Original Article

Post-weaning isocaloric hyper-soybean oil versus a hyper-carbohydrate diet reduces obesity in adult rats induced by a high-fat diet

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The aim of this study was to investigate the effects of a post-weaning isocaloric hyper-soybean oil diet on later obesity and explore the underlying mechanisms. In the present study, newborn male Wistar rats were weaned on d 24, divided into CON (control), HC and HSO groups. CON was assigned to AIN-93G diet (a hyper-carbohydrate diet, for short HC diet) during the entire experiment. HC and HSO were fed with HC and isocaloric hyper-soybean oil (HSO) diet for 3 wk respectively, fed with HC diet for 2 wk successively, finally administrated high fat diet (HF) for 6 wk to induce obesity. On 3,5,11wk, the body weight, body fat content, blood glucose, blood lipid, serum insulin and leptin levels and obesity-related gene (CPT-1, FAS, UCP2, UCP3) expression levels in rats were detected. It was shown that body weight, body fat content, blood glucose and blood lipid, serum insulin and leptin levels in HSO were down-regulated on 3 and 5wk, therefore were significantly reduced on 11wk vs. HC. The CPT-1, UCP2, UCP3 gene expressions were up-regulated but FAS were down-regulated persistently in HSO. The study indicated that an early isocaloric HSO diet may reduce later obesity risk and reduce blood lipid and glucose abnormalities in adulthood via persistently influencing insulin and leptin sensitivity and permanent regulation of obesity-related gene expressions.

Key Words: post-weaning, HSO diet, adult obesity, programming, gene expression

Introduction

Obesity is widely viewed as a major public health problem in developed as well as in several developing countries.[1-2] The findings that obesity can increase the risk of mortality and morbidity from hypertension, dyslipidemia, type 2 diabetes, stroke, coronary heart disease, and cancer significantly have made body-weight regulation an important health issue in the 21st century.[3]

Obesity is a multifactorial disease that develops from the interaction between genotype and the environment. Although genetic factors can contribute to the development of obesity, the rapid increase in the global prevalence of obesity suggests that common environmental and lifestyle factors may be promoting and exacerbating the problem.[4] Nutrition is a very important environmental factor in obesity prevalence. For many years, the adult nutrition has been viewed as the key risk factor of obesity. In recent decades, the effect of early nutrition on later obesity has been focused. Increasing epidemiological and experimental data support that perinatal nutrition plays an important role in adulthood obesity. Data about effect of birth anthropometry on adult obesity generally showed that there is a U-shaped relationship, with both low and high birth weights predicting later adiposity.[5-7] Breastfeeding may help prevent childhood overweight as reported by William.[8] Animal models also supported the hypothesis that prenatal and early postnatal nutrition permanently affect later obesity.[9-10] Vickers reported that subjecting pregnant rats to severe food restriction (feeding only 30% of ad libitum intake) promotes profound intrauterine growth retardation in their offspring.[11] These growth-retarded pups grown up to be hyperphagic and developed pronounced central adiposity, when provided with a hypercaloric diet. The fetus and infants appear to respond to insults during the prenatal and postnatal period through the process of "nutritional programming," which has short-term survival advantages but may have a long-term disadvantage in that it is associated with cardiovascular disease, hypertension, type 2 diabetes, and later obesity.

Most of the studies cited above deal with an altered intrauterine environment (mostly malnourishment), or infant nutrition resulting in the altered growth pattern of the neonate and subsequently in adult-onset obesity. However, very few studies have monitored the effect of dietary experience during early after weaning (during this period mammal exhibit rapid growth and development also) on rats adiposity during maturation.

