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Insulin resistance precedes glucose intolerance and hyperleptinaemia in high-fat simple carbohydrate-fed C57BL/6J mice

Insulinooporność poprzedza nietolerancję glukozy i hiperleptynemię u myszy C57BL/6J otrzymujących karmę wysokotłuszczową i zawierającą cukry proste

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Abstract

Introduction: Very few systematic studies are done during the onset and progression of metabolic syndrome in suitable animal models. In this paper we present the effect of High-Fat Simple Carbohydrate (HFSC) feed on the metabolic hormones in C57BL/6J mice to understand the sequence of events leading to impairment of glucose homeostasis.
Material and methods: One-month-old male C57BL/6J mice were fed with control (C group) and HFSC (T group) feed (n = 30 each) respectively for five months. The glucose tolerance was studied by Oral Glucose Tolerance Test (OGTT) whereas serum insulin and leptin were quantified using ELISA kits, and serum cortisol was quantified using CLIA kits.
Results: Insulin resistance index and HOMA-IR levels were higher in the mice of group T as compared to age-matched mice of group C within one month and significantly higher after and five months of feeding. The total area under the glucose tolerance test curve (AUC) and the insulin curve (AUC ins) was found to significantly increase in the mice of T group as compared to the mice of C group as early as two months of feeding and was elevated after 5 months post feeding. Comparison of the Matsuda index revealed that pancreatic beta cell function was significantly lower in mice of T group as compared to mice of C group by five months of feeding. Leptin levels fluctuated during the 1st–4th month and by the 5th month significant hyperleptinaemia was detected. There was no significant change in cortisol levels in mice of group T as compared to mice of group C after five months of feeding.
Conclusions: HFSC feed induces insulin resistance by the first month and progressively impairs glucose tolerance, resulting in hyperleptinaemia by the fifth month in male C57BL/6J mice. **(Endokrynol Pol 2016; 67 (6): 592–598)**

Key words: *insulin resistance; oral glucose tolerance; cortisol; leptin; HFSC diet; C57BL/6J mice; metabolic syndrome; Matsuda index; pancreatic islet function*

Streszczenie

Wstęp: Dostępnych jest bardzo niewiele badań systematycznych oceniających wystąpienie i progresję zespołu metabolicznego na odpowiednich modelach zwierzęcych. W niniejszej pracy przedstawiono wpływ podawania myszom C57BL/6J karmy wysokotłuszczowej i zawierającej cukry proste (HFSC, *High Fat Simple Carbohydrate*) na sekwencje zdarzeń prowadzących do zaburzeń homeostazy glukozy.
Materiał i metody: Jednomiesięcznym samcom myszy C57BL/6J podawano przez 5miesięcy karmę kontrolną (grupa C) lub HFSC (grupa T) (n = 30 w każdej grupie). Tolerancję glukozy oceniono na podstawie doustnego testu tolerancji glukozy (OGTT, *oral glucose tolerance test*), natomiast stężenia insulin i leptyny w surowicy oznaczono, używając metody ELISA, a do oznaczenia stężenia kortyzolu w surowicy użyto metody CLIA.
Wyniki: Wskaźnik insulinooporności HOMA-IR był wyższy u myszy z grupy T niż u dobranej pod względem wieku myszy z grupy C już w ciągu pierwszego miesiąca, a po 3 i 5 miesiącach diety HFSC różnice były istotne statystycznie. Całkowite pole pod krzywą (AUC, *area under the curve*) w teście tolerancji glukozy oraz pole pod krzywą insuliny (AUC ins) zwiększyło się istotnie u myszy z grupy T w porównaniu z myszami z grupy C, co było widoczne już po 2 miesiącach podawania karmy HFSC i było podwyższone przez 5 miesięcy od zakończenia podawania tej karmy. Porównanie wskaźnika Matsudy wykazało, że po 5 miesiącach czynność komórek beta trzustki była istotnie upośledzono u myszy z grupy T w porównaniu z myszami z grupy C. Stężenia leptyny wahały się w okresie 1.–4. miesiąca, a po 5 miesiącach wykryto istotną hiperleptynemię. Po 3 miesiącach nie stwierdzono istotnych zmian stężeń kortyzolu u myszy z grupy T w porównaniu z grupą C.
Wnioski: U samców myszy C57BL/6J dieta HFSC wywołała insulinooporność już po pierwszym miesiącu, a następnie powodowała stopniowe pogarszanie tolerancji glukozy, co po pięciu miesiącach doprowadziło do hiperleptynemii. **(Endokrynol Pol 2016; 67 (6): 592–598)**

