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# INFLUENCE OF DIETARY FATS ON SERUM PHOSPHOLIPID FATTY ACID COMPOSITION AND ITS RELATION TO OBESITY IN ANIMALS

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*Dietary fat and its relation to obesity has been a controversial issue for many years. Experimental data shows that most, though not all animals, which consume a high fat diet, will become obese. However, the effect of fatty acids on animal obesity has not been studied in detail. In order to evaluate the effects of low versus high fat diet on serum phospholipids fatty acids composition a 4-wk study was conducted on male Wister rats. The rats were fed low-fat (10% energy) and high-fat (46% energy) foods containing constant proportions of fatty acids. Control group C was fed a standard laboratory diet (polyunsaturated/saturated (P/S) fatty ratio 1.3), group M was fed a standard laboratory diet supplemented with margarine (P/S ratio 0.95), and the diet of the SL group was additionally supplemented with a sunflower oil-lard (1:1) mixture (P/S ratio 1.3).*

*All lipid supplemented hyperenergetic diets caused an increase in the average daily energy intake. Both the final and the daily body weight gain were significantly higher in M and SL groups than in group C. Additionally, serum triglyceride levels, LDL-cholesterol and total cholesterol were also significantly higher in M and SL groups when compared to the control group. Serum phospholipids fatty acids varied in response to total dietary fat. A significant decrease in saturated fatty acids (SFA) content (16:0 and 18:0) and an increase in monounsaturated fatty acid (MUFA) content (18:1, n-9) was found in the M group when compared to both C and SL groups. In the SL group, SFA content (18:0) was higher and MUFA content (18:1, n-9) was lower than in group C. Polyunsaturated fatty acids (PUFA) content showed an increase in both experimental groups. The PUFA/SFA ratio was higher in the M group than in the C and SL groups. Our study suggests that the amount of dietary fat has a greater influence on obesity than the effects of the type of fat consumed. However, depending on the type of fat present in the diet the differences were observed in the composition of serum PL fatty acid suggesting that both total fat and individual fatty acids have to be considered when reaching conclusions about the effect of dietary fat and obesity in animals.*

*Key words: dietary fat, phospholipids fatty acids, animal obesity, animal model*

## INTRODUCTION

A high-fat (HF) diet is known to induce obesity both in animals and in humans (Astrup *et al*., 1994; Bray and Popkin, 1998). Although the etiology of obesity is complex, dietary factors, and in particular the consumption of a high-fat (HF) diet, are its major risk factors. The length of a high fat diet also has a strong influence on obesity (Hill *et al*., 1992). There are experimental data showing that most, but not all, animals consuming a high fat diet will become obese. A number of mechanisms have been postulated for this difference, including differential sensitivities to the intestinal peptide, bombesin, and to individual fatty acids (Bray, 2000). However, a causal role of dietary fat and individual fatty acids in animal obesity has never been well documented and this is so in part because of inadequate animal models and experimental animal diets (Huang *et al*., 2004). Considerable experimental evidence links dietary fat intake with the development of cardiovascular diseases and insulin resistance (Fukuchi *et al*., 2004). Dietary fats can modulate the serum phospholipid fatty acid composition (Willett, 1998). Alterations in these lipid species are of special interest because serum phospholipids mirror the tissue phospholipid status and these functional and pathological consequences can be correlated (Pan and Storlien, 1993). It has been proposed in the early work of Murata *et al*. (1982) that the net initial increase in the arterial concentration of PL rich in saturated fatty acids may be an early step in the development of artherogenesis. Additionally, clinical studies in humans and animals show that *trans* fatty acids can increase insulin resistance and development of diabetes. The differences in response of inflammatory signals and of insulin resistance to different fatty acids indicate that not all fatty acids are the same (Haag and Dippenaar, 2005). It has been documented that not only fat quantity, but also the type of fat consumed affects weight gain. Saturated fatty acids (SFAs) have been shown to produce higher rates of weight gain when compared to other types of fatty acids (Al-Othman, 2000). On the other hand, polyunsaturated fatty acids (PUFA) have been reported to be negatively correlated to body fat in humans, but same fatty acids induce obesity in animals, even in the second generation (Pellizzon *et al*., 2002; Taylor and Poston, 2007).