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Table 1. Diet compositions

Ingredient	*HC* g/kg	HSO g/kg	HF g/kg
Casein	200	200	200
Cornstarch	523	395	423
L-cystine	4.6	4.6	4.6
Cellulose	35.0	35.0	35.0
Sucrose	100	100	100
Fat	100 （soybean oil）	225 （soybean oil）	200 (lard)
Vitamin mix, AIN-93G	10.0	10.0	10.0
Mineral mix, AIN-93G	35.0	35.0	35.0
Choline bitartrate	2.5	2.5	2.5
Carbohydrate energy (%)	60.5	42.9	46.0
Protein energy (%)	18.8	16.4	16.9
Fat energy (%)	20.7	40.7	37.1

Moreover it is more important to get isocaloric different composition diets for human. We hypothesize the early post-weaning is a sensitive period to nutritional programming either. The aim of this study is to investigate whether post-weaning dietary experience including isocaloric hyper-soybean oil and hyper-carbohydrate diet can influence later obesity. The underlying mechanism was explored owing to that the specific mechanisms responsible for nutritional programming remain elusive.

Materials and methods

Study design and animal diet

Timed pregnant Wistar rats (Weitonglihua Experimental Animals Co. Ltd., China) were housed in individual plastic shoebox cages with free access to both food and water, and were maintained at 22°C with a 12-h light and dark cycle. The number of male pups per litter was standardized to seven males within 24 h of birth to minimize variation among litters. Male pups were weaned on d 24 and housed in wire-mesh hanging cages at an ambient temperature of 22°C with a 12-h light: dark cycle, have free access to water. These pups were allocated to three groups, CON (n=24), HC (n=24) and HSO (n=24). In the present study, AIN-93 growth diet regarded as high-carbohydrate diet, for short HC, since the energy percentage provided by carbohydrate is as high as 60.51%. CON was fed HC in the whole experiment. HC and HSO were fed with HC and HSO for 3 weeks respectively, the latter diet contains soybean oil as high as 22.5g providing 40.68% (kilocalories) per 100g diet. The diet intake was administrated to make all pups get same energy per kilogram body weight during the intervention of the 3 weeks. After 2 weeks of free access to HC diet successively, HC and HSO both were fed with HF (high fat), which is made of lard containing 39% saturated fat, for 6 weeks to induce obesity. Food intake and body weight were determined twice each week. The major difference between HC and HSO diets consists in the soybean oil content (10.0 and 22.5 g respectively) and polyunsaturated fatty acid (n-3 plus n-6) content (6.21 and 13.97 g respectively) per 100 g diet.

Blood and tissue specimen collections

At 3, 5, 11wk, 8 rats selected randomly from each group were fasted overnight and hocused with sodium pentobarbital and sacrificed during 8:00 to 9:00 AM next day. Blood was collected into heparinized vacutainers. Tissues were removed and weighed including the liver, gastrocnemius, perirenal and epididymal fat pads. Liver, gastrocnemius, perirenal adipose tissues slices were frozen in liquid nitrogen and remained at -80°C.

FAS, CPT-1, UCP2,UCP3 gene expression

FAS, CPT-1, UCP2, UCP3 mRNA levels from liver, liver, perirenal adipose tissue, gastrocnemius respectively were determined by a reverse transcription-polymerase chain reaction (RT-PCR) method. Total RNA from 100 mg samples isolated by using TRIzol reagent (Invitrogen Company, USA) according to the manufacturer's protocol. Concentrations of RNA were measured spectrophotometrically at A260. cDNAs were synthesized by RT from total RNA (2μg) using Reverse Transcriptase XL (AMV), RNase inhibitor, and random primer oligo dT (TaKaRa

Table 2. The primers sequences used in RT-PCR

Gene	Sequence	Length of Products	Melting temperature	Annealing temperature	Extension temperature
		bp	°C	°C	°C
FAS	U†:5′-TAGTGCGTGGTCGTATTCA-3′ D ‡:5′-TCTTCTGCCAGGGAGTTG-3′	274	94	60	72
CPT-1	U:5′-TATGTGAGGATGCTGCTTCC-3′ D: 5′-CTCGGAGAGCTAAGCTTGTC-3′	629	94	60	72
UCP2	U:5′-TAAAGCAGTTCTACACCAAGGG-3′ D: 5′-CGAAGGCAGAAGTGAAGTGG-3′	360	94	58	72
UCP3	U:5′-GGGTGTTGGGAAGATAGAA -3′ D: 5′-AGAAGGCGGAAGAGCAGA -3′	312	94	60	72
β-actin	U:5′-TCTACAATGAGCTGCGTGTG-3′ D: 5′-GGTCAGGATCTTCATGAGGT-3′	314	94	50	72

† U, upstream; ‡ D, downstream.