Słowa kluczowe: *insulinooporność; doustny test tolerancji glukozy; kortyzol; leptyna; dieta HFSC; myszy C57BL/6J; zespół metaboliczny; wskaźnik Matsudy; czynność wysp trzustkowych*

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Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All the experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India with approval from the Institutional Animal Ethics Committee (Sanction No. SAC/IAEC/110/2011, 30th March 2011).

Introduction

The rampant mushrooming of fast food outlets has led to a change in dietary patterns across the globe. Most of the food items available are categorically rich in fat and simple carbohydrates. Food influences hormones such as insulin [1, 2] and leptin [3, 4]. Insulin and leptin, both anorexic hormones, are known to suppress appetite [5]. Among the animal models used for nutritional studies, C57BL/6J mice seem to respond better to hormonal changes caused by diet. High-fat diet-fed C57BL/6J mice develop obesity, hyperglycaemia, hyperinsulinaemia, and impaired glucose-stimulated insulin secretion [6], as well as peripheral and central leptin insensitivity [7]. Paradoxical observations exist with respect to the role of leptin and insulin resistance. Leptin appears to act in both insulin sensitisation and resistance phenotype [8]. Most of the studies are based on feeding a high-fat diet that does not mimic the present day fast foods. Recognising the importance of simple carbohydrates in weight gain and other associated complications of glucose homeostasis, we specially formulated and prepared High-Fat Simple Carbohydrate Diet (HFSC) feed in our laboratory.

HFSC-fed C57BL/6J male mice progressively gained body weight, developed hyperglycaemia, obesity, hypercholesterolaemia, and hypertriglyceridemia during a five-month study [9]. Given the observed increase in body weight, we wanted to study the leptin profile in the developed animal model due to its anorectic mode of action in body weight regulation. Also, the glucose levels that were studied by us primarily focused on fasting blood glucose. This did not provide us with an insight into tolerance of the animal model to the glucose flux that could be obtained by a glucose tolerance test. Along with insulin, cortisol also plays an important role in maintaining the glucose levels of the body by stimulating gluconeogenesis as well as in fat, carbohydrate, and lipid metabolism [10]. To get a better understanding of the observed disrupted biochemical profile, in the present investigation we have tried to assess the timeframe of disruption of glucose homeostasis in C57BL/6J mice in response to the HFSC feed. In this study we evaluated the glucose tolerance as well as insulin, cortisol, leptin, and pancreatic insulin function in experimental C57BL/6J mice during a five-month period.

Material and methods

Animals used

Pathogen-free, one-month-old, male C57BL/6J mice obtained by breeding (breeder stock procured from the National Institute of Nutrition, Hyderabad, India) were used for the experimental study. Male mice were chosen for the study because the energy balance-related pathways are affected due to hormonal variations associated with oestrous cycle [11–13]. Also, the body composition and body fat distribution differs in males and females wherein females have higher percentage of body fat and less abdominal fat compared to males [14]. The mice were divided into control and test groups (n = 30 each) and were fed with control feed and high-fat simple carbohydrate feed respectively at the rate of 5 g/day according to Indian National Science Academy regulations [15].

The control feed consisted of wheat flour (79% w/w), infant milk spray (Amul, India) (16% w/w) (used as the source of proteins and vitamins) and soya bean oil (4.67% v/w), whereas the HFSC feed contained porcine fat (13% w/w), sucrose (10% w/w), corn starch (7% w/w), and infant milk spray (Amul, India) (70% w/w). Total energy content from control feed was 3582.369 Kcal/kg and from HFSC feed was 5064.581 Kcal/kg [16]. The mice were provided with fresh food and water, both *ad libitum*, daily. All the experiments were carried out according to CPCSEA India guidelines with approval from the Institutional Animal Ethics Committee (sanction No. SAC/IAEC/110/2011, 30th March 2011). These guidelines are in accordance with the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education issued by the New York Academy of Sciences Ad Hoc Committee on Animal Research.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed every month in accordance with Buetner et al. (2006) [17]. The mice were starved for 16 hours prior to the procedure. A glu-

cose load (1 g/kg body weight) was orally administered to each mouse. The blood was collected by tail vein bleeding, and blood glucose levels were then measured at 0 (before glucose administration) 30, 60, 90, and 120 minutes post glucose load using a glucometer (One touch Horizon, India). The serum obtained was used for insulin analysis.

