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EFFECTS OF SATURATED AND POLYUNSATURATED FAT ENRICHED DIET ON THE SKELETAL MUSCLE INSULIN SENSITIVITY IN YOUNG RATS

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The purpose of this study was to evaluate the effect of diets enriched with fat containing different amounts (30% or 60% total energy) of either saturated (SF) or polyunsaturated fatty acids (PUFA) on glucose tolerance in vivo and insulin sensitivity of glucose utilisation by the soleus muscle in vitro. Young rats were maintained for 28 days after weanling on diets containing either sunflower oil (PUFA), animal fat (butter + lard – SF) or the standard laboratory chow (controls). The sunflower oil added to a diet in moderate quantities (30% total energy) improved the total glucose tolerance, while the diets containing high percentage of PUFA (60%) and moderate or high contents of SF caused impairment of glucose tolerance. The diet with 30% of total energy derived from sunflower oil increased the in vitro sensitivity of glucose utilisation to insulin by the soleus muscle, while in contrast, higher proportions of energy given in a form of PUFA as well as the diets enriched with animal fat impaired the sensitivity of this process to insulin. It is also important to note that the synthesis of glycogen in muscles taken from the rats fed high percentage of saturated fatty acid was found unresponsible to insulin. It is concluded that composition of dietary fat has a profound effect on carbohydrate tolerance and the response of muscle glucose metabolism to insulin. It seems likely that this effect may be at least partly mediated by changes in locally produced prostaglandins.

Key words: *fat diet, glucose tolerance, insulin sensitivity, polyunsaturated fatty acids, saturated fatty acids*

INTRODUCTION

A high-fat diet is known to impair glucose tolerance in man (1). Glucose intolerance can also be induced by feeding laboratory animals the fat enriched diet for a short period of time (2), which seems to be, at least partly, caused by insulin resistance of peripheral tissues (3, 4).

An inhibitory effect of fatty acids on the rates of glucose utilization and oxidation by the rat heart was first reported 25 years ago by Randle et al. (5) who suggested that this effect plays an important role in the control and maintenance of blood glucose level. This finding became an important part of the concept of the glucose/fatty acid cycle in regulation of glucose metabolism (5). Subsequently it was shown that an increased intake of fat, particularly of saturated fat, results in a decrease of glucose utilisation rate by the whole animal (6). It was assumed that this effect is the result of an increase in the plasma level of non-esterified fatty acids (NEFA) which, *via* the glucose/fatty acid cycle, decrease the rate of glucose utilization. Furthermore, feeding animals a high fat diet often results in obesity which is associated with a marked impairment of glucose tolerance (4, 7, 8). There is also some evidence that this effect may be due to a reduced rate of insulin-mediated glucose utilization by skeletal muscles (6, 7, 8). Such mechanism remains in agreement with the concept of the glucose/fatty acid cycle. However, an alternative explanation seems also likely. Feeding fat diet, containing a high content of either saturated or unsaturated fatty acids for a longer period of time, may cause changes in the fatty acid composition of membrane phospholipids, and in turn alter the type E of prostaglandins produced in the cell membranes. It has been proved in the studies performed using an isolated skeletal muscle that prostaglandins can modify sensitivity of glucose transport to insulin (9). It may be assumed, therefore, that a change in the composition of membrane phospholipids could alter the sensitivity of glucose utilisation to insulin by muscle cells.

In studies performed in human subjects a correlation has been ascertained between fat intake and the risk of developing the type II diabetes mellitus (10) characterised by insulin resistance. This impairment of insulin action can be caused, at least partly, by a decrease in insulin sensitivity of skeletal muscles due to a change in the membrane phospholipid composition and an altered content of local modulatores such as e.g. prostaglandins.

Experiments have been, therefore, performed to establish whether feeding rats various fat enriched diets influences the insulin sensitivity of glucose conversion to lactate (glycolysis) and glucose conversion to glycogen (glycogen synthesis) in the isolated soleus muscle preparation of the rat. The rate of the former process is known to be dependent on the rate of glucose transport to the cell, which is insulin sensitive (11, 12). In addition a glucose tolerance test (GTT) was performed in rats to find out to what extent an enhanced content of fat in diets influences blood glucose concentration in response to glucose load.