Biotech Company, Dalian, China), and incubated for 10 minutes at 37°C, 1 hour at 42°C then 2 minutes at ice in 20 μL reaction medium made up of 5×Buffer 4.0μl, 10 mM dNTP 2.0 μL, 50 pmol oligo dT, and 10 U AMV RTase (Promega Biotech Company, USA). The PCR amplifications were performed in 25 μL medium containing 10×ExTaq Buffer（Mg2＋）2.5μL, 2.5 mM dNTP 2.0 μL, 20 pmol sense and antisense primers, and 1.75 U Taq DNA polymerase (Promega Biotech Company, USA). PCR amplification was carried out after RT reactions, using selective oligonucleotide primers listed in table 2.

PCR reactions were performed using 1μg of RT product according to the reaction condition listed in Table 2 in the PTC-100PCRTM PCR equipment (MJ Research Inc, USA). The RT-PCR products were analyzed by 1.2% agarose gel electrophoresis, and intensity of bands was compared with imaging of ethidium bromide staining and analyzed by Chemi ImagerTM 4000 (Alpha Innotech Corp, USA).

Blood and serum assays

Blood glucose is measured with blood glucose meter (Johnson & Johnson Company, USA), at the same time, blood was collected and centrifuged at 3000rpm 15min to obtain serum, which was frozen at -80°C to measure insulin and leptin (Linco Company, USA) with radioimmunoassay (RIA). The leptin and insulin RIA kits, containing precoated tubes, are used (DL Company, USA). On the other hand, pups' serum was assayed colorimetrically for total cholesterol (TC) and high-density lipoprotein cholesterol (HDL), triglycerides (TG) (Zhongsheng Biotech Company, China).

Statistical analysis

Data were analyzed using the procedure of SPSS version 11.0. Results are given as means ± SD. Data from pups were analyzed based on post-weaning diet. Group differences were considered as statistically significant at $p< 0.05$.

Table 3. Summary of body composition and metabolic parameters from experiment rats

Parameter	CON	HC	HSO
BW (g)			
0wk	56.9±11.1	56.8±11.0	57.2±8.30
3wk	109±19.1	108±19.1	107±19.2
5wk	145±11.6	144±11.6	151±17.7
11wk	307±17.9*	373±14.1	316±18.9*
Body fat content (g/100g body weight)			
3wk	0.96±0.22	0.97±0.21	0.92±0.24
5wk	1.93±0.53	1.92±0.54	1.67±0.40
11wk	2.59±0.13*	5.69±0.17	4.24±0.24*
Fasting blood glucose (mmol/L)			
3wk	3.80±0.43	3.70±0.33	3.50±0.51
5wk	5.61±0.71	5.59±0.61	4.91±0.92
11wk	5.76±0.11*	6.38±0.45	5.78±0.30*
Serum TG (mmol/L)			
3wk	1.43±0.39	1.44±0.29	0.78±0.31
5wk	0.52±0.06	0.53±0.07	0.47±0.14
11wk	0.77±0.04*	1.25±0.04	0.81±0.03*
Serum TC (mmol/L)			
3wk	4.12±0.97	4.13±0.96	3.41±0.91
5wk	2.53±0.13	2.54±0.12	2.66±0.13
11wk	1.22±0.19	1.86±0.15	1.50±0.14*
Serum HDL (mmol/L)			
3wk	0.70±0.27	0.69±0.24	0.80±0.22
5wk	0.41±0.05	0.40±0.09	0.51±0.14
11wk	0.79±0.14*	0.66±0.07	0.72±0.06
Fasting serum Insulin (μIU/mL)			
3wk	2.27±0.78	2.28±0.77	2.01±0.47
5wk	3.86±0.77	3.87±0.75	3.43±0.83
11wk	6.67±0.66*	11.6±0.53	7.10±0.43*
ISI			
3wk	-0.91±0.18	-0.93±0.15	-0.82±0.14
5wk	-1.32±0.11	-1.33±0.14	-1.21±0.13
11wk	-1.58±0.04*	-1.87±0.03	-1.61±0.05*
Fasting serum leptin (ng/mL)			
3wk	0.21±0.06	0.22±0.05	0.24±0.03
5wk	0.39±0.08	0.40±0.07	0.35±0.06
11wk	3.77±0.37*	8.70±0.34	6.09±0.10*

†n= 8 per group. ‡Data are ± SD.§ ISI= -Log（Fasting Serum Insulin× Fasting Blood Glucose）*, $p<0.05$ vs. HC.