Insulin analysis

The serum insulin analysis was performed using an Ultra-Sensitive Mouse Insulin ELISA kit according to the manufacturer's instruction (Crystal Chem Inc., USA).

Homeostatic Model of Assessment-Insulin Resistance (HOMA-IR) was calculated according to the given formula [18]:

$$\text{HOMA-IR} = \text{Glucose [mmol/L]} * \text{Insulin [m/U/mL]}$$

The **Matsuda index** of insulin sensitivity was calculated as follows, with glucose and insulin values in mmol/L and pmol/L, respectively [18]:

$$10{,}000/(G_0 \times I_0 \times G_m \times I_m)^{0.5}$$

where G_0 and I_0 are pre-meal values for insulin and glucose, respectively, and G_m and I_m are mean post-meal values during the first 120 minutes of the glucose tolerance test.

Total area under the curve of both glucose and insulin was calculated during 0-120 minutes following oral liquid glucose load [18].

The ratio of AUC (ins/glc) and Matsuda index provides an insight into the β cell function [19].

Leptin analysis

Leptin was analysed from the serum obtained by tail vein bleeding using Mouse Leptin ELISA kit according to the manufacturer's instructions (RayBio®, USA).

Cortisol

Serum cortisol was analysed by Chemiluminescence Immuno assay (CLIA) technique by Bioscan Diagnostics and Health Care Centre (Kadri, Shivabaugh, Karnataka, India).

Statistical analysis

The obtained data was statistically analysed using Graph Pad InStat Version 3.1. Specifically, the OGTT data (post Students t-test) was further subjected to Bonferroni correction in order to calculate the correct error because after each month of OGTT the mice were sacrificed for further analysis in the study. For example, for the fifth month, only those values below 0.01 were considered significant ($p < 0.05/5 = 0.01$). Accordingly, the corrections were applied for the other months as well. The statistical approach was confirmed by a statistician, Dr Mariamma Philip (Assistant Professor, NIMHANS, Bangalore, India).

Results

HFSC feed significantly increased fasting blood glucose in the mice of group T as compared to the mice of group C ($p < 0.05$) after one month of feeding. However, oral glucose tolerance was unaffected (i.e. the ability of mice of group T to bring the blood glucose levels to normal levels was not affected) at the end of one month of feeding with HFSC diet (Table I). Glucose tolerance was found to be significantly impaired after two months of feeding the HFSC diet. The blood glucose levels were significantly ($p < 0.025$) higher in the mice of group T after 0 minutes and 30 minutes of glucose load as compared to the mice of group C (Table I). This impairment only worsened in the mice of group T throughout the feeding regimen as compared to the mice of group C, shown by significantly higher levels of the blood glucose levels of mice of group T at 0 minutes ($p < 0.001$), 30 minutes ($p < 0.01$), 60 minutes ($p < 0.01$), and 120 minutes ($p < 0.01$) post glucose load after three months of feeding (Table I). After four and five months of feeding, significantly ($p < 0.01$ and $p < 0.001$) higher impaired glucose tolerance was observed until 120 minutes of feeding with the blood glucose levels rapidly becoming very high in the mice of group T as compared to the mice of group C (Table I). The total area under the glucose curve (AUC glc) (Fig. 1) and total area under the insulin curve (AUC ins) (Fig. 2) were found to significantly increase in the mice of group T as compared to the mice of group C as early as within two months of feeding and was elevated until five months of feeding. Comparison of the Matsuda index revealed that pancreatic beta-cell function was significantly lower in mice of group T as compared to mice of group C from one month, and worsened by five months of feeding (Fig. 3). The ratio of AUC (ins/glc) and Matsuda index (Fig. 4) further confirms that the pancreatic beta-cell function was affected from the first month and deteriorated by fifth month.

