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# Substituting Dietary Polyunsaturated Fat with Monounsaturated Fat Increases Insulin Sensitivity in Cultured Rat Hepatocytes

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## ABSTRACT

*Increased dietary fat intake generally, and saturated fat specifically, will lead to impairment of insulin action and development of metabolic syndrome. The aim of this study was to find out changing of hepatic glucose output in dependence of high monounsaturated and high polyunsaturated fat diet and possible direct action of insulin in hepatocytes. Hepatocytes were isolated by a collagenase perfusion technique and cultured for 24 h in M 199 serum-free medium. The glucose production in hepatocytes isolated from rats on high polyunsaturated fat diet, as well as those isolated from rats on high monounsaturated fat diet was significantly higher than in standard controls. Insulin significantly decreased glucose production but only in hepatocytes obtained from rats on high monounsaturated fat diet. On the other side energy expenditure was significantly higher in rats on high monounsaturated fat diet and significantly lower in rats on high polyunsaturated fat diet comparable with animals on standard diet. Monounsaturated fat in Mediterranean diet could have beneficial effect on the development of the metabolic syndrome by increasing insulin sensitivity and energy expenditure.*

**Key words:** *insulin, insulin resistance, glucose production, gluconeogenesis, metabolic syndrome*

## Introduction

Insulin resistance is a common phenomenon in Type 2 diabetes and metabolic syndrome<sup>1</sup>. The metabolic syndrome is a collection of associated conditions such as dyslipidemia, high blood pressure, impaired glucose tolerance and tendency to develop fat around the abdomen<sup>2</sup>. A recent report estimated that 115 million people in Western countries suffer from metabolic syndrome<sup>3</sup>. Differences in genetic background, dietary habits, levels of physical activity, population age and sex structure, levels of over and under nutrition, may influence the prevalence of metabolic syndrome. The relationship between Mediterranean diet and metabolic syndrome has been investigated in several studies. Several aspects of this dietary pattern have been shown a beneficial effect on the development of the metabolic syndrome. The mechanism of reduction all-cause mortality are not fully understood but there are strong evidence to support that omega-3

fatty acids intake are associated with lower levels of blood pressure, triglycerides and other factors associated with metabolic syndrome<sup>4</sup>. Daily olive oil consumption is also characteristic of this diet.

Total lipid intake may be high, but the ratio of mono-unsaturated to polyunsaturated and saturated fats is much higher than in other type of diets. High fat feeding induces insulin resistance, but the type of dietary fat is thought to be important in this regard. Rats on high saturated fat diet had a greater hepatic glucose production and more intensive insulin resistance comparable with rats on standard diet<sup>5</sup>. Increased hepatic glucose output correlates well with fasting glucose levels and is the main cause of fasting hyperglycemia in Type 2 diabetes and metabolic syndrome<sup>6</sup>. We tried to find out does increase of hepatic glucose output depends on percentage of mo-

nounsaturated and polyunsaturated fat consumed during early experimental period before development of manifesting symptoms of diabetes. We measured rate of glucose production in hepatocytes isolated from rat fed with high monounsaturated fat from olive oil and high polyunsaturated fat from sunflower oil and found striking differences in rate of glucose production as well as in insulin action.

## Materials and Methods

In all experiments, male adult Wistar rats, each weighing 180–245 g. were used. Rats were housed individually in wire cages in a temperature-controlled room ( $21 \pm 1$  °C), on 12h light-dark cycle, with free access to food and water for three weeks. By energy, standard food contained 57% carbohydrates, 32% protein and 11% fat. High fat diet we prepare mixing 30% of sunflower oil or 30% of olive oil to standard food. In both cases high fat diet contained 30% carbohydrates, 16% protein and 54% fat.

The principles of animal care (NIH publication No. 85-23, revised 1985) were followed.

Hepatocytes were isolated by a modified collagenase-perfusion technique<sup>7</sup>. The rats were anaesthetized with Phenobarbital (10 mg/100 g body weight) and calcium-free Swim's S-77 medium containing collagenase (0.5 g/L) was used for liver perfusion through a portal cannula. Usually more than 90% of cells excluded trypan blue as the measure of viability. After washing twice with the same collagenase-free medium, the cells were suspended to a final concentration of a one million cells per mL M199 serum-free medium. Three mL of cell suspension was placed in 60-mm Petri dishes previously coated with collagen. Culture dishes were kept at 37 °C in an atmosphere of 5% CO<sub>2</sub> and 95% air (CO<sub>2</sub> incubator Heraeus, Hanau, Germany). The culture medium was replaced with fresh medium 4 hours later to remove unattached cells and hepatocytes were incubated for the next 24 hours in the M199 serum-free medium.