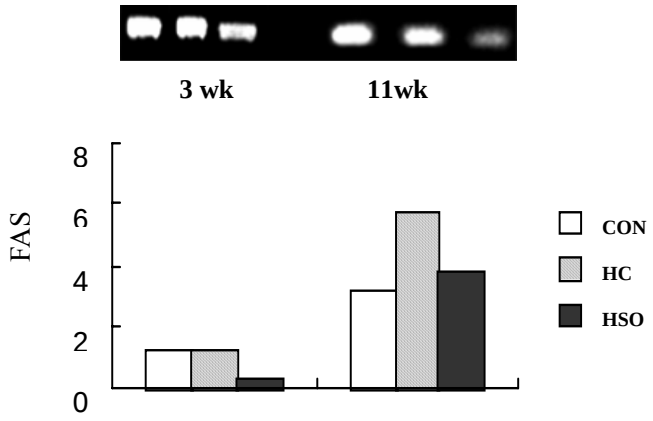

Figure 1. Effect of post-weaning nutrition on the hepatic FAS gene expression. The columns represent normalized FAS mRNA levels by β-actin. CON: group fed on HC diet in the whole experiment; HC: group fed with HC diet after weaning for 3wk,then administrated high-fat diet in adult life; HSO: group were fed on isocaloric HSO diet for 3wk after weaning, then fed on high-fat diet in later life. FAS: fatty acid synthase. 3wk: FAS mRNA at 3 weeks of early diet intervention; 11wk: FAS mRNA at 6 weeks of high-fat diet.

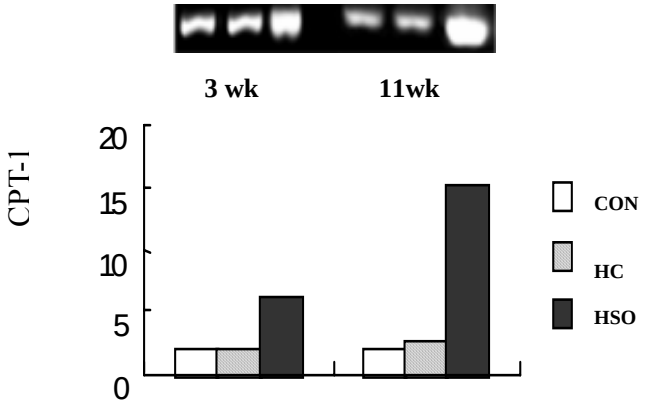

Figure 2. Effect of post-weaning nutrition on the hepatic CPT-1 gene expression. The columns represent normalized CPT-1 mRNA levels by β-actin. CON: group fed on HC diet in the whole experiment; HC: group fed with HC diet after weaning for 3wk, then administrated high-fat diet in adult life; HSO: group were fed on isocaloric HSO diet for 3wk after weaning, then fed on high-fat diet in later life. CPT-1: carnitine palmitoyl transferase-1. 3wk: CPT-1 mRNA at 3 weeks of early diet intervention; 11wk: CPT-1 mRNA at 6 weeks of high-fat feeding.

Ethics

The experiment is approved by Harbin Medical University Animal Ethics Committee and carried out under the supervision of the committee.