The aim of this study was to examine changes of phospholipid class distribution and fatty acid profile serum phospholipids in animals fed obesity inducing experimental diets.

## MATERIALS AND METHODS

Male Wister rats, aged 8 weeks and at an average weight of 200 g, were divided into three groups (n = 6) and housed individually in a temperature controlled room. The control group C, was fed a commercial nonpurified diet (Veterinarski zavod, Subotica, Serbia), containing (w/w) 17.2% protein, 60.9% carbohydrates, 3.7% fat with a polyunsaturated/saturated (P/S) fatty acid ratio of 1.3, fiber 5.6%, and an adequate amount of vitamins and minerals (ash 7.6%). One gram represented an estimated metabolic energy of 14,9 kJ with 10% derived from fat. The second group (SL) was fed a standard diet supplemented with a

20 g % w/w sunflower oil-lard (1:1) mixture containing a fatty acid spectrum with a P/S ratio similar to the control diet. SL diet was composed of: protein 13.7%, fat 23.7%, carbohydrates 48.7%, fiber 4.5%, and an adequate amount of vitamins and minerals (ash 6.1%). One gram represented an estimated metabolizable energy of 19,7 kJ with 46% being derived from fat. The third group (M) was fed a diet identical to the SL diet but the sunflower oil-lard mixture was replaced with margarine (20 g% w/w) providing a P/S ratio of 0.95. Fatty acid composition of each diet is given in Table 1.

Table 1. Fatty acid composition in experimental diets (mol %)

| Fatty acid | C group | M group | SL group |
|---|---|---|---|
| 16:0 | 19.62 | 9.24 | 16.84 |
| 16:1 | 0.62 | 0.10 | 0.80 |
| 18:0 | 7.31 | 7.27 | 8.77 |
| 18:1 | 36.53 | 66.34 | 40.00 |
| 18:2 | 32.32 | 14.98 | 30.88 |
| 18:3 | 1.63 | 0.43 | 1.20 |
| 20:4 | 0.41 | 0.57 | 0.34 |
| SFA[#] | 26.93 | 16.81 | 25.60 |
| MUFA | 37.15 | 66.44 | 40.79 |
| PUFA | 34.35 | 15.97 | 32.41 |
| PUFA/SFA | 1.3 | 0.95 | 1.30 |

[#]SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids

The rats maintained a 12 hr light/dark cycle for 4 weeks and had free access to water. The control group food was restricted to 18 g/d while experimental groups were fed *ad libitum.* At the end of the four-week experimental period, all animals fasted overnight and were sacrificed. Samples of blood were taken by heart puncture using a heparinized syringe. Serum was separated by centrifugation, collected and stored at -40$^{o}$C until further processing.

All experimental protocols were approved by the Institute Ethical Office and designated by the Animals (Scientific Procedures) Act.

*Analytical procedures*

Serum lipids were extracted with chloroform-methanol mixture (2:1 v/v), following a procedure described previously by Ristić-Medić *et al*., 2003 and Ristić *et al*., 2006. During the extraction procedure, lipids were protected against oxidation by an adding 10 mg/100 mL butylated hydroxitoluene to the solvents. The total PL was determined by the method of Zilversmith (Zilversmih and Davis,

1950). One aliquot of serum (containing 10 mg lipid phosphorus) was spotted on thin layer glass plates precoated with a 0.25 mm layer of silica H and florisil (9:1). PL were separated by a one-dimensional thin-layer chromatography (TLC) system by using solvents (chloroform: methanol: 20% ammonia) in to the following fractions: sphingophospholipids (SPL), lysophosphatidylcholine (LL), phosphatidylcholine (PC), phosphatidylethanolamine (PE). PL fractions were aspirated from the plates and analyzed for phosphorus content.