After having been 24h in culture, the medium was removed and cells were incubated in glucose-free Hanks-Hepes medium, containing 10 mmol/L pyruvate, without hormones (control) or with insulin (80 nmol/L). The glucose production was measured by incubating the cultures in glucose-free Hanks-Hepes medium with addition 10 mmol/L pyruvate. The glucose was determined enzymatically with glucose oxidase. The incubation medium was removed and hepatocytes were washed three times with cold saline and frozen immediately in liquid nitrogen. The cells were digested in 0.2 N NaOH and an aliquot were taken for the determination of protein. The amount of protein was determined by the Lowry et al. method<sup>8</sup>.

Albumin bovine, glutamine, HEPES, M199 medium, Swim's S-77 medium, insulin, was obtained from Sigma; Collagenase CLS II (131 U/mg) was purchased from Worthington; Collagen R was purchased from Serva.

Perfusion medium is a Swim's S-77 medium containing 2.2 g NaHCO<sub>3</sub> and 585 mg glutamine per liter.

Incubation medium is a M199 medium containing the following additions per liter: 2 g albumen, 900 mg L-glutamine and 2.2 g NaHCO<sub>3</sub>.

The data are expressed as means  $\pm$  SEM. Statistical significance was evaluated by Student's t-test.  $P < 0.01$  was considered statistically significant.

## Results

Body weight changes of the Wistar rats on three different dietary protocols are shown on Figure 1. Both the rats on high fat diets and those on a standard diet had a same body weight at the beginning of the experimental period. The increase in body mass was almost linear and without any significant differences between rats on high monounsaturated fat diet and control group on standard diet. After three weeks period final body weight was 25% higher comparable with body weight at the beginning of experiment in animals on high monounsaturated fat diet as well as in control animals on standard diet. On the other side increase in body weight was 30% in animals on high polyunsaturated fat diet. The dietary intake was measured daily during whole three weeks period. It was very stable in a range between 27 and 29 g per day in animals on standard diet. This provided sufficient energy for various body functions as well as for steady growth. The dietary intake was significantly lower in rats on high fat diets (Figure 2). In animals on high monounsaturated

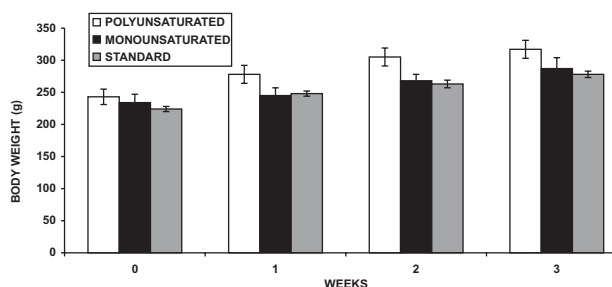


Fig. 1. Body weight (g) changes of the Wistar rats fed ad libitum with high polyunsaturated fat diet, high monounsaturated fat diet and control standard diet during three weeks period. Each value is the mean  $\pm$  SEM of 6 animals.

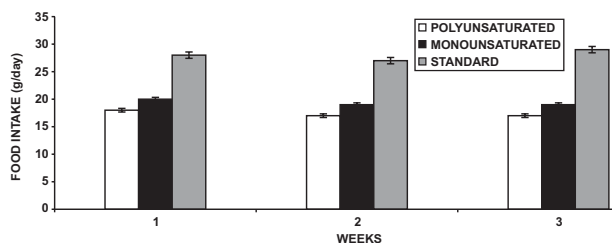


Fig. 2. Food intake (g/day) of the Wistar rats fed ad libitum with high polyunsaturated fat diet, high monounsaturated fat diet and control standard diet during three weeks period. Each value is the mean  $\pm$  SEM of 6 animals.

