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EFFECT OF DIETARY CARBOHYDRATE SOURCE AND TYPE ON THE CONCENTRATIONS OF LIPOLYSIS-ENHANCING HORMONES IN RATS

Mariola Friedrich

Department of Human Nutrition Physiology, Agricultural University, Szczecin

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The effects of isocaloric substitution of dietary carbohydrates from whole cereal grains with carbohydrates from white flour and sucrose on body weight gain, body chemical composition, fatty acid profiles, and concentrations of corticosterone and thyroxine (T4), two hormones enhancing or promoting lipolysis, were followed experimentally.

The change in source and type of dietary carbohydrates was found to be accompanied by a reduction in the blood corticosterone concentration (from 115.3±54.6 to 44.9±29.8 ng/mL) in males only; the reduction could have facilitated adipose tissue accumulation (body weight gain from 1.22±0.97 g per 100 g standard diet to 1.68±0.58 g per 100 g modified diet). As, however, the females receiving the modified diet did show increased adipose tissue accumulation as well (from 1.32±0.47 to 2.23±0.80 g per 100 g feed consumed), with only a slight corticosterone concentration increase (from 52.2±20.0 to 67.4±336.7 ng/mL), it seems that the adipose tissue accumulation mechanism in females must have been different. No significant effect of the modified diet on thyroxine concentration was detected.

INTRODUCTION

It is already well known that triacylglycerol biosynthesis is enhanced not only by a surplus dietary energy, but also by a diet enriched with processed, refined, and/or simple carbohydrates which affect certain metabolic pathways [Chicco *et al.*, 2003]. The composition of a diet, has been reported as resulting in adipose tissue accumulation [Dreon *et al.*, 1988; Romieu *et al.*, 1988].

The research on effects of isocaloric substitution of dietary carbohydrates from whole cereal grains with carbohydrates originating from white flour and sucrose, has supplied data that show a modified diet to result in a statistically significant body weight gain, both in absolute values and when converted to 100 g diet consumed and 100 g body weight [Friedrich & Piech, 2000; Friedrich *et al.*, 2002]. Such a diet has also been demonstrated to affect the amount and distribution of adipose tissue, including the perivisceral one [Friedrich & Piech, 2000; Friedrich & Mateńczuk, 2001] and to change the adipose tissue fatty acid profile [Friedrich & Mateńczuk, 2001]. The causes of those changes may be sought in, *i.a.* lipogenic activity of insulin secretion, stimulated by diet composition [Pelikanova *et al.*, 1989], glucose metabolic disorders [Bjorntorp, 2000; Chicco *et al.,* 2003], and in deficiencies of minerals and vitamins involved in carbohydrate-lipid metabolism [Eder & Kirchgessner, 1994; Enomoto *et al.*, 1998; Faure *et al.*, 1997; Friedrich *et al.*, 2002; Korhle, 1994].

However, the few reports on the effects of dietary fatty acid types on secretion, transport, and tissue retention of adrenal and thyroid hormones [Kennedy *et al.*, 1994; Rosołowska-Huszcz *et al.*, 2001; Sarel & Widmaier, 1995; Smith *et al.*, 1993; Vallette *et al.*, 1991] that play a key role in lipolysis [Freake *et al.*, 1989; Strack *et al.*, 1995] are conducive to asking if a similar effect could be exerted by fatty acids released from body lipids and if their effects could be similar to those produced by identical fatty acids supplied with diet.

When trying to address that question, it was decided to follow effects of isocaloric substitution of dietary carbohydrates from whole cereal grains with carbohydrates originating from white flour and sucrose on body weight gain, body composition, and corticosterone and thyroxine (T_4) concentrations, the activity of the latter two hormones being described as enhancing or promoting lipolysis.

MATERIALS AND METHODS

The experiment (approved by the Local Ethical Commission) involved 60 rats (males and females) aged 8 months (the females had already offspring). The rats were randomly assigned to one of two experimental groups of 30 individuals each (15 males and 15 females), fed *ad libitum* a pelletised feed mix manufactured by GRSP Miłosław. Group I was fed a standard feed (diet I), while Group II was fed a modified feed in which whole wheat grains were substituted with wheat flour and sucrose; the two components replaced 50% of corn grains and 30% of barley groats (diet II). Composition of the feeds used in the experiment is shown in Table 1. The rats drank clean water left to stand for a period of time beforehand.

