Effect of dietary fats on the development of insulin resistance in relation to PPARγ activation and leptin concentration in rats

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Abstract: Insulin resistance usually precedes the onset of type 2 diabetes. Therefore, studying factors that may influence the development of insulin resistance and their mechanisms of action is of huge importance. The aim of the present study was to investigate the effect of dietary fat type on the development of insulin resistance in relation to PPARγ activation and leptin concentration. Male Sprague–Dawley rats were assigned into maize starch-fed or fructose-fed group. The rats consumed diets containing olive oil, maize oil or sheep tallow for 10 weeks. Serum glucose, insulin and leptin concentrations were then determined. HOMA-IR was calculated as an index of insulin resistance. PPARγ activation was assessed using a PPRE-based ELISA system. Sheep tallow produced the highest HOMA-IR in the maize starch-fed group. In the fructose-fed group, rats consuming olive oil were the only rats to show significantly higher HOMA-IR and insulin concentration compared to the maize starch-fed group. Neither PPARγ activation nor leptin concentration was affected by dietary fat type. In conclusion, dietary fat type, in the maize starch-fed group, altered insulin resistance by mechanisms independent of affecting PPARγ activation or leptin concentration with sheep tallow showing a deleterious effect.

1. Introduction

Insulin resistance is a risk factor of developing both of prediabetes and type 2 diabetes [1]. The role of dietary fat type in the development of insulin resistance has been widely studied, yet findings produced are rather inconsistent. It is generally accepted that saturated fatty acids (SFA) increase the risk of insulin resistance [2-5]; nevertheless, some studies failed to support this [6-8]. n-3 polyunsaturated fatty acids (PUFA) may be beneficial in animals but not in humans [9, 10], whereas the role of n-6 PUFA is more controversial [11]. The effect of monounsaturated fatty acids (MUFA) is yet to be fully elucidated. Some [12-16] but not all [17-19] epidemiological and intervention studies have found a favourable impact with respect to insulin resistance.

The mechanisms linking dietary fat quality to insulin resistance are not fully understood; nonetheless, the effect of dietary fat type is believed to be mediated, at least partially, through the fatty acid composition of cell membranes [20]. More recent experimental data also point towards other mechanisms that involve direct regulatory effects on transcriptional activity and adipocytokines [21, 22]. In this study we examined the effect of dietary fat type on the ligand-activated transcription factor, peroxisome proliferator-activated receptor gamma (PPARγ), and the adipocytokine, leptin.

PPARγ is a member of the peroxisome proliferator-activated receptors (PPAR) family. This family of transcription factors has fatty acids and lipid-derived compounds as natural ligands [23] and it plays crucial role in glucose and lipid metabolism [24]. Several lines of evidence, which are reviewed by Rangwala and Lazar [25], suggest that PPARγ activation causes insulin sensitization. PPARγ promotes the storage of fat, increases adipocyte differentiation and regulates the transcription of numerous genes resulting in insulin sensitization [26, 27]. In fact, thiazolidinedione derivatives, the most extensively employed insulin-sensitizing drugs, have been found to possess a high affinity for PPARγ [28]. Similarly, it has been found that several fatty acids can directly bind PPARγ at physiological concentrations [26-28]. Results regarding the potency of various fatty acids in activating PPARγ are inconsistent [11, 21, 29]. Moreover, discrepancies may exist when the effect of diets rather than the effect of particular isolated fatty acids is studied [21].

Fatty acids in general are considered weak activators compared to drugs, which are used once the disease has already developed, despite this, fatty acids can influence transcriptional activity over

decades [21] causing considerable effects on insulin sensitivity and this could be of clinical interest.

Leptin, a product of the ob gene, is speculated to play a role in the pathogenesis of insulin resistance as it has been found to desensitize the activity of insulin most notably in the white adipose tissue [30]. Several studies have found that leptin is associated with insulin resistance independent of fat mass [31-33]. In addition, it has been found that leptin may be one of the factors predicting the degree of insulin resistance [34] and that baseline leptin concentration can predict diabetes [35]. However, the regulation of leptin is not completely understood with the majority of data suggesting that the size of the adipose tissue is a key regulator [36]. The effect of different dietary fat types on leptin is not known. Conflicting results have been reported by both human and animal studies [22, 37-41].

Therefore, the present study aimed at investigating the effect of different dietary fat types on the development of insulin resistance as well as examining possible mechanisms of action of dietary fats including its effect on PPARγ activation and leptin concentration using a high-fructose diet rat model that supplies olive oil, maize oil or sheep tallow as the sole source of fat for 10 weeks.