Serum lipids were fractionated by TLC using hexane-diethyl ether-acetic acid (87:12:1, v/v) as a solvent. The PL fractions were scraped into glass tubes and transmethilated with 2M NaOH-methanol (heated at 85$^{o}$C for 1 h) and 1M sulfuric acid-methanol esters (heated at 85$^{o}$C for 2 h). The fatty acid methyl esters were then analyzed by gas chromatography using a Varian GC (model 3400, Varian Associates) as previously described (Tepšić *et al*., 1998).

Individual fatty acid methyl esters in the sample were identified from the retention times of authentic standards (Sigma Chemical Company) and/or polyunsaturated fatty acids (PUFA)-2 mixtures (Supelco, Bellefonte). Peak areas were determined with a Varian 4290 integrator, and the results were expressed as percentages of total identified fatty acids.

*Statistical analyses*

All results are expressed as means ± SD. Effects of the experimental diets were examined using a two-way ANOVA. If a significant effect was identified, differences between individual diet pairs were compared using the paired Student's t-test.

## RESULTS AND DISCUSSION

Data presented in Table 2 illustrates the energy intake and weight gain of experimental animals. Even though experimental groups were fed *ad libitum* there was no significant difference in food intake when compared with the control group. Average daily energy intake and the percent of energy derived from fat were significantly higher in both M and SL groups. Final body weight and daily body weight gain were also significantly higher in these groups. Our results demonstrate that the total caloric intake in experimental groups was significantly higher than in control rats. Rats fed with margarine (M group) had body weights that were not different from rats fed sunflower oil-lard (SL). The excessive energy intake caused by supplemented fat resulted in weight gain and fat deposition in experimental animals. It was shown that energy density of foods could be the key element on daily energy intake and induction of animal obesity (Stubbs *et al*., 1995; Prentice and Poppit, 1996).

The serum triglyceride levels and total and LDL-cholesterol were significantly higher in both experimental groups. However, total serum phospholipids and the cholesterol/phospholipid ratio increased in both experimental groups, though a significant increase was noted only for phospholipids (Table 3).

Table 2. Energy intake and weight gain of experimental animals

| | C group | M group | SL group |
|---|---|---|---|
| food (g/day) | 18±0 | 21.48±2.94 | 21.57±3.48 |
| average energy intake (kJ/day) | 267.64±0 | 422.82±57.91* | 424.13±67.96* |
| energy derived from fat (kJ/day) initial | 26.76±0 | 194.50±26.64* | 198.87±33.36* |
| body mass (g) | 190±29 | 205±28 | 191±23 |
| final body mass (g) | 276±6 | 424±43* | 418±48* |
| daily weight gain (g) | 2.85±0.32 | 7,52±1,08* | 7.08±0.56* |
| Efficacy Weight gain (g/MJ) | 1.06±0.30 | 1.68±0.12* | 1.78±0.12* |

Data: means ± S.D
*Significantly different ($p<0.01$) from the control

Table 3. The effects of experimental diets on serum triglyceride, total and HDL cholesterol and total phospholipid levels (mmol/L)

| | C group | M group | SL group |
|---|---|---|---|
| triglyceride | 0.82±0.08 | 1.01±0.14* | 1.02±0.06* |
| total cholesterol | 1.71±0.13 | 2.43±0.30** | 2.28±0.90** |
| HDL-cholesterol | 0.94±0.07 | 1.75±0.18** | 1.56±0.14** |
| total phospholipids | 0.94±0.07 | 2.21±0.24* | 2.34±0.16** |
| cholesterol/ phospholipids | 0.89±0.07 | 1.14±0.23 | 0.97±0.07 |

Data: means ± S.D.
*Significantly different ($p<0.05$) from the control
**Significantly different ($p<0.001$) from the control

The effect of different diets on serum PL content and distribution is shown in Table 4. LL and PC content increased in the margarine fed group while an increase of all four PL fractions was found in the group consuming sunflower oil-lard mixture. SPL/PC ratio showed a tendency to decrease in both experimental groups.