Using the methods described by Gawęcki and Jeszka [1995], the feeds were analysed for their contents of basic components: crude protein, lipids, dry matter, and ash.

Author's address for correspondence address: Mariola Friedrich, Faculty of Food Sciences and Fisheries, Agricultural University, ul. Papieża Pawła VI/3, 71-459 Szczecin; tel.: (48 91) 425 04 44; e-mail: friedrich@tz.ar.szczecin.pl

TABLE 1. The composition of diets (%).

Component	Diet I	Diet II
Wheat	20	–
Wheat flour (type 500)	–	20
Corn grain	20	10
Barley grits	15	10.5
Sucrose	-	14.5
Wheat bran	10	10
Milk substitute	15	15
Meat-bone meal	8	8
Soybean 44%	5	5
Dried green forage	5	5
Fodder falk	1	1
Polfamix „F"	1	1

TABLE 2. The chemical composition of diets (%).

Component	Diet I	Diet II
Protein	13.4	13.1
Fat	6.0	6.0
Carbohydrates	66.8	67.1
Dry matter	93.0	93.3
Ash	6.9	7.0
Dietary fibre		
Hemicellulose	10.31	5.69
Cellulose	6.09	5.67
Lignin	1.42	1.31
Gross energy (kJ/g)	17.2	17.2
Metab. energy (kJ/g)	15.7	15.7

Carbohydrate contents were calculated from differences between the feed dry weight and a sum of the basic component contents. The dietary fibre content was determined with ANKOM 220 (Table 2). The feed gross and metabolic energy contents were calculated using standard energy conversion factors.

The experiment took 6 weeks; the amount of feed consumed was being calculated during the progress of the experiment and the rats were weighed once a week. On termination of the experiment, the rats were put to sleep with an anaesthetic and blood was drawn from the heart. In the serum obtained, assays of glucose, triacylglycerol, and total cholesterol contents were run using enzymatic and colorimetric methods (a closed system involving an Integra biochemical analyser with reagents manufactured by Roche). The serum corticosterone assays were conducted radioimmunologically, with a 125I RIA Corticosterone Kit for mice and rats (ICN Biomedicals, Inc., California). The serum thyroxine (T_4) concentration was assayed radioimmunologically with a T4-RIA-PROP reagent kit (POLATOM, Świerk).

Chemical assays of the rat body were carried out on homogenised shoulder blade and thigh muscles, in which concentrations (%) of crude protein [Kjeldahl's technique, 2100 Kjeltec analyser (Foss Tecator)], raw fat (Soxhlet technique; dissolvent – petroleum ether, Sotex analyser (Foss Tecator)], dry matter (drying at 100–105°C to constant weight), and ash (a 6 h combustion at 550°C) were determined.

The pericardial and peri-intestinal fat was removed immediately after the blood had been collected, and weighed to 0.001 g.

The data were processed statistically – test t-Students, with the Statistica® computer software.

RESULTS

The wheat flour- and sucrose-containing diet was found to result in significantly higher body weight gains, both absolute and per 100 g feed consumed, compared to the control. The effect in males was accompanied by increased

TABLE 3. The effect of diets on changes in body weight, pericardial and peri-intestinal adipose tissue accumulation in male and female rats (n=15, x±SD).

Trait	Sex	Diet I	Diet II	Significance of differences: Diet	Significance of differences: Interaction diet x sex
Fodder consumption per day (g)	Males	27.0±1.0	26.7±1.1	-	
	Females	22.1±2.3	21.1±1.3	-	-
Body weight growth (g)	Males	18.7±5.8	26.7±9.2	**	
	Females	10.9±3.7	19.5±6.6	**	**
Body weight growth per 100g diet (g)	Males	1.22±0.97	1.68±0.58	**	
	Females	1.32±0.47	2.23±0.80	**	*
Pericardial fat per 100 g body mass (g)	Males	0.026±0.015	0.031±0.022		
	Females	0.023±0.006	0.017±0.006	-	-
Pericardial fat per 100 g diet (g)	Males	0.010±0.006	0.011±0.006		
	Females	0.009±0.002	0.006±0.002	-	-
Peri-intestinal fat per 100 g body mass (g)	Males	1.074±0.333	1.184±0.306		
	Females	1.928±0.336	1.715±0.291	-	-
Peri-intestinal fat per 100 g diet (g)	Males	0.380±0.146	0.407±0.114	-	
	Females	0.759±0.138	0.631±0.093	*	*

accumulation of pericardial and peri-intestinal fat, the increase amounting to 19 and 10%, respectively (Table 3). At the same time, the muscle fat content was reduced by 7.4% (Table 4). On the other hand, the females showed 26 and 11% reductions in the amount of pericardial and periintestinal adipose tissue, respectively, the muscle fat content remaining unchanged.