The HOMA-IR levels of the mice of group T were higher than the mice of group C within one month and significantly higher after three months ($p < 0.001$) and five months ($p < 0.001$) of feeding (Table II). An interesting observation was that the HOMA-IR of mice of group T was lower after five months of feeding as compared to HOMA-IR after three months of feeding of the same group. Corresponding analysis of the insulin levels using the serum samples obtained during OGTT showed that the insulin levels of the mice of group T were sig-

Table I. *Blood glucose levels in male C57BL/6J MICE following oral glucose load during five-month study*

Tabela I. *Stężenia glukozy we krwi samców myszy C57BL/6J po doustnym obciążeniu glukozą w trakcie 5-miesięcznego badania*

	Control [mg/dL]					Test [mg/dL]				
Month	**0 minutes**	**30 minutes**	**60 minutes**	**90 minutes**	**120 minutes**	**0 minutes**	**30 minutes**	**60 minutes**	**90minutes**	**120minutes**
1 mo	94.25 ± 7.36	158.6 ± 9.02	136.8 ± 7.42	112.8 ± 3.23	95.8 ± 1.02	161.33 ± 18.49*	169.6 ± 5.17	152 ± 10.75	136.8 ± 10.42	104 ± 4.50
2 mo	97.25 ± 1.03	173 ± 22.35	165.8 ± 13.38	106.8 ± 2.35	95.4 ± 0.97	165 ± 12.46#	228 ± 9.97**	187.8 ± 7.79	118.4 ± 4.94	117.6 ± 8.19
3 mo	89.5 ± 2.15	133.83 ± 5.79	120.83 ± 2.53	103 ± 4.86	89 ± 2.28	131.83 ± 5.3***	171.86 ± 10.86**	159 ± 9.82**	133.6 ± 4.55	125.66 ± 1.62**
4 mo	91.83 ± 4.23	137.16 ± 9.39	110.33 ± 2.67	93.66 ± 3.27	88.83 ± 2.93	141.16 ± 7.78**	196.83 ± 9.1***	178.33 ± 7.72***	174 ± 14.28***	154.16 ± 13.42***
5 mo	98.25 ± 0.86	185.8 ± 4.65	169.8 ± 2.39	127.25 ± 3.32	97.6 ± 0.92	164.6 ± 3.20***	255.4 ± 13.94**	282.8 ± 22.59***	225.6 ± 11.47***	207.6 ± 12.33***

Data is represented as mean ± S.E.M. of 5–6 independent values
*p < 0.05, **p < 0.01; ***p < 0.001 Test as compared to the respective age-matched control; #p < 0.025 Test as compared to the respective age-matched control

Figure 1. *Total area under the glucose curve. C — mice fed with control diet, T — mice fed with HFSC diet*

Rycina 1. *Całkowite pole pod krzywą stężenia glukozy. C — myszy otrzymujące karmę kontrolną, T — myszy otrzymujące karmę HFSC*

Figure 2. *Total area under the insulin curve. C — mice fed with control diet, T — mice fed with HFSC diet*

Rycina 2. *Całkowite pole pod krzywą stężenia insuliny. C — myszy otrzymujące karmę kontrolną, T — myszy otrzymujące karmę HFSC*

Figure 3. *Matsuda index after five months of feeding hfsc feed. C — mice fed with control diet, T — mice fed with HFSC diet*

Rycina 3. *Wskaźnik Matsuda po 5 miesiącach stosowania karmy HFSC. C — myszy otrzymujące karmę kontrolną, T — myszy otrzymujące karmę HFSC*