Results and discussion

The study firstly investigated the effect of an isocaloric hyper-soybean oil diet rich in polyunsaturated fatty acid after weaning on later obesity, and explored the underlying mechanisms via dynamically detecting the expression of key genes related to obesity. Table 3 showed that, compared with HC, the levels of body weight, body fat content, blood glucose, serum triglyceride and total cholesterol in HSO were down-regulated at 3wk and significantly reduced at 11wk when feeding on high-fat diet. It is found that the serum HDL level in HSO is higher than HC. It is indicated that post-weaning isocaloric HSO diet intake can protect rats from adiposity in adulthood,

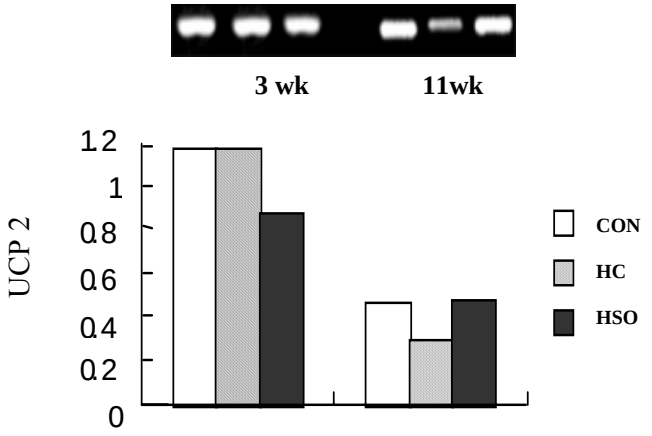

Figure 3. Effect of post-weaning nutrition on the adipose UCP2 gene expression. The columns represent normalized UCP2 mRNA levels by β-actin. CON: group fed on HC diet in the whole experiment; HC: group fed with HC diet after weaning for 3wk,then administrated high-fat diet in adult life; HSO: group were fed on isocaloric HSO diet for 3wk after weaning, then fed on high-fat diet in later life. UCP2: uncoupling protein 2. 3wk: UCP2 mRNA at 3 weeks of early diet intervention; 11wk: UCP2 mRNA at 6 weeks of high-fat diet.

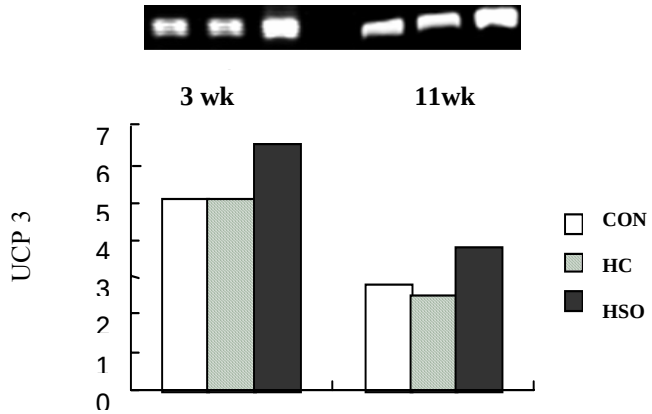

Figure 4. Effect of post-weaning nutrition on the muscular UCP3 gene expression. The columns represent normalized UCP3 mRNA levels by β-actin. CON: group fed on HC diet in the experiment; HC: group fed with HC diet after weaning for 3wk,then administrated high-fat diet in adult life; HSO: group fed on isocaloric HSO diet for 3wk after weaning, then fed on high-fat diet in later life. UCP3: uncoupling protein 3. 3wk: UCP3 mRNA at 3 weeks of early diet intervention; 11wk: UCP3 mRNA at 6 weeks of high-fat diet.

improve blood lipid and glucose metabolic abnormalities and that early post-weaning may be the sensitive period of metabolic programming.

It is well known that obesity is associated with insulin and leptin resistance. Leptin, which is primarily expressed by adipose tissue, as an anti-obesity hormone, can promote energy expenditure and inhibit fat accumulation. Obesity in humans and rodents is almost always associated with a resistance to, rather than a deficiency of, leptin.[12] Hyperinsulinaemia, a marker of lower insulin sensitivity, is one of the earliest metabolic indicators of obesity, closely correlated to abnormal blood glucose.[13] From table 3, it is demonstrated that the fasting serum leptin level was reduced at 3wk, 5wk and significantly reduced at 11wk in HSO vs. HC. Table 3 also showed that insulin sensitivity index level was increased at 3wk, 5wk and significantly increased at 11wk in HSO vs. HC. From

above results, it can be concluded that the leptin and insulin sensitivity was increased after early HSO intervention, this action was further amplified by feeding HF diet in later life, therefore glucose and lipid metabolism abnormalities are inhibited significantly.