TABLE 4. The effect of diets on body composition of male and female rats (n=15, x±SD).

Component (%)	Sex	Diet I	Diet II
Fat	Males	4.47±0.37	4.14±0.41
	Females	6.32±0.32	6.30±0.33
Total protein	Males	22.3±0.20	21.9±0.22
	Females	20.9±0.16	21.0±0.18
Dry matter	Males	26.4±0.42	26.6±0.44
	Females	27.9±0.39	27.6±0.42
Ash	Males	1.14±0.03	1.13±0.03
	Females	1.27±0.04	1.15±0.05

The females fed the modified diet showed also a significant increase in the blood glucose and triacylglycerol concentrations, while both sexes revealed an increased total cholesterol content (Table 5).

Concentrations of the hormones assayed showed the modified diet to have significantly affected the corticosterone concentration in males only. Their blood concentration of the hormone was reduced by 61% (Table 5). The modified diet produced a different effect in females whose blood corticosterone concentration was observed to increase (by 9%). The modified diet's effect on the thyroxine (T4) concentration was non-significant in both sexes, but a slight reduction (by 3% in males and by 6% in females) of the hormone concentration was recorded.

DISCUSSION

The rat diet modified by substituting whole cereal grains with flour and sucrose was found to be conducive to increased body weight gains and to increased adipose tissue accumulation. The fat tissue was deposited not only peripherally in the body (females), but also inside (males).

Generally, lipogenesis involves transformation of glucose and participation of indirect transformation compounds such as pyrogronate, lactate, and acetyl-CoA in lipids. In rats, this pathway is particularly active in the adipose tissue and in the liver. The entire lipogenesis is controlled by the nourishment level and also by diet composition. In the present experiment, the two key compounds were the easily available starch and sucrose which, rapidly digested and inducing release of increased amounts of glucose, caused secretion of insulin activating acetyl-CoA carboxylase, responsible for carbohydrate transformation into acetyl-CoA. The intensity of lipogenesis is augmented by fructose, present in sucrose, which enters the pathway bypassing the control stage, *i.e.* phospho-fructokinase glycolysis, and stimulates metabolic pathways in liver that lead to intensified synthesis of fatty acids, their esterification, VLDL release, and blood triacylglycerol concentration increase.

In the experiment described, dietary effects on glucose, triacylglycerol, and total cholesterol concentrations was more pronounced in females than in males, but interactions between dietary and sex-related effects on the compounds analysed were observed only in the triacylglycerol contents. Levi and Werman [1998] contended that the type of carbohydrates consumed produced no effect on concentrations of the compounds in the blood of males; a contrary opinion was expressed by Faure *et al.* [1997] who claimed the effect to be significant in both sexes. The adverse changes observed in the experiment described (increased concentrations of glucose, triacylglycerols, and total cholesterol in the blood) could have been enhanced also by the amount of dietary fibre, reduced in the modified diet, as well as lower contents of vitamins and minerals that play a vital role in carbohydrate-lipid metabolism [Enomoto *et al.*, 1998; Faure *et al.*, 1997; Friedrich *et al.*, 2002; Gerhardt & Gallo, 1998; Korhle *et al.*, 1994].

However, the diet composition-induced intensified lipid synthesis does not explain adipose tissue accumulation which, in the experiment presented, was manifested as significantly higher body weight gains per 100 g feed consumed.

It is known at present that metabolic activity of the adipose tissue, particularly that surrounding the internal organs, is very substantial and that the tissue triacylglycerols constantly release fatty acids. Lipolysis of the accumu-

TABLE 5. The effect of diets on the concentrations of selected components in blood serum of male and female rats (n=15, x±SD).