2. Material and Methods

Animals

Adult male Sprague–Dawley rats ($n = 36$; body weight 141-170 g) were purchased from the Experimental Animal Unit of Al-Yarmouk University (Irbid, Jordan). The animals were acclimatized for 2 weeks before the experiment. They were housed individually in plastic cages with stainless steel wire-mesh bottom under controlled temperature ($22 \pm 2°C$) and 12–h light–dark cycle. All experimental procedures involving animals were approved by the Institutional Animal Ethics Committee and conducted in compliance with the guidelines for animal use.

Diets

Six experimental diets were prepared, three of them contained maize starch as the main source of carbohydrate (normal diets) but differed in their fat type (10% w/w olive oil, maize oil or sheep tallow), while in the other three, maize starch was replaced with fructose to induce insulin resistance (high-fructose diets). The experimental diets were freshly prepared once a week and stored at −18 °C to avoid rancidity. The composition of all experimental diets is described in Table 1. Dietary supply of vitamins, minerals and protein were in accordance with the dietary recommended allowances for rats from the American Institute of Nutrition (AIN)-93M [42]. The major fatty acid composition of the dietary fats used in the experimental diets was determined by gas-liquid chromatography (GLC) and is shown in Table 2.

Experimental protocol

At the beginning of the experiment, animals weighed about 185 g and they were assigned into maize starch-fed group, consuming normal diets, or fructose-fed group, consuming high-fructose diets. Each group was further divided into three subgroups ($n = 6$) differing in the type of fat used (olive oil, maize oil or sheep tallow). During the experimental period, which lasted for 10 weeks, animals were fed ad libitum. Body weight and food intake were monitored weekly. On the termination day and after an overnight fast, animals were anesthetized using chloroform. Blood was collected by performing cardiac puncture and serum was isolated and stored as multiple aliquots at −18 °C until analysis. Finally, epididymal fat pads were excised, rinsed, blotted, weighed, immediately immersed in liquid N_2 and stored at −80 °C until analysis.

Biochemical analysis

Serum glucose concentration was determined by the glucose oxidase method using a commercial kit (Glucose liqicolor, Human Gesellschaft für Biochemica und Diagnostica mbH, Germany). Serum insulin and leptin concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits (Linco, St. Charles, MO, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as an index of insulin resistance using the equation of Mathews et al. [43]:

HOMA-IR = fasting insulin (μU/ml) × fasting glucose (mmol/l) / 22·5

DNA binding activity of PPARγ

The DNA binding activity of PPARγ was determined using the ELISA-based TransAM PPARγ transcription factor assay kit (Active Motif, Carlsbad, CA, USA). This ELISA-based method detects and quantifies PPARγ using a 96-well plate to which an oligonucleotide containing the peroxisome proliferator response element (PPRE) has been immobilized. Active PPARγ binds this PPRE and is detected through the use of an antibody directed against PPARγ. Addition of a secondary antibody conjugated to horseradish peroxidase provides a colorimetric readout that is quantified by spectrophotometry.

One hundred fifty milligrams of epididymal fat was homogenized manually on ice with 75 μl complete lysis buffer (lysis buffer AM1,

Table 1 Composition of the experimental diets

Ingredient	Normal diets (g/kg)	High-fructose diets (g/kg)
Maize starch	600	-
Fructose	-	600
Casein	140	140
Sucrose	60·3	60·3
fat[1]	100	100
Fibre	50	50
Mineral mix (AIN-93M-MX)	35	35
Vitamin mix (AIN-93M-VX)	10	10
DL-Methionine	2·2	2·2
Choline bitartrate	2·5	2·5
Tert-Butylhydroquinone	0·008	0·008
Total energy (kJ/kg)	1748·9	1748·9
Carbohydrate (% total energy)	64·9	64·9
Protein (% total energy)	13·6	13·6
Fat (% total energy)	21·5	21·5

[1] Olive oil, maize oil or sheep tallow
AIN American Institute of Nutrition

Table 2 Major fatty acid composition of the dietary fats used in the experimental diets

Fatty acid (g/100g total fatty acids)	Olive oil	Maize oil	Sheep tallow
10:0	ND	ND	0·2
12:0	ND	0·2	0·3
14:0	ND	ND	3·2
16:0	13·1	11·2	20·2
16:1n-7	ND	ND	0·3
18:0	2·6	1·7	16·6
18:1n-9	69.7	29·5	39·4
18:2n-6	10·8	53·9	2·8
18:3n-3	0·6	0·8	0·4
20:0	0·4	0·4	0·2
Sum SFA	16·1	13·5	40·7
Sum MUFA	69·7	29·5	39·7
Sum PUFA	11·4	54·7	3·2
Unsaturated fatty acids:SFA ratio	5·0	6·2	1·1
PUFA:SFA ratio	0·7	4·1	0·1