Fig 1 showed that the FAS (fatty acid synthase) mRNA level in HSO was lower than HC both at 3wk of HSO diet intervention and 11wk when were fed with HF diet. It is well known that FAS is a key lipogenic enzyme that catalyzes all of the reactions involved in the synthesis of long-chain saturated fatty acid, palmitate, from acetyl CoA, malonyl CoA, and NADPH by the action of its seven active sites. FAS content in tissue influences fat store of body. FAS concentration in lipogenic tissues such as liver and adipose tissue, being dramatically induced upon refeeding a high carbohydrate, fat-free diet after fasting.[14] However, PUFA (polyunsaturated fatty acid) administration to cultured rat hepatocytes rapidly inhibits the transcription of genes encoding FAS.[15] The results from this experiment suggest that the protective role of after weaning HSO diet may be linked with its higher PUFA level which reduced the FAS expression and inhibited lipogenesis permanently.

In the experiment, the CPT-1 (carnitine palmitoyl transferase-1) mRNA level was induced by isocaloric hyper-soybean oil diet and persisted to 11wk when being administrated HF diet (Fig 2). CPT-1 is a pace-setting enzyme for fatty acid oxidation, its content reflects the lipolysis level in mammal.[16] It was reported that the CPT-1 activity was reduced in obese human skeletal muscle.[17] C75**,** a CPT-1 agonist, treatment made diet-induced obese rats loss a 50% greater weight via increasing adipocytes and hepatocytes fatty acid oxidation, thereby Thupari concluded that CPT-1 can be regarded as a therapeutic target for obesity.[18] In addition, in primary hepatocytes from fetal rats CPT-1 mRNA is induced by PUFA, whether saturated or not.[19] Therefore, it might be postulated the PUFA increasing CPT-1 mRNA and lipolysis level may play an important role in post-weaning HSO prevention later adiposity.

Fig 3 and 4 showed that the adipose UCP2 and muscular UCP3 expressions permanently increased in the HSO group vs. HC. UCPs (uncoupling proteins) are mitochondrial proton transporters that uncouple oxidative phosphorylation by dissipating the proton gradient across the membrane, and convert the stored energy to heat and increase thermogenesis and reduce the development of obesity. UCP2 and UCP3 are two proteins, which were found to play important role in animal and human obesity in UCP family. Obesity-prone strain of mice, C57Bl/6J, fails to show an increase in UCP2 expression in response to a high-fat diet, whereas an obesity-resistant strain increases expression twofold.[20-21] UCP3 is expressed predominantly in skeletal muscle and has been associated with whole-body energy metabolism. It is demonstrated that obesity resistant animals may initially resist weight gain when placed on a high energy diet through a greater induction of muscle UCP3, but obesity-prone animals show no this action.[22] These findings viewed UCP2 and 3 as important potential candidates in the regulation of mammalian energy stores. It is shown that no response of UCP2 expression to treatment with a saturated fatty acid, whereas the polyunsaturated fatty acids (PUFA) significantly up-regulated UCP2 expression.[23] It is suggested that HSO diet may prevent later obesity via persistently through inducing the adipose UCP2 and muscular UCP3 gene expression to increase energy expenditure. The action may be related to higher PUFA level in HSO.

In summary, it is demonstrated that feeding on isocaloric HSO diet rich of PUFA after weaning contributed to prevent later adiposity, and this action may be correlated with permanently altering the lipid and energy metabolism key enzymes gene expressions, increasing leptin and insulin sensitivity, that may be the potential mechanism underlying for nutritional programming. It is of paramount interest to further distinguish the effects of PUFA such as n-3 and n-6 PUFA and to search for an optimal post-weaning diet model to prevent later obesity.

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Abbreviations

HC diet, hyper-carbohydrate diet; HSO diet, hyper-soybean oil diet; RIA, radioimmunoassay; TC, total cholesterol; HDL, high-density lipoprotein cholesterol; TG, triglycerides; FAS, fatty acid synthase; CPT-1, carnitine palmitoyl transferase-1; UCP, uncoupling protein; PUFA, polyunsaturated fatty acid.

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