Component	Sex	Diet I	Diet II	Significance of differences	
				Diet	Interaction diet x sex
Corticosterone (ng/mL)	Males	115.3±54.6	44.9±29.8	**	
	Females	52.2±20.0	67.4±36.7	-	**
Thyroxine (T4) (ng/mL)	Males	34.2±4.3	33.3±3.4	-	
	Females	34.9±3.9	32.9±4.2	-	-
Glucose (mg/dL)	Males	171.0±53.0	175.7±54.2	-	
	Females	147.4±19.7	182.9±21.3	**	-
Triacyloglycerols (mg/dL)	Males	139.3±49.0	159.2±57.1	-	
	Females	182.1±72.3	241.6±76.8	**	*
Total cholesterol (mg/dL)	Males	42.2±10,1	57.3±8.6	**	
	Females	46.7±8.9	64.1±12.3	**	-

lated adipose tissue reserves is due to, *i.a.* adrenaline, noradrenaline, glucagon, corticotrophin (ACTH), alpha- and beta-melanotrophin (MSH), thyreotrophin (TSH), growth hormone (GH), and vasopressin (AVP). Their lipolytic activity has been shown to involve activation of the cellular hormone-dependent lipase. To achieve this effect, the hormones need the presence of glycocorticoides and thyroid hormones which, as such, do not particularly increase lipolysis, but enhance the lipolytic activity of the hormones listed above [Freake *et al.*, 1989; Strack *et al.*, 1995].

In the present experiment, the relationship between diet composition, lipolysis enhancing hormones, and adipose tissue accumulation was found only in males who showed a corticosterone concentration reduction as high as 61%. This could suggest that reduction in the cortisol concentration below the regular level was also conducive to adipose tissue accumulation. The opinion in vogue at present holds that the accumulation of adipose tissue, particularly on the visceral, is due to hormone disorders, particularly high levels of insulin [Chicco *et al.*, 2003; Bjorntorp, 2000] and cortisol [Bjorntorp, 2000] as well as low concentrations of sexual hormones and of the growth hormone [Bjorntorp, 1997].

On the other hand, considering the results obtained and the few reports showing the fatty acid type to be capable of affecting the secretion, transport, and tissue retention of adrenal and thyroid hormones [Rosołowska-Huszcz *et al.*, 2001; Vallette *et al.*, 1991], it seems that the fatty acid composition of perivisceral adipose tissue may affect those processes as well.

That this is possible is confirmed by a significant increase in the total cholesterol in males, at an absence of changes in the concentration of glucose, the increase content of which in the blood triggers the onset of a number of processes resulting in enhanced retention of cholesterol esters [o'Rourke *et al.*, 2002]. Sarel & Widmaier [1995], who studied isolated rat cells, found an increase in oleic acid concentration to block corticosterone synthesis; this may indirectly, by inhibiting transformation, result in an increase in the total cholesterol blood concentration. The effect may be augmented by the inhibitory effect of oleic acid on somatotropine secretion observed by Kennedy *et al.* [1994] in their study on isolated rat hypophysal cells, as shown by Bjorntorp [1997], somatotropin content reduction enhances accumulation of perivisceral adipose tissue.

Why, however, no corticosterone reduction was observed in females? Both the females and males kept on the modified diet showed significantly higher body weight gains (per unit feed consumed), compared to the control (standard diet), it seems that the adipose tissue accumulation mechanism in the experimental females could have been related to lipogenic insulin effect, which is indicated also by the significantly higher concentrations of glucose, triacylglycerols, and corticosterone. The increase in the blood glucose concentration observed was directly related to the dietary starch and sucrose, the components of high glycaemic indices. After six weeks on the modified diet, such a high glucose concentration could have also resulted from a decreased sensitivity to the controlling effect of insulin, the phenomenon always accompanied by increased threshold of sensitivity to insulin and hyperinsulinaemia [Chicco *et al.*, 2003; Faure *et al.*, 1997]. Triacylglycerol concentration under physiological conditions was demonstrated to decrease with the amount of glucose offered, and that a high triacylglycerol content, despite glucose supply, is always indicative of, *i.a.* poor glucose tolerance [Chicco *et al.*, 2003].

The high glucose concentrations could have been also favoured by the observed increase in the corticosterone concentration that inhibited glucose uptake in non-hepatic tissues. That insulin was involved in the adipose tissue accumulation in females is also supported by the significant increase in the triacylglycerol concentration they showed. Insulin intensifies synthesis of triacylglycerols which are antagonistic towards insulin and still more adversely affect carbohydrate tolerance.