In our study, differences were observed in serum PL fatty acid composition, as shown in Table 5. A significant decrease in saturated fatty acid (SFA) content (both 16:0 and 18:0) and an increase in monounsaturated fatty acid (MUFA) content (18:1 n-9) were found in the M group when compared to both the C and the SL groups. The content of SFA (18:0) was higher and MUFA (18:1 n-9) was lower in the SL group when compared to the control. Polyunsaturated fatty acids (PUFA) content increased in both experimental groups. N-6 PUFA was higher in both the M and the SL group. N-3 PUFA content and n-3/n-6 ratio also increased in these groups though reached statistical significance only in the SL group. However, the PUFA/SFA ratio was higher in the M group than in the C and SL groups. It is well known that fat deposition and changes in serum PA composition are important factors in the pathogenesis of a variety of obesity-related disorders, including diabetes, insulin resistance, and hyperlipidemia, which affect the health of animals as well humans (Bray *et al*., 2002).

Table 4. Content and distribution of serum phospholipid fractions

| PL fraction | C group | | M group | | SL group | |
|---|---|---|---|---|---|---|
| | % | mg/g | % | mg/g | % | mg/g |
| LPC ^ | 12.63±0.43 | 0.24±0.01 | 12.69±0.51 | 0.28±0.03* | 11.93±1.19 | 0.28±0.03* |
| SPL | 26.41±1.42 | 0.51±0.03 | 25.43±0.56 | 0.55±0.05 | 25.26±1.19 | 0.59±0.04** |
| PC | 50.46±1.46 | 0.97±0.04 | 51.72±1.81 | 1.14±0.13* | 52.20±1.99 | 1.22±0.10** |
| PE | 10.47±0.95 | 0.20±0.03 | 10.85±0.75 | 0.24±0.04 | 10.68±1.11 | 0.25±0.01 |
| SPL/PC | | 0.52±0.05 | | 0.48±0.03 | | 0.48±0.03 |

Data: means ± S.D
^ phosphatidylcholine (PC), phosphatidylethanolamine (PE), sphingophospholipids (SPL), lysophosphatidylcholine (LPC)
* Significantly different ($p<0.05$) from the control
** Significantly different ($p<0.001$) from the control

In summary, our results suggest that both the amount and the type of dietary fat can affect body weight and body composition, and thus induce obesity. However, the amount of dietary fat plays a more significant role. Additionally, our research suggests that both total fat and individual fatty acids have to be considered when reaching conclusions about dietary fat and obesity in animals. Our future research will examine the mechanisms underlying individual fatty acid effects on diet-induced obesity in animals.

Table 5. Fatty acid composition of serum phospholipids

| Fatty acid | C group | M group | SL group |
|---|---|---|---|
| 16:0 | 27.92±1.19 | 17.53±1.21* | 22.52±1.33** |
| 18:0 | 26.97±0.44 | 20.77±1.81** | 28.21±2.07** |
| 16:1 | 0.36±0.09 | 0.16±0.02** | 0.29±0.13 |
| 18:1 n-9 | 6.27±0.63 | 19.45±0.95** | 4.65±0.51** |
| 18:2 n-6 | 16.06±0.97 | 17.17±1.55 | 14.83±1.17 |
| 20:3 n-6 | 0.92±0.22 | 1.18±0.10* | 0.52±0.11** |
| 20:4 n-6 | 12.78±1.52 | 13.77±0.86 | 21.87±2.41** |
| 22:4 n-6 | 0.28±0.08 | 0.30±0.04 | 0.43±0.10** |
| 18:3 n-3 | 0.33±0.07 | 0.19±0.02** | 0.19±0.05** |
| 20:5 n-3 | 0.49±0.11 | 0.18±0.04** | 0.19±0.09* |
| 22:5 n-3 | 1.09±0.18 | 0.77±0.11** | 0.71±0.09* |
| 22:6 n-3 | 4.67±0.66 | 5.56±0.36** | 4.46±0.62 |
| Σ SAT* | 54.78±1.29 | 38.21±2.39** | 50.73±3.07* |
| Σ MUFA | 6.64±0.66 | 19,59±0,91** | 4.95±0.54* |
| Σ PUFA | 36.63±1.14 | 39,02±2,12* | 43.18±2.98** |
| Σ n-6 | 30.05±1.07 | 32,42±2,03* | 37.65±2.93c** |
| Σ n-3 | 6.58±0.55 | 6,61±0,35 | 5.54±0.42** |
| n-6/n-3 | 4.40±0.47 | 4,92±0,35 | 6.83±0.72** |
| PUFA/SFA | 0.67±0.03 | 1,03±0,12** | 0.86±0.11** |

