8	30.9 ± 4.6	31.7 ± 8.4	30.0 ± 3.8	26.6 ± 6.3	ns
25.0 mM glucose	57.0 ± 6.2	46.9 ± 6.1	53.2 ± 8.2	58.7 ± 7.7	52.4 ± 11.5	ns
11.1 mM glucose + 0.1 μM GLP-1	95.5 ± 13.2	80.0 ± 15.3	75.9 ± 10.7	78.9 ± 8.8	60.4 ± 12.2	ns

Legend: Perifusion experiments were performed as described in the methods section. Results are presented as total area under the curve (AUC (ng/ml/20 min), mean ± SEM) for stimulated periods respectively. There were 10 islets from one animal in each perifusion chamber, which is referred to as one experiment (n = 7-9; perifusion experiments per group per stimuli). The insulin responses to low glucose were low and comparable (not shown). ns: not significant compared to group 1 (control). The experiment design for the five groups is provided in Table 1.

Weight gain and food consumption

The offspring were marked individually at the age of 4 wk, and the individual weights were determined at the age of 4 and 14 wk. Table 3 shows the average weight development in the different groups. No differences were found in the average weight between the experimental groups and the control group at any time point.

Food consumption in both the mothers and the offspring was monitored throughout the study, and no differences between the groups were found at any time point (data not shown).

Blood samples

Blood was sampled at week 8 for determination of glucose, insulin, and leptin and prior to islet isolation (age 14 wk) for determination of insulin and as an estimation of glucose level over time, glycated hemoglobin (Table 4). We did not detect any differences for any of these measurements for groups 2-5 compared to the control group.

Incubation experiments

Results from the incubation experiments are presented as total insulin concentration in the medium after 1 h incubation (Table 5). In brief, isolated islets of Langerhans were incubated for 1 h with the different stimulatory agents. Subsequently, the incubation medium was collected and later analyzed for insulin concentration. As shown in Table 5 there was a significantly lower insulin response in group 5 compared to the control group when the islets were challenged with 11.1 mM glucose supplemented with 0.4 mM palmitate or 0.1 μM GLP-1. There was a tendency towards lower insulin responses for group 5 compared to the control

Linear regression analysis was performed with the insulin responses as the dependent variable, clustered per animal and stimuli, and analyzed for interactions of mother's diet, nursing mother's diet and the offspring's diet post weaning (and cross-interactions between these periods). The linear regression analysis showed a significant negative correlation between high fat diet fed to the mother during gestation and the capability to secrete insulin in the offspring (coef: -3.1, 95% CI: -6.1 to -0.03, p = 0.048). High fat content in the nursing mothers' diet was positively correlated with insulin secretory capacity in the offspring later in life (coef: 4.4, 95% CI: 0.9 to 7.8, p = 0.01). Interestingly, we did not detect any significant association between the diet fed to the offspring post weaning and the capability to secrete insulin from the isolated pancreatic islets 14 wk after birth (coef: 0.5, 95 % CI: -2.6 to 3.7, p = 0.7).

Perifusion studies

The insulin response during perifusion was calculated as area under the curve (AUC) using the trapezoidal method [21]. As can be seen in Table 6, the insulin response was higher when stimulated with 25.0 mM than with 11.1 mM glucose, and even higher when GLP-1 (0.1 μM) was added at 11.1 mM glucose. There were no independent differences in insulin secretion calculated as AUC between any of the groups and the control group (group 1) in response to any of the applied stimuli. The insulin secretion was low and constant in response to low basal glucose (2.0 mM, data not shown).

Regression analyses were performed with all insulin responses per animal for all 2-minute fractions clustered per individual animal and stimuli, and analyzed for interactions of the mother's diet, nursing mother's diet and the offspring's diet post weaning (and cross

interactions between these periods). This analysis showed no correlation between high fat diet fed to the mother during gestation and the capability of the offspring's pancreatic islet to secrete insulin during dynamic perifusion experiments (coef: -0.2, 95% CI: -0.7 to 0.3, p = 0.4). We found no correlation between high fat content in the nursing mothers' diet (coef: 0.2, 95% CI: -0.3 to 0.7, p = 0.4) or in the diet fed to the offspring post weaning (coef: -0.1, 95% CI: -0.5 to 0.3, p = 0.5), and the capability to secrete insulin from the isolated pancreatic islets during perifusion later in life.

Insulin biosynthesis

Insulin secretion, measured as insulin concentration in the incubation media during the biosynthesis experiment was similar in all groups for the different stimuli (Table 7). Biosynthesis of insulin was measured as incorporation of 4, 5-^{3}H(N)-leucine, and did not differ between the groups (Table 7). Total insulin content in islets was not statistically significant different among the groups.