In addition to another mechanism of adipose tissue deposition, the females differed from males in the adipose tissue deposition sites. It seems that sex hormones played a role in the differences. In his studies on testosterone and estrogen effects on adipose tissue distribution in the body, Bjorntorp [1997] demonstrated estrogens to control adipocyte metabolism similarly to testosterone, but the effects involve other regions of the adipose tissue. Moreover, Zamboni *et al.* [1992] found post-menopausal women to suffer from android obesity more frequently than did the pre-menopausal women showing regular estrogen levels.

No statistically significant diet-induced effect on concentration of thyroxine, another lipolysis-enhancing hormone, could be revealed. The slight reduction of its concentration could have, however, contributed to the observed decrease in the muscle fat content. The thyroxine concentration reduction as such could have resulted from the modified diet containing less selenium. Its presence in Type I deiodinase is crucial for the formation of triiodinethyronine (T_3) [Korhle, 1994]. The thyroxine reduction could have also been caused by insulin concentrations in the rat blood, as Gabarrou *et al.* [2000] demonstrated that insulin concentrations were inversely correlated with concentrations of T_3.

CONCLUSIONS

1. As shown by the data obtained, replacement of dietary carbohydrates originating from whole cereal grains with those provided by flour and sugar resulted in significantly higher body weight gains.
2. The effect in males was accompanied by a reduction in the blood corticosterone concentration, which could have enhanced adipose tissue accumulation.
3. Increased adipose tissue accumulation in the females fed the modified diet was accompanied by a slight increase in the corticosterone concentration and a significant increase in concentrations of glucose and triacylglycerols, which indicates a different mechanism of adipose tissue accumulation.
4. No significant effect of the modified diet on thyroxine concentration was observed.

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WPŁYW ZMIAN ŹRÓDŁA I RODZAJU WĘGLOWODANÓW W DIECIE NA STĘŻENIA HORMONÓW AKTYWUJĄCYCH LIPOLITYCZNE CZYNNIKI WEWNĄTRZWYDZIELNICZE U SZCZURA

Mariola Friedrich

Zakład Fizjologii Żywienia Człowieka, Akademia Rolnicza, Szczecin

Doświadczenie przeprowadzono na 60 szczurach (samce i samice) w wieku 8 miesięcy. Zwierzęta podzielono losowo na dwie grupy żywieniowe (po 30 sztuk w każdej – 15 samców i 15 samic), żywione *ad libitum* granulowaną mieszanką wyprodukowaną przez GRSP w Miłosławiu. Grupa I otrzymywała mieszankę podstawową, grupa II mieszankę zmodyfikowaną, w której w stosunku do mieszanki podstawowej, pełne ziarna pszenicy zastąpiono mąką pszenną, a 50% ziarna kukurydzy i 30% kaszy jęczmiennej – sacharozą. Do picia zwierzęta otrzymywały czystą, odstaną wodę.

Doświadczenie trwało 6 tygodni, w trakcie których na bieżąco obliczano ilość spożytej paszy, a raz na tydzień kontrolowano masę ciała zwierząt. Po zakończeniu doświadczenia pobierano krew z serca i w uzyskanej surowicy oznaczano stężenia: glukozy, triacylogliceroli, cholesterolu całkowitego, kortykosteronu i tyroksyny (T4). Przeprowadzono również analizę składu chemicznego ciała oraz określono ilość tłuszczu okołonarządowego. Analiza uzyskanych wyników pozwoliła na stwierdzenie, że zamiana obecnych w diecie węglowodanów, których źródłem były pełne ziarna zbóż na mąkę i cukier, spowodowała istotnie większe przyrosty masy ciała badanych zwierząt (tab. 3) ale tylko u samców towarzyszył temu spadek stężenia kortykosteronu we krwi (tab. 5). U samic na diecie zmodyfikowanej zwiększonemu gromadzeniu tkanki tłuszczowej towarzyszył niewielki wzrost stężenia kortykosteronu, przy istotnym wzroście stężenia glukozy i triacylogliceroli (tab. 5). Nie stwierdzono istotnego wpływu zastosowanej diety na stężenie tyroksyny.