fat diet daily food intake was 30% lower than in control group. In spite of this increase in body mass was identical like in control animals. In animals on high polyunsaturated fat diet daily food intake was 40% lower than in control group and the increase in body mass was even higher than in control group (Figure 1). If we compare energy intake expressed as kJ per rat consumed during day most differences between experimental groups and control group disappeared (Figure 3). There are not any significant differences in energy intake between high polyunsaturated fat diet and control group on standard diet. On the other side energy intake in animals on high monounsaturated fat diet was 12% higher than in control animals. The highest rate of glucose production in control cultures was in first hour and was approximately 30 nmol per mg of protein, in second hour was 20 nmol and only 12 nmol in third hour (Figure 4). In cultured hepatocytes isolated from rats on high polyunsaturated fat diet, there was an almost linear increase of glucose production throughout the all 3-hours period and in first hour was 29%, in second hour 50% and in third hour 69% higher than in control cultures. In cultured hepatocytes isolated from rats on high monounsaturated fat diet, glucose production was also significantly higher than in standard control and in first hour was 29%, in second hour 40% and in third hour 53% higher than in control cultures. Insulin in a concentration of the 80 nmol/L significantly decreased glucose production but only in hepatocytes isolated from rats on high monounsaturated fat diet (Figure 4).

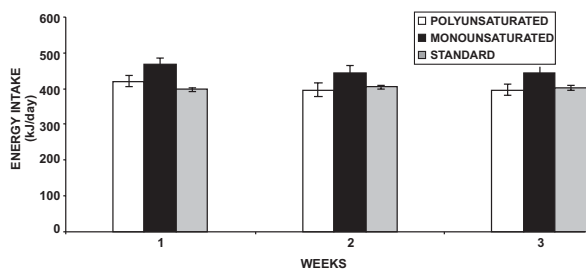


Fig. 3. Energy intake (kJ) of the Wistar rats fed ad libitum with high polyunsaturated fat diet, high monounsaturated fat diet and control standard diet during three weeks period. Each value is the mean  $\pm$  SEM of 6 animals.

## Discussion

The present study demonstrated that the increase in body mass was linear and without any significant differences between high monounsaturated fat diet and control group on standard diet (Figure 1). Stability of body weight over long period of time requires that animal's energy intake be balanced with energy expenditure. In young animals intake of energy persistently exceeds expenditure and the excess of energy is used for the steady growth. Of course if energy intake exceeds needs for steady growth, the most of this difference is stored as a fat. This probably happens in animals on high polyunsat-

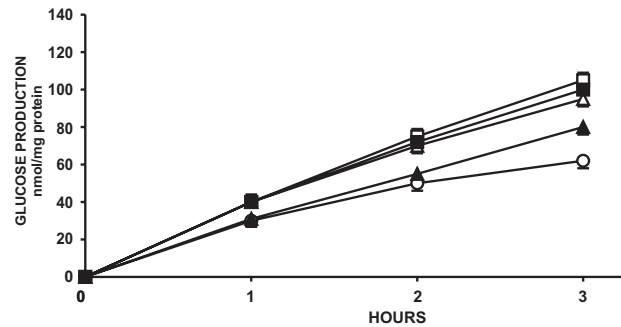


Fig. 4. Glucose production (nmol/mg prot) in cultured hepatocytes incubated in glucose-free Hanks-Hepes medium in presence 10 mmol/L pyruvate, isolated from rats on high polyunsaturated fat diet without (-□-), or with insulin (80 nmol/L) (-■-), or from rats on monounsaturated fat diet without (-△-) or with insulin (-▲-) and from rats on standard control diet (-○-). Each point is the mean  $\pm$  SEM for 6 plates in two separates experiments.

urated fat diet since increase of body weight was significantly higher than in control animals on standard diet. Interestingly, animals on high polyunsaturated fat diet consumed 40% less food comparable with animals on standard diet, but their energy intake was identical (Figure 2 and 3). In spite of this, increase of body weight was significantly higher in animals on high polyunsaturated fat diet presumably because of less energy expenditure in metabolic processes or lower muscle activity or both, what actually would lead in adiposity. This adiposity, particularly central adiposity, would lead to the impairment of insulin action. The association of obesity with type 2 diabetes and metabolic syndrome has been recognized long time ago<sup>9</sup>. Major link between obesity, Type 2 diabetes and metabolic syndrome is insulin resistance which is characterized by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output. These functional defects may result from impaired insulin signaling in all three target tissues<sup>10</sup>. The basic glucose production in hepatocytes isolated from rats on high polyunsaturated as well as on high monounsaturated fat diet was significantly higher comparable with controls on standard diet (Figure 4). We already showed similar effect of high saturated fat diet on glucose production<sup>11</sup>. The hepatic glucose production represents a sum of the glucose derived from glycogen stores and that originated from gluconeogenesis. In glucose-free medium hepatocytes produce glucose mainly from glycogen during the first hour of incubation. The glycogen stores are limited and exhausted after one hour in hepatocytes in vitro<sup>12</sup>. Than glucose supply relies on its synthesis from non-glucidic precursors such as lactate or pyruvate<sup>13</sup>. The process of the gluconeogenesis was activated during the first hour of incubation in hepatocytes and it became the main process for glucose production during second and third hour<sup>12</sup>. Presumably high free fatty acid oxidation in hepatocytes isolated from rats on high fat diets promoted gluconeogenesis via production of ATP, NADH and acetylCoA<sup>14</sup>. Fatty acid oxidation