Discussion

In the present study, we used *in vitro* incubation and perifusion of isolated islets of Langerhans to investigate the influence of a high content of saturated fat in the maternal diet on the insulin secretory capacity in the offspring. We report a differential effect of the mothers' diet composition during gestation and nursing periods on islet insulin secretion in the offspring of rats. We found a negative correlation between a diet rich in fat fed to mothers during gestation and a positive correlation between nursing mothers' high fat diet and the insulin secretory capacity in the offspring.

The quite extreme dietary intervention we performed did not affect the body weight development in the different groups. We also found comparably average birth weights between the groups. Neither did the maternal diet affect their reproduction capacity, judged as total litter weight, sex, or number of offspring. These findings confirm studies with selective malnutrition [4-6], e.g. protein deprivation and administration of saturated fatty acids to pregnant rats [12, 22]. The

Table 7. Insulin responses and biosynthesis of insulin

Medium	Group 1	Group 2	Group 3	Group 4	Group 5	p-value
Insulin biosynthesis (dpm/10 islets)						
2.0 mM glucose	53.7 ± 8.7	38.2 ± 6.9	43.4 ± 5.7	50.3 ± 17.2	39.9 ± 6.0	ns
11.1 mM glucose	151.6 ± 22.8	110.7 ± 14.9	123.0 ± 14.3	121.4 ± 26.2	132.2 ± 27.2	ns
2.0 mM glucose +0.4 mM palmitate	51.0 ± 11.6	38.0 ± 8.9	31.6 ± 7.7	38.4 ± 10.9	45.4 ± 9.2	ns
11.1 mM glucose +0.4 mM palmitate	154.6 ± 19.9	122.0 ± 21.0	131.3 ± 9.4	106.8 ± 17.2	120.6 ± 18.1	ns
Insulin concentration in supernatant (ng/ml per 10 islets)						
2.0 mM glucose	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	ns
11.1 mM glucose	2.6 ± 0.4	1.8 ± 0.3	3.0 ± 0.4	2.4 ± 0.5	2.2 ± 0.2	ns
2.0 mM glucose +0.4 mM palmitate	0.5 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.1	ns
11.1 mM glucose +0.4 mM palmitate	3.3 ± 0.3	2.6 ± 0.4	2.6 ± 0.2	2.3 ± 0.4	2.3 ± 0.2	ns
Total insulin (mg/ml per 10 islets)	553.0 ± 43.0	530.0 ± 42.0	546.0 ± 34.0	513.0 ± 29.0	499.0 ± 26.0	ns

Legend: After 90 minutes incubation with the indicated stimulatory agents, the supernatant was removed and later analyzed for insulin concentration. The islet insulin biosynthesis was analyzed as described in the methods section. Values are mean ± SEM. Ten islets from each animal were used in the experiments (n = 4-6 animals per group). Total insulin reflects average insulin content per 10 islets. ns: not significant compared to group 1 (control). The experiment design for the five groups is provided in Table 1.

low and comparable levels of leptin between the experimental groups several weeks post-weaning is consistent with other reports on animals after high-fat feeding during lactation [23].

At termination of the study, we had expected some degree of diabetic metabolic disorder, at least in animals in groups 2 and 3, which received the fat-rich diet for the last 10 wk. Our findings are somewhat in contrast with other reports where high-fat feeding of rats for several weeks has been reported to reduce the glucose-stimulated insulin secretion [24]. Although not statistically significant, our results indicate a tendency towards higher fasting insulin levels in these fat fed groups (2 and 3) compared to the control group at the end of the study, presumably reflecting development of a pre-diabetic state. This tendency corroborates other reports, where a positive association between intake of saturated fat and hyperinsulinemia has been found [25-27]. Interestingly, Siemelink *et al.* [12] demonstrated that the nutritional fatty acid balance during development programs pancreatic responsivity, observable as early as at 12 wk of age i.e. a maternal diet rich in SAFA compared to a diet rich in unsaturated fat caused an increased insulin response at week 12 in the offspring of the SAFA group [12]. The level of blood glucose, determined both as glucose in the first part and as the level of glycated hemoglobin at termination of our study, were not different between the SAFA- and CHO-fed groups, indicating normal glycemic control in all groups at the termination of the study. The animals in groups 2 and 3 were fed a fat-rich diet at termination of the study, and we have previously [17] reported that animals in these groups have reduced insulin sensitivity compared to the control group. Thus, the fat-fed animals must have been able to compensate for the increased insulin resistance by increasing insulin secretion.

We compared the insulin secretory capacity for the isolated islets using both static incubation and dynamic perifusion experiments. For each stimulus, we compared the insulin responses in experimental groups with the control group (group 1). We only detected statistical differences in insulin secretion during static incubation with palmitate (0.4 mM) and GLP-1 (0.1 μM) in the presence of 11.1 mM glucose. Thus, for these stimuli, islets from group 5 had lower insulin secretory capacity than the control group. The offspring in group 5 were borne by mothers on a SAFA-rich diet, but were switched immediately after birth to nursing mothers on a carbohydrate-rich diet, and continued on the same diet after weaning.