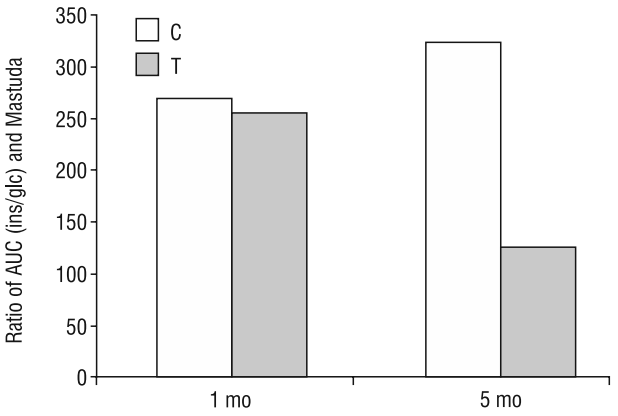


Figure 4. *Effect of HFSC diet on pancreatic β-cell function during the five-month feeding regimen. C — Mice fed with control diet, T — Mice fed with HFSC diet*

Rycina 4. *Wpływ karmy HFSC na czynność komórek beta trzustki w trakcie 5-miesięcznego badania. C — myszy otrzymujące karmę kontrolną, T — myszy otrzymujące karmę HFSC*

nificantly higher than the mice of group C ($p < 0.01$) as early as within one month of feeding but only 30 minutes post glucose load (Table III). Significantly higher levels of insulin were observed in the mice of group T at all time points (0 minutes [$p < 0.001$], 30 minutes [$p < 0.01$], and 120 minutes [$p < 0.025$]) after five months of feeding as compared to the mice of group C (Table III).

In our study the leptin levels were lower in mice of group T, although not significantly after a month of feeding as compared to mice of group C (Fig. 5), indicating that the body's sensitivity to leptin was not lost yet. However, leptin levels in the mice of group T fluctuated during the second to the fourth month period, reaching significantly ($p < 0.05$) higher concentration than the mice of group C after feeding for five months (Fig. 5). Also, the cortisol levels did not show any significant change in mice of group T as compared to mice of group C after five months of feeding (Table IV).

Discussion

A previous study from our laboratory has shown that HFSC feed could induce features of metabolic syndrome like obesity, hyperglycaemia, hypercholesterolaemia, and hypertriglyceridaemia in male C57BL/6J mice [9]. The increased levels of fasting blood glucose that were noted in our study could indicate hepatic insulin resistance because this glucose is derived from glycogenolysis from the liver [20, 21]. Glucose tolerance was found to be significantly impaired after two months of feeding with the HFSC diet because there was a failure to clear the high blood glucose levels post glucose load. This impairment only worsened in mice of group T throughout the feeding regimen as compared to mice of group C. Our results are in agreement with a previous study [22]; however, glucose intolerance was much more severe in the current study. This could be due to hepatic insulin resistance, muscle insulin resistance, and impaired insulin secretion [20]. High Insulin levels observed in our study further confirms it.

The total area under the glucose tolerance test curve (AUC) and the insulin curve (AUC ins) was found to significantly increase in the mice of group T as compared to the mice of group C as early as after two months of feeding and was elevated until five months of feeding implying impaired glucose tolerance. This could be due to insulin resistance triggered by HFSC feed. Several studies [23] show that insulin sensitivity is reduced in human subjects with reduced glucose tolerance. HOMA-IR further confirmed insulin resistance in our animal model. The increased levels of insulin in the mice of group T after five months of feeding as compared to mice of group C indicate that the mice of group T had OGTT-stimulated hyperinsulinaemia as compared to mice of group C. A study reported that 54% of obese children and adolescents have higher fasting or OGTT-stimulated hyperinsulinaemia [24]. This supports our study. Insulin resistance was also observed in prediabetic patients, which increased in parallel to waist circumference [25]. Pancreatic beta cell function as revealed by Matsuda index was significantly lower in mice of group T as compared to mice of group C by one month and worsened by five months of feeding. The ratio of AUC (ins/glc) is an insulin secretion index whereas the Matsuda index is an OGTT-stimulated index and provides an insight into the insulin sensitivity [19]. The product of these two indices is a measure of

Table II. *Homeostatic model of insulin resistance analysis (HOMA-IR) of male C57BL/6J mice during the five-month study*

Tabela II. *Analiza danych modelu homeostatycznego oceny insulinooporności (HOMA-IR) u samców myszy C57BL/6J w trakcie 5-miesięcznego badania*