played a permissive role on gluconeogenesis and provided the liver with co-factors necessary for an efficient gluconeogenesis, which could lead to fasting hyperglycemia in vivo. It has become conventional wisdom that fasting hyperglycemia is directly related to an increase in hepatic glucose production, but beside the hepatic glucose production in absolute term, it is much more important does liver respond normally on various hormonal signals. In this regard it was very important finding in our experiments that insulin did not inhibit glucose production in hepatocytes isolated from rats on high polyunsaturated fat diet. This inability of insulin to control the activity of gluconeogenic enzymes contributed even more to an increased hepatic glucose output and elevated blood glucose levels. Direct insulin effects on glucose production in the liver, especially on gluconeogenesis, are highly dependent on the level of glucose in the medium. In all our experiments glucose production was measured in a glucose free medium. We already showed very weak insulin effect on the glucose production in hepatocytes isolated from rats on a standard diet which were cultured in vitro and incubated in glucose-free medium<sup>12</sup>. So finding that insulin significantly and strongly decreased glucose production in hepatocytes isolated from animals on high monounsaturated fat could be presumably a result of increased insulin sensitivity (Figure 4). One of the possible

mechanisms for the signaling defects in obesity may be increased expression and activity of several protein tyrosine phosphatases, which dephosphorylate and thus terminate signaling propagated through tyrosyl phosphorylation events. Protein tyrosine phosphatase 1B and leukocyte antigen-relating phosphatase have been shown to dephosphorylate the insulin receptor and IRS-1 in vitro<sup>15</sup>. Mice in which protein tyrosine phosphatase 1B has been knocked out have increased insulin sensitivity and increased energy expenditure<sup>16</sup>. Same mechanism of increased insulin sensitivity could be operative in rats on high monounsaturated fat diet since they had a higher energy expenditure comparable with control animals on standard diet as well as with rats on polyunsaturated fat diet.

Our results clearly demonstrated that monounsaturated fat in Mediterranean diet could have a beneficial effect on the development of the metabolic syndrome increasing insulin sensitivity and energy expenditure.

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### ZAMJENA DIJETALNIH VIŠESTRUKO-NEZASIĆENIH MASNOĆA SA JEDNOSTRUKO-NEZASIĆENIM MASNOĆAMA POVEĆAVA INZULINSKU OSJETLJIVOST U KULTURI ŠTAKORSKIH HEPATOCITA

### SAŽETAK

Povećani unos masnoća, a posebno zasićenih masti, dovodi do smanjenja inzulinskog učinka i razvitka metaboličkog sindroma. Cilj istraživanja je otkriti promjene u proizvodnji glukoze iz jetre u ovisnosti o dijeti bogatoj jednostruko-nezasićenim i višestruko-nezasićenim masnoćama i moguće direktne učinke inzulina u hepatocitima. Hepatociti su izolirani pomoću kolagenaze i kultivirani 24 sata u M 199 mediju bez dodatka seruma. U hepatocitima izoliranim iz štakora na dijeti bogatoj višestruko-nezasićenim masnoćama, kao i u onima izoliranim iz štakora na dijeti bogatoj jednostruko-nezasićenim masnoćama, proizvodnja glukoze bila je signifikantno veća nego u standardnim kontrolama.

Inzulin je signifikantno smanjio proizvodnju glukoze ali samo u hepatocitima dobivenim iz štakora na dijeti bogatoj jednostruko-nezasićenim masnoćama. S druge strane potrošnja energije bila je signifikantno veća u štakora na dijeti bogatoj jednostruko-nezasićenim masnoćama, a signifikantno manja u štakora na dijeti bogatoj višestruko-nezasićenim masnoćama u usporedbi sa životinjama na standardnoj dijeti. Jednostruko-nezasićene masnoće u mediteranskoj prehrani mogle bi imati blagotvorno djelovanje na razvitak metaboličkog sindroma, povećavajući inzulinsku osjetljivost i potrošnju energije.