Although not significant for the other conditions, there was a tendency towards reduced insulin responses from group 5 compared to the control group for all the experimental conditions. It should be noted, however, that our data derived from experiments of the static system was not normalized for cell number, cellular protein, or intracellular insulin which would have been of interest. When we determined insulin secretory capacity using perifusion experiments, we were unable to detect any significant differences to the stimuli applied including palmitate and GLP-1. However, a tendency towards reduced insulin secretion responses to palmitate and GLP-1 in group 5 was discernable.

One may suspect that a biochemical imbalance occurs which can only partly be compensated. This hypothesized imbalance is not reflected in detectable changes in the measured parameters, but may reflect a condition which would further develop over time.

We applied linear regression analysis to explore the impact of the different diets during gestation, nursing, and post weaning on β-cell function in the offspring. The regression model used the insulin responses from all incubation studies as a dependent variable (clustered per animal and stimuli), and analyzed for interaction with mothers' diet during gestation and nursing, and the diet applied to the offspring post weaning. This analysis showed a significant negative association between a high content of SAFA in the diet of mothers during gestation and the capability to secrete insulin in the offspring at the age of 14 wk. Furthermore, we detected a positive association between a high fat content in the nursing maternal diet and the capability of the offspring to secrete insulin. Surprisingly, we did not detect any independent effect of the diet applied to the offspring post-weaning and the capability to secrete insulin during the incubation studies.

Our findings indicate that high SAFA in the maternal diet during gestation negatively influences the insulin secretion capacity in the offspring later in life, whereas the same diet applied during the nursing period increases the insulin secretion capacity and thus compensates for the negative influence during gestation. The insulin secretion capacity in the offspring is, in our study, not influenced by a high saturated fat diet from the time of weaning until the age of 14 wk.

All together, our results demonstrate a reduced capability to secrete insulin from islets originating from animals that have experienced a high fat environment during gestation and changed to a high carbohydrate environment during nursing and post weaning.

Neither the level of insulin biosynthesis nor the total insulin content differed between the control group and any of the experimental groups. This indicates that whatever the mechanisms behind the reduced insulin secretory capacity is, it should be found downstream from insulin synthesis, and other studies indicate the oxidative capacity of the mitochondria to be the place to search [28].

We used a rat model without genetic predisposition to diabetes and attempted to induce a pre-diabetic state by dietary manipulation. One could argue that the experiments should have waited until the animals had reached a higher age to show a possible metabolic derangement. At present, it is not known if this would have been the case. The results reported here derive from *in vitro* studies in collagenase-treated islets. It can not be ruled out that *in vivo* studies, with intact islet architecture and blood supply, might give different results regarding the insulin secretory capacity.

Glucose is a primary energy source for the fetus [29]. The placenta is not permeable to insulin, but is highly permeable to glucose through glucose transporters [29]. Mothers fed a diet rich in saturated fatty acids are likely to be relatively insulin-resistant and thus to have a compensatory increased level of insulin, and a higher level of blood glucose.

We hypothesize that an expected higher glucose level in the fetus during the gestation period induces a change in the "set point" in the fetal β-cells and thus a higher level of glucose is needed to stimulate the insulin secretion in the offspring of fat-fed mothers. Offspring nursed by mothers on a SAFA-rich diet get a high fat load through the breast milk [30] and these offspring may develop insulin resistance and compensate by hypertrophy of the β-cells. These animals are thus better equipped, as the result of β-cell hypertrophy during late part of organogenesis, for the challenge they experience if they develop further insulin resistance later in life.

When offspring of fat-fed mothers are switched to nursing mothers on a CHO diet (group 5), the glucose offered to the offspring is relatively reduced compared to glucose levels in their biological fat-fed mothers milk. Consequently, the postulated compensatory β-cell hypertrophy does not take place and the animals thus have a lower insulin secretory potential later in life. Therefore, it seems that it is not only the high-fat diet per se being detrimental for the insulin secretory capacity in the offspring, but possibly also timing.

In conclusion, our results indicate that a high-fat diet fed to rats during gestation induces a reduced insulin secretory capability in the adult offspring. Further, a high-fat diet fed to nursing mothers induces increased capability to secrete insulin in the adult offspring. The differential effect on the insulin secretory capacity in the offspring depending on the time period from conception to adult life where a high-fat diet has been introduced is extremely interesting and, to our knowledge, has not been demonstrated previously. Our results underline the importance of applying a nutrient-balanced diet during pregnancy and the nursing period. Further studies are needed to clarify effects of dietary fat in animals pre-disposed to developing diabetes.

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