ND not detected, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

dithiotheritol and protease inhibitor cocktail, added as indicated by the manufacturer's instructions). After standing for 30 min on ice, the homogenates were centrifuged at 10,000 g for 30 min at 4 °C. The infranatant (below the fat cake) of each homogenate was collected and the protein concentration was determined using a Bradford assay (Bio-Rad, München, Germany). Equal amounts (5 μg) of protein were used in the assay according to the manufacturer's instructions.

Statistical analysis

Data analysis was performed using statistical analysis software (SAS version 9; SAS Institute Inc., Cary, NC, USA). Statistical significance was assessed by two-way ANOVA followed by the least significant difference (LSD) test. Data were presented as means with standard errors of the mean (SEM). A probability of $P \leq 0·05$ was accepted as being statistically significant.

3. Results

Body weight, weight gain, food intake, food efficiency ratio and epididymal fat weight

In the maize starch-fed group, dietary fat type did not induce changes in body weight, weight gain, food intake, food efficiency ratio and epididymal fat weight ($P \geq 0.05$; Table 3). On the other hand, in the fructose-fed group, weight gain and food efficiency

ratio were lowest in rats consuming sheep tallow ($P \leq$ 0.05; Table 3), whereas body weight, food intake and epididymal fat weight were not affected by dietary fat type ($P \geq 0.05$; Table 3).

Serum glucose and insulin concentrations

Dietary fat type did not affect glucose concentration in either maize starch-fed or fructose-fed group ($P \geq 0.05$; Table 3). Whereas dietary fat type influenced insulin concentration in the maize starch-fed group with sheep tallow showing a significantly higher insulin concentration compared to olive oil ($P \leq 0.05$; Table 3). In contrast, insulin concentration was not influenced by dietary fat type in the fructose-fed group ($P \geq 0.05$; Table 3). Noteworthy, rats consuming olive oil, in the fructose-fed group, were the only rats to show significantly higher insulin concentration compared to the maize starch-fed group ($P \leq 0.05$; Table 3).

Insulin resistance as determined by HOMA-IR

The degree of HOMA-IR in the maize starch-fed group was highest in rats consuming sheep tallow compared to rats consuming either olive oil or maize oil ($P \leq 0.05$; Table 3). In a different manner, the degree of HOMA-IR in the fructose-fed group was significantly higher in rats consuming olive oil compared to rats consuming maize oil ($P \leq 0.05$; Table 3). Noteworthy, in the fructose-fed group, rats consuming olive oil were the only rats to show significantly higher HOMA-IR compared to the maize starch-fed group ($P \leq 0.05$; Table 3).

DNA binding activity of PPARγ and serum leptin concentration

Dietary fat type did not induce modifications in DNA binding activity of PPARγ or leptin concentration in either maize starch-fed or fructose-fed group ($P \geq 0.05$; Table 3).

4. Discussions

High-fructose diet has been widely used in animals to induce several metabolic disorders including hyperglycaemia, hyperinsulinaemia, hypertriglyceridaemia, glucose intolerance and insulin resistance [44]. Surprisingly, after 10 weeks of high-fructose feeding, hyperinsulinaemia and insulin resistance, measured by HOMA-IR, were not established in our study except in the rats consuming olive oil. This is evident as insulin concentration and HOMA-IR were not significantly higher in the other two dietary fats compared to their controls in the maize starch-fed group.

In fact, two previous studies which used high-fructose diet to induce insulin resistance did not show significant changes in glucose and insulin

concentrations after 4 weeks of high-fructose feeding despite the presence of insulin resistance, measured by the intravenous insulin tolerance test (ITT) [45, 46]. In another study, which lasted for 8 weeks, high-fructose diet induced hyperinsulinaemia and insulin resistance, measured by the steady-state plasma glucose (SSPG) method, without hyperglycaemia [47]. In addition, a recent study by Chen et al. [48] has found that insulin resistance and hyperinsulinaemia developed only after 6-8 weeks of fructose feeding but without hyperglycaemia. Further feeding with high-fructose diet revealed hyperglycaemia without significant hyperinsulinaemia. Indeed, the duration of fructose feeding besides other factors, such as the fat type and content of the diet and the method used to measure insulin resistance can affect the metabolic glucose and insulin concentrations along with the degree of insulin resistance.