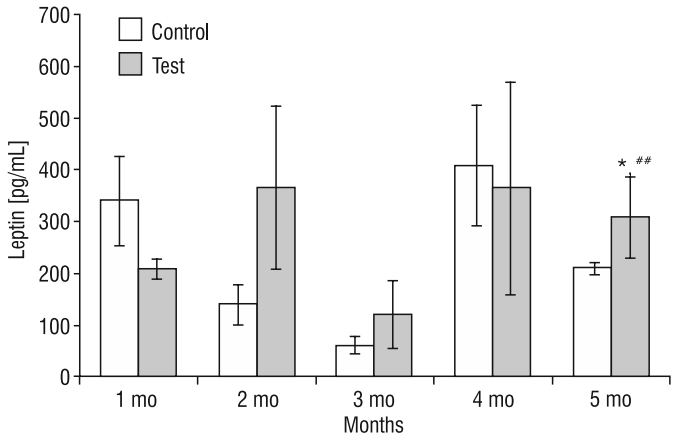
	0 Day	1 mo	3 mo	5 mo
Control	1.86 ± 0.009	2.35 ± 0.37	2.61 ± 0.31	3.007 ± 0.46
Test	1.86 ± 0.03	4.13 ± 0.84	33.92 ± 4.12***	18.22 ± 1.39***

Data is represented as mean ± S.E.M. of 5-6 independent values. *** $p < 0.001$ Test as compared to the respective age-matched control

Table III. *Insulin levels of male C57BL/6J mice following oral glucose load during the five-month study*
Tabela III. *Stężenia insulin u samców myszy C57BL/6J po doustnym obciążeniu glukozą w trakcie 5-miesięcznego badania*

	Control [μIU/mL]			Test [μIU/mL]		
	0 minutes	30 minutes	120 minutes	0 minutes	30 minutes	120 minutes
1 mo	10.29 ± 1.72	8.58 ± 0.70	11.07 ± 1.60	16.95 ± 3.44	17.42 ± 1.85**	16.06 ± 1.95
5 mo	12.41 ± 1.92	17.59 ± 2.62	16.88 ± 1.78	44.91 ± 3.32***	60.37 ± 6.77**	76.01 ± 18.88*

Data is represented as mean ± S.E.M. of 5-6 independent values. *p < 0.05, **p < 0.01, ***p < 0.001 Test as compared to the respective time-matched control



Data is represented as Mean ± S.E.M. of 5–6 independent values
* p < 0.05 5 mo Test as compared 5 mo Control; ##p < 0.01 5 mo Test compared to 1 mo Test

Figure 5. *Effect of HFSC diet on leptin on male C57BL/6J MICE. Control — mice fed with control diet, Test — mice fed with HFSC diet*
Rycina 5. *Wpływ karmy HFSC na stężenie leptyny u samców myszy C57BL/6J. Control — myszy otrzymujące karmę kontrolną, Test — myszy otrzymujące karmę HFSC*

Table IV. *Cortisol levels of male C57BL/6J after five months of feeding HFSC diet*
Tabela IV. *Stężenia kortyzolu u samców myszy C57BL/6J po 5 miesiącach stosowania karmy HFSC*

	Control	Test
Cortisol [μg/dL]	8.58 ± 0.62	9.76 ± 0.49

pancreatic β-cell function. This combination is distinct for different degrees of glucose tolerance. It is found to decrease from Normal Glucose Tolerance to Impaired Glucose Tolerance to Diabetes. In our study, the insert insulin of group T had reduced values as compared to mice of group C, indicating decreased pancreatic β-cell function. This is further supported by histopathological observations in the pancreas of HFSC-fed C57BL/6J mice in our previous study [16].