MATERIAL AND METHODS

The experiments were carried out on young male Wistar rats (bred at the National Institute of Food and Nutrition in Warsaw, Poland). Their initial body weight after weanling was 47 ± 4 g. They were divided into five main groups according to composition of diets on which they were maintained for 28 days: 1) a control group fed a standard stock diet, 2) animals fed a diet containing 30% of energy as a polyunsaturated fat (sunflower seed oil, PUFA 30), 3) rats fed a diet containing 60% energy as polyunsaturated fat (PUFA 60), 4) a group fed a diet containing 30% energy as saturated fat (butter and lard, 3:2 on a weight basis, SF 30) and 5) rats fed a diet containing 60% energy as saturated fat (SF 60). The animals of the control group were fed Murigran granulated stock diet (Bacutil rec. nr 1/113980).

All diets had similar energy content estimated on a weight basis (1860 Kcal/100 g for the experimental diets and 1867 Kcal/100 g for the stock diet).

Glucose tolerance test was performed in the overnight starved rats on the 25th day of the dietary regime as described by Budohoski et al. (13). A subcutaneus dose of glucose ($1\,g \times kg^{-1}$) was given as a 50% (w/v) solution. Blood samples (20 µ) from the tail artery were taken before glucose administration and then every 15 min within the subsequent period of 2 h following glucose load. Blood glucose concentration was measured according to the method of Bergmeyer et al. (14).

All animals were sacrificed on the 29th day of the experiment by decapitation after 12 h fasting. Immediately after sacrifice, soleus muscles from both hindlimbs were isolated and dissected longitudinally into two halves of similar weight (15). The strips were then used for incubation and measurement of the rates of lactate production and glycogen synthesis at various insulin concentrations. The muscle strips were first preincubated for 15 min in the modified Krebs-Ringer bicarbonate buffer containing 1.5% (w/v) deffated bovine serum albumin, and then transfered to the fresh medium containing in addition to the preincubation medium $0.25\,\mu Ci \times ml^{-1}$ of [$U^{-14}C$] glucose and insulin at various concentrations (from 1 to $10000\,\mu U \times ml^{-1}$). The flasks with muscle samples were gassed continuously with O_2/CO_2 (19:1) both during the preincubation period and for the first 45 min of the 60 min incubation (16). At the end of the incubation muscle strips were removed from the medium and freeze clamped. [$U^{-14}C$] glucose incorporation into glycogen was assessed as described by Espinal et al. (16). Samples of the incubation medium were used for enzymatic determination of lactate concentration according to the same author (16).

Muscle insulin sensitivity was expressed as the concentration of insulin in the incubation medium required to produce the half maximal stimulation of lactate production or glycogen formation. The results were obtained from a computer transformation of the relationship between insulin concentration in the medium and the magnitude of the response into a log-logit plot as described by Stupnicki (17).

Obtained data were checked by means of the two-way analysis of variance, followed by Duncan test. The null hypothesis was rejected when $p < 0.05$. The results are expressed as means $\pm$ SEM throughout the paper.

RESULTS

The effect of fat-enriched diets on the total glucose tolerance (*Fig. 1*) was found to depend on amount of fat and its origin. The PUFA 30 diet caused an improvement of glucose tolerance, whereas the same type of the diet but containing higher proportion of fat (60%) resulted in a marked decrease of GTT.