Due to the duration of our study, it might be reasonable to expect the absence of hyperinsuliaemia and the presence of hyperglycaemia and insulin resistance, since after 10 weeks of fructose feeding, a progressive state of insulin resistance might cause β-cells failure and impaired insulin secretion, this was previously seen in rats fed high-fructose diet for 10-12 weeks [48]. However, hyperinsuliaemia and insulin resistance were observed only in the rats consuming olive oil, where the absence of hyperglycaemia, hyperinsuliaemia and insulin resistance was observed in the rats consuming either maize oil or sheep tallow.

Even though HOMA-IR is not the gold standard method to measure insulin resistance, as it relies only on the fasting glucose and insulin concentrations, and even that some data suggest that it offers little or no advantage over the fasting insulin concentration alone [49], it is still questionable whether the high-fructose diet efficiently induced insulin resistance in the fructose-fed group and this might imply that it is not a reliable group to further study the effect of dietary fats, therefore, the following discussion will focus on the maize starch-fed group.

In the maize starch-fed group, sheep tallow nicely showed a significant higher HOMA-IR. Indeed, in terms of dietary fat type, it is accepted that SFA, relative to MUFA and PUFA, appear to be more deleterious with respect to fat-induced insulin resistance [5]. The results of human studies consistently show that the fatty acid composition of body tissues (serum lipids, phospholipid in erythrocyte membranes, triglyceride in adipose tissue, phospholipid in skeletal muscle membranes) reflects, at least in part, the fat composition of the habitual diet [50]. Indeed, when newly detected noninsulin-dependent diabetes mellitus patients were

Table 3 Body weight, weight gain, food intake, food efficiency ratio, epididymal fat weight and metabolic variables for rats fed olive oil, maize oil and sheep tallow for 10 weeks

	Maize-starch-fed group			Fructose-fed group		
	Olive oil	Maize oil	Sheep tallow	Olive oil	Maize oil	Sheep tallow
Body weight (g)	385·9 ± 11·5	377·0 ± 11·5	389·5 ± 6·5	372·3 ± 10·2	383·0 ± 2·9	342·9 ± 16·3
Weight gain (g)	200·3[a] ± 12	191·6[a] ± 11·4	203·9[a] ± 6·4	187·3[a] ± 8·2	197·7[a] ± 4·5	157[b] ± 15·3
Food intake (d/day)	16·9 ± 0·4	16·3 ± 0·4	17·4 ± 0·2	16·5 ± 0·4	16·3 ± 0·2	16·1 ± 0·5
Food efficiency ratio[1]	0·170[a] ±0·007	0·170[a] ±0·007	0·170[a] ±0·005	0·160[a] ±0·004	0·170[a] ± 0·005	0·140[b] ± 0·009
Epididymal fat weight (g)	7·8 ± 1·0	5·1 ± 0·6	7·0 ± 0·3	6·3 ± 1·0	5·6 ± 0·5	5·3 ± 0·6
Glucose (mmol/l)	4·75 ± 0·35	4·69 ± 0·56	7·00 ± 0·70	6·88 ± 0·50	6·54 ± 0·73	7·92 ± 0·85
Insulin (µU/ml)	11·55[c] ± 1·61	12·42[bc] ± 3·09	18·49[ab] ± 2·16	21·18[a] ± 2·65	15·12[abc] ± 1·24	15·59[abc] ± 2·70
HOMA-IR	2·53[c] ± 0·50	2·79[c] ± 0·93	5·38[ab] ± 0·87	6·48[a] ± 0·91	3·69[bc] ± 0·32	5·32[ab] ± 1·13
leptin (ng/ml)	5·60 ± 1·17	3·47 ± 0·63	5·22 ± 0·44	6·17 ± 1·28	4·87 ± 0·57	3·31 ± 0·59
PPARγ activation (OD)	0·022 ± 0·006	0·034 ± 0·007	0·051 ± 0·015	0·056 ± 0·012	0·044 ± 0·007	0·051 ± 0·012

Values are means ± SEM ($n = 6$)

[a,b,c] Mean values within a raw with unlike superscript letters are significantly different ($P \leq 0.05$)

[1] Weight gain/food intake

HOMA-IR homeostasis model assessment of insulin resistance, *PPARγ* peroxisome proliferator-activated receptor gamma, *OD* optical density

investigated and compared with healthy controls, they had considerably higher proportions of SFA and lower proportions of linoleic acid in the serum cholesterol esters [51