Leptin is associated with insulin resistance in patients with metabolic syndrome [26]. Insulin is known to stimulate leptin release [27]. In our study, leptin levels were found to be lower in mice of group T after one month of feeding, but not significantly as compared to mice of group C, indicating that sensitivity to leptin has not been lost. A study reported no difference in the leptin levels of pre-diabetic patients as compared to normal patients [26]. This supports our finding. However, leptin levels were significantly higher in mice of group T than mice of group C after feeding for five months. A contrasting report was found in a human study wherein no difference was observed in the leptin levels of metabolic syndrome and non-metabolic syndrome patients [28]. This could also be due to differences in diets consumed. Diets were found to have varied effects on leptin, as discussed subsequently. Long-term intake of a high-fat diet was found to positively correlate with plasma leptin levels in male rats [6] whereas a short-term, moderately high-fat diet was found to reduce circulating leptin levels [7]. Also, the type of dietary fats seems to affect plasma leptin levels [29]. In our study, insulin secretion was not found to be modulated by hyperleptinaemia even though leptin is known to inhibit insulin secretion in the pancreas via leptin receptors on the pancreatic β-cells [30]. This could be due to polymorphisms in the leptin receptors or leptin resistance in the pancreas, which needs to be evaluated.

There was no significant change in cortisol levels in mice of group T than mice of group C after five months of feeding. Higher cortisol levels were reported to reduce insulin secretion [31]. However, in our study insulin secretion, as stated earlier, was higher in mice of group T than mice of group C, indicating loss of regulation.

Conclusions

High incidence of metabolic syndrome worldwide warrants more focussed research for elucidating the causative factors. Among other factors, fast foods, which are energy dense, could also play a role in the aetiology of the syndrome. In the present investigation we have tried to systematically evaluate the time frame of disruption of glucose homeostasis by feeding HFSC feed (which mimics calorie-rich fast foods) to male C57BL/6J mice. Our study shows that HFSC feed induces glucose intolerance within two months of feeding probably by inducing insulin resistance and pancreatic beta cell dysfunction. Hyperleptinaemia was also noted in HFSC-fed C57BL/6J mice, which further shows that our animal model displays essential features of metabolic syndrome. Further studies on the hypothalamus adrenal axis in this model might shed more light onto the cell signalling mechanisms contributing to this syndrome. These observations may have clinical significance, especially with changing nutritional patterns globally.