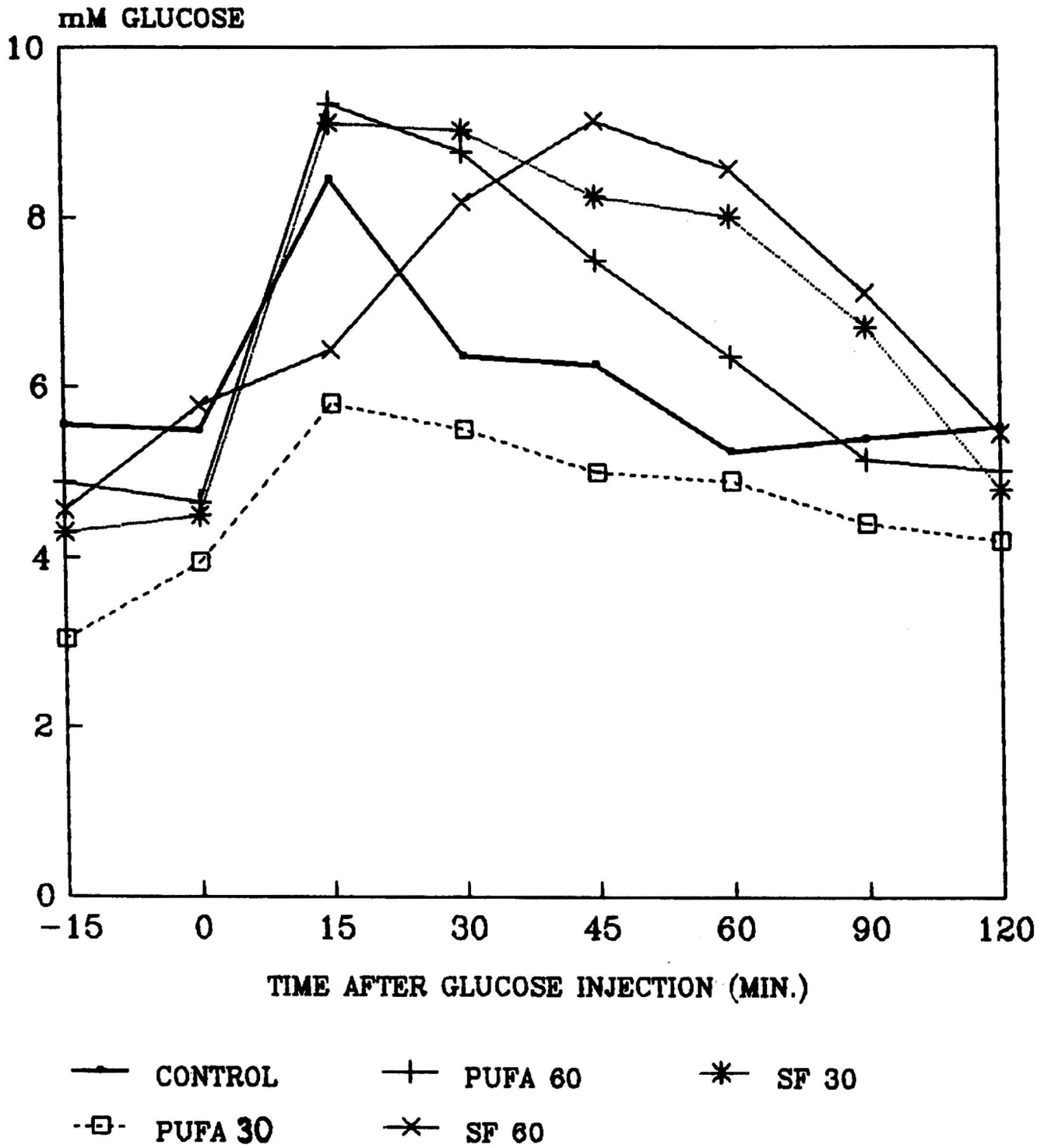

Fig. 1. The effect of 28 days diet containing 30% PUFA, 60% PUFA, 30% SF, 60% SF and standard laboratory chow on subcutaneus glucose tolerance test. Each point represents the mean value of 10 samples taken fom 10 animals.

Feeding rats the diet containing SF irrespectively to its content (30% or 60% of the energy) produced a significant impairment of glucose tolerance.