Data: means ± S.D.
#SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids
*Significantly different ($p<0.05$) from the control
**Significantly different ($p<0.01$) from the control

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## UTICAJ MASTI U OBROKU NA MASNE KISELINE U SERUMSKIM FOSFOLIPIDIMA I GOJAZNOST ŽIVOTINJA

TEPŠIĆ VESNA, PAVLOVIĆ MIRJANA, RISTIĆ-MEDIĆ DANIJELA, RISTIĆ VANJA, LEKIĆ N, TEPŠIĆ JASNA, DEBELJAK-MARTAČIĆ JASMINA, MILIĆEVIĆ M i GLIBETIĆ MARIJA

SADRŽAJ

Uticaj masnih kiselina u ishrani i njihov efekat na pojavu gojaznosti kod životinja je kontraverzno pitanje već dugi niz godina. Uticaj pojedinačnih masnih kiselina na pojavu gojaznosti kod životinja nije do sada detaljnije proučavan. Sa ciljem da se ispita efekat dijeta sa niskim ili visokim sadržajem masti na pojavu gojaznosti, izvršena su istraživanja na Wister pacovima u trajanju od 4 nedelje. Pacovi su hranjeni eksperimentalnim dijetama koje su sadržale standardan, nizak (10%) ili visok (46%) energetski unos poreklom od masti. Grupa kontrolnih životinja (C) je hranjena standardnom laboratorijskom dijetom (odnos polizasićenih/zasićenih masnih kiselina/PUFA, P/S 1.3), grupa M sa standardnom dijetom sa dodatkom margarina (P/S 0.95) i grupa SL sa dijetom kojoj je dodata mešavina 1:1 suncokretovog ulja i masti (odnos P/S 1.3). Obe dijete (M i SL) su uzrokovale povećanje u prosečnom dnevnom energetskom unosu. U grupi M i SL, registrovano je značajno povećanje u telesnoj težini kontinuirano tokom eksperimenta. Nivo serumskih triglicerida, LDL kao i ukupnog holesterola su bili značajnije povećani u M i SL grupi u poređenju sa kontrolom. Promene u profilu serumskih masnih kiselina fosfolipida su zavisile od sastava masti u eksperimentalnoj dijeti. Kod eksperimentalnih životinja grupe M primećeno je značajno smanjene serumskih zasićenih masnih kiselina (SFA, 16:0 i 18:0) kao i porast monozasićenih masnih kiselina (MUFA, 18:1, n-9) u poređenju sa SL i C grupom. Odnos PUFA/SFA je bio veći u M u odnosu na SL i C grupu. Rezultati naših istraživanja ukazuju da količina masti u ishrani životinja ima veći uticaj na porast telesne mase nego vrsta prisutne masnoće. Promene u sastavu serumskih masnih kiselina fosfolipida u odnosu na tip masti u eksperimentalnim dijetama ukazuju na ulogu pojedinačnih masnih kiselina u procesu nastajanja gojaznosti kod životinja i te mehanizme treba dalje istraživati.