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References

1. Bielohuby M, Sisley S, Sandoval D et al. Impaired glucose tolerance in rats fed low-carbohydrate, high-fat diets. Am J Physiol Endocrinol Metab 2013; 305: E1059–1070. doi: 10.1152/ajpendo.00208.2013
2. Woods SC, Lutz TA, Geary N et al. Pancreatic signals controlling food intake; insulin, glucagon and amylin. Phil Trans R Soc B 2006; 361: 1219–1235.doi:10.1098/rstb.2006.1858
3. Jenkins AB, Markovic TP, Fleury A et al. Carbohydrate intake and short-term regulation of leptin in humans. Diabetologia 1997; 40: 348–351. doi:10.1007/s001250050686
4. Dagogo-JS, Fanelli C, Paramore D et al. Plasma leptin and insulin relationships in obese and non-obese humans. Diabetes 1996; 45: 695–698. doi:10.2337/diab.45.5.695
5. Hamman A, Matthaei S. Regulation of energy balance by leptin. Endocrinol Diabetes 1996; 104: 293–300. doi:10.1055/s-0029-1211457
6. Ahrén B, Scheurink AJ. Marked hyperleptinemia after high-fat diet associated with severe glucose intolerance in mice. Eur J Endocrinol 1998; 139: 461–467. doi: 10.1530/eje.0.1390461
7. Lin S, Thomas TC, Storlien LH et al. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. Int J Obes 2000; 24: 639–646.
8. Ceddia RB, Koistinen HA, Zierath JR et al. Analysis of paradoxical observations on the association between leptin and insulin resistance. Faseb J 2002; 16:1163–1176.
9. D'Souza SS, Asha A. A systematic study of biochemical profile during the induction and development of an animal model for metabolic syndrome. IOSR J Pharm Biol Sci 2014; 9: 109–113.
10. Hoehn K, Marieb EN. Human Anatomy & Physiology. Benjamin Cummings. San Francisco 2010. doi: 10.1371/journal.pone.0094698
11. Asarian L. Membrane estrogen receptors and energy homeostasis. J Neurosci 2006; 26: 11255–11256. doi: 10.1523/jneurosci.3717-06.2006
12. Fernandez-Fernandez , Martini AC, Navarr V et al. Novel signals for the integration of energy balance and reproduction. Mol Cell Endocrinol 2006; 254–255: 127–132.
13. Hill JW, Elmquist JK, Elias CF. Hypothalamic pathways linking energy balance and reproduction. Am J Physiol Endocrinol Metab 2008; 294: E827–E832. doi:10.1152/ajpendo.00670.2007.
14. Ellacott KJG, Morton GJ et al. Assessment of feeding behavior in laboratory mice. Cell Metab 2010; 12: 10–17. doi: 10.1016/j.cmet.2010.06.001
15. Guidelines for care and use of animals in scientific research. Indian National Science Academy: New Delhi 2000.
16. D'Souza SS, Sri Charan Bindu B, Ali MM et al. Nutritional profile of High Fat Simple Carbohydrate Diet used to induce metabolic syndrome in C57BL/6J mice. Journal of Nutrition & Intermediary Metabolism 2016; 3: 41–49. doi: http://dx.doi.org/10.1016/j.jnim.2015.12.334
17. Buettner R, Parhofer KG, Woenckhaus M et al. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. J Mol Endocrinol 2006; 36: 485–501. doi:10.1677/jme.101909
18. Matthews DR, Hosker JP, Rudenski AS et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412–419. doi: 10.2337/db14-0116
19. Retnakaran R, Shen S, Hanley AJ et al. Hyperbolic relationship between insulin secretion and sensitivity on oral glucose tolerance test. Obesity 2008; 16: 1901–1907. doi: 10.1038/oby.2008.307
20. Abdul-Ghani MA, Matsuda M, Ballas B et al. Muscle and liver insulin resistance indexes derived from oral glucose tolerance test. Diabetes Care 2007; 30: 89–94. doi: 10.2337/dc06-1519
21. Winzell MS, Ahre´n B. The High-Fat Diet-Fed Mouse: A model for studying mechanisms and treatment of impaired glucose tolerance and Type 2 Diabetes. Diabetes 2004; 53: S215–S219. doi:10.2337/diabetes.53.suppl_3.S215
22. Fraulob JC, Ogg-Diamantino R et al. A mouse model of Metabolic syndrome: Insulin resistance, fatty liver and non alcoholic fatty pancreas disease in C57/BL6 mice fed a high fat diet. J Clin Biochem Nutr 2010; 46: 212–223. doi: 10.3164/jcbn.09-83.
23. Jensen CC, Cnop M, Hull RL et al., American Diabetes Association GENNID Study Group. β-Cell Function Is a Major Contributor to Oral Glucose Tolerance in High-Risk Relatives of Four Ethnic Groups in the U.S. Diabetes 2002; 51: 2170–2178. doi:10.2337/diabetes.51.7.2170
24. Buczyńska AZ, Klimek K, Pedras MF et al. Are metabolic syndrome and its components in obese children influenced by the overweight status or the insulin resistance? Endokrynol Pol 2011; 62: 102–108.
25. Bilir BE, Güldiken S, Tunçbilek N et al. The effects of fat distribution and some adipokines on insulin resistance Endokrynol Pol 2016. doi: 10.5603/EP.a2016.0023. [Epub ahead of print]
26. Lichnovska R, Gwozdziewiczova S, Chlup R et al. Serum leptin in the development of insulin resistance and other disorders in the metabolic syndrome. Biomed Papers 2005; 149:119–126. doi: 10.5507/bp.2005.014
27. Seufert J. Leptin effects on pancreatic beta-cell gene expression and function. Diabetes 2004; 53:152–158. doi:10.2337/diabetes.53.2007.S152
28. Gnacińska M, Małgorzewicz S, Szydłowska WL et al. The serum profile of adipokines in overweight patients with metabolic syndrome. Endokrynol Pol 2010; 61: 36–41.
29. Cha MC, Peter JHJ. Dietary fat type and energy restriction interactively influence plasma leptin concentration in rats. Lipid Res 1998; 39: 1655–1660.
30. Kieffer TJ, Habener JF. The adipoinsular axis: effects of leptin on pancreatic beta-cells. Am J Physiol 2000; 278: E01–E14.
31. Delaunay F, Khan A, Cintra A et al. Pancreatic beta cells are important targets for the diabetogenic effects of glucocorticoids. J Clin Invest 1997; 100: 2094–2098. doi:10.1172/JCI119743.