Sensitivity of LA production and glycogen synthesis to insulin measured in the soleus muscle *in vitro* was affected differently by the applied fat-enriched diets (*Tab. 1*). The PUFA 30 diet caused an increase in the rates of LA production at 10 and $100\,\mu U \times ml^{-1}$ of insulin in comparison with controls without any effect on the rates of glycogen synthesis. The concentration of

Table 1. The effect of different diets on lactate and glycogen production in the rat soleus muscle incubated in different insulin concentrations.

INSULIN CONC. ($\mu U \times ml^{-1}$)	STOCK DIET	SUNFLOWER OIL-DIET (PUFA) 30%	60%	ANIMAL FAT-DIET (SF) 30%	60%
	LACTATE PRODUCTION ($\mu mol \times g^{-1} \times h^{-1}$)				
1	7.29+/−0.21 (10)	6.18+/−0.07 (10)	5.69+/−0.10 (10) *	8.03+/−0.22 (10) $^{+}$	8.46+/−0.31 (10) $^{+}$
10	7.72+/−0.26 (10)	8.32+/−0.26 (10) *	6.07+/−0.08 (10) *	8.55+/−0.21 (10)	8.72+/−0.41 (10) $^{+}$
100	10.36+/−0.25 (10)	11.42+/−0.30 (10) *	7.12+/−0.21 (10) *	8.93+/−0.07 (10) *$^{+}$	8.91+/−0.26 (10) *$^{+}$
1000	12.93+/−0.27 (10)	13.66+/−0.32 (10)	14.25+/−0.76 (10)	11.41+/−0.30 (10)	10.12+/−0.42 (10) *$^{+}$
10000	13.32+/−0.31 (10)	14.52+/−0.396 (10)	16.11+/−0.52 (10) *	13.83+/−0.25 (10)	12.51+/−0.64 (10) $^{+}$
ECso	110.0+/−9.0 (10)	40.0+/−3.0 *	525.0+/−81.0 (10) *	1400.0+/−130.0(10) *$^{+}$	1125.0+/−150.0(10) *$^{+}$
	GLYCOGEN SYNTHESIS ($\mu mol \times g^{-1} \times h^{-1}$)				
1	1.29+/−0.09 (10)	1.11+/−0.07 (10)	1.20+/−0.06 (10)	3.93+/−0.42 (10) *$^{+}$	3.76+/−0.54 (10) *$^{+}$
10	1.42+/−0.16 (10)	1.22+/−0.06 (10)	1.20+/−0.06 (10)	4.55+/−0.21 (10) *$^{+}$	4.29+/−0.69 (10) *$^{+}$
100	2.36+/−0.05 (10)	2.27+/−0.16 (10)	2.01+/−0.09 (10)	4.93+/−0.47 (10) *$^{+}$	3.21+/−0.78 (10) *
1000	3.93+/−0.17 (10)	3.06+/−0.22 (10)	3.09+/−0.14 (10)	5.01+/−0.30 (10) *$^{+}$	4.65+/−0.25 (10) *
10000	3.82+/−0.21 (10)	3.52+/−0.09 (10)	3.67+/−0.29 (10)	4.83+/−0.65 (10) *$^{+}$	5.12+/−0.96 (10) *
EC50	108.0+/−10.0 (10)	96.0+/−13.0 (10)	96.0+/−15.0 (10)	n. m.	n. m.

Results are presented as means +/−S.E.M. with the number of animals (n) in parentheses.
n. m. — no measurable
* — $p < 0.05$ in comparison with control values
$^{+}$ — $p < 0.05$ in comparison PUFA 30 with SF 30 or PUFA 60 with SF 60

insulin that caused the half maximum stimulation of LA formation (ED_{50}) was markedly decreased by feeding the PUFA 30 as compared to controls (40 vs. 110 $\mu U \times ml^{-1}$ of insulin). Therefore sensitivity of this process to insulin in the soleus taken from the animals of this group was increased. The maximum response to insulin was, however, not affected. In contrast, the PUFA 60 diet caused a decrease in LA production at 1, 10 and 100 $\mu U \times ml^{-1}$ of insulin, resulting in a decreased sensitivity of this process to insulin with a ED_{50} value 500 $\mu U \times ml^{-1}$. Moreover, responsiveness of this process to insulin was increased. There was no effect of this diet on glycogen synthesis or its sensitivity to insulin.

In the soleus muscle taken from the rats fed a SF 30 diet, there was a considerable decrease in the sensitivity of LA formation to insulin in comparison with the control diet (1400 vs. 110 $\mu U \times ml^{-1}$). Moreover, the saturated fat diet increased the rate of glycogen synthesis at all insulin concentrations. An increase in insulin concentration from 1 to 10000 $\mu U \times ml^{-1}$ produced an increase in the glycogen synthesis only by 1 μM glucose $\times h^{-1} \times g^{-1}$ w.w., therefore this process did not show any sensitivity to insulin (*Table 1*).

The insulin sensitivity of the rate of glucose conversion to lactate was also markedly decreased in the soleus muscle taken from rats maintained for 28 days on the SF 60 diet (*Table 1*) in comparison with the control rats (ED_{50} 1125 ± 150 vs. $110 +/- 9$ $\mu U \times ml^{-1}$, *Table 1*). The responsiveness of this process to insulin was diminished as compared to that found in the animals fed polyunsaturated fat. However, it did not differ from the value in control animals (*Table 1*).

Similary to SF-30 diet, the rates of glycogen synthesis were increased at all concentrations of insulin and the sensitivity of this process to insulin was unmeasurable (*Table 1*).

DISCUSSION

The important finding of this study is that in rats both the *in vivo* glucose tolerance and insulin sensitivity of the rate of glucose utilisation to insulin in the soleus muscle in vitro are decreased in young animals maintained for 1 month on diets containing either a high proportion of saturated fat (30% and 60% energy) or a high proportion of polyunsaturated fat (60% energy). It is of interest that feeding animals a diet enriched with a smaller amount of PUFA 30 resulted in a marked increase of the insulin sensitivity of glucose conversion to lactate in the isolated soleus muscle. It can be explained by the fact that the sunflower seed oil contains a high proportion of the linoleic acid which might increase the rate of formation of homo-linoleic and arachidonic

acids (18) – the precursors of prostaglandins E_1 and E_2, respectively. As it has been shown previously (9) an addition of prostaglandin E_1 or E_2 to the incubation medium markedly improves the insulin sensitivity of glucose conversion to lactate in the soleus muscle preparation. Thus, it seems possible that the benificial effect of the diet to which some polyunsaturated fat was added on the muscle insulin sensitivity is due to the increased level of prostaglandins in this muscle. It is then tempting to suggest that a decrease in the sensitivity of this process to insulin after feeding the rats the saturated fat diet was caused by a decrease in the rate of production of prostaglandins of the E series.

When, however, an extra amount of polyunsaturated fat is added to the diet the increase in the FFA oxidation might occur. The latter process is known to cause an inhibition of glucose utilisation and glucose oxidation via the glucose/fatty acid cycle (18). An elevation of the plasma FFA concentration in the animals fed the polysaturated fatty acid containing diet (71% KJ) was previously reported (19). Thus it seems possible that when a diet contains a large amount of fat, the plasma fatty acid concentrations increase and the resulting inhibition of glucose utilisation causes the tissue insulin resistance. If this is the case then the postulated effect may, at least in the skeletal muscles, override the beneficial effect of prostaglandins produced from polyunsaturated fatty acid resulting in a marked decrease in insulin sensitivity of LA production when rats were fed SF 60 diet.

It is of interest to note that sensitivity of glycogen synthesis hardly changed in the experimental animal groups fed the polyunsaturated fat whilst it decreased dramatically in the muscles of animals fed saturated fat (both SF 30 and SF 60). This could be due to the fact that this process has been already maximally stimulated by high concentration of saturated fatty acid in the presence of minimal concentration of insulin.

Summarizing: The effect of fat diet on glucose tolerance and muscle insulin sensitivity depends not only on the type of fat (polyunsaturated or saturated) but also on the proportion of energy in the form of fat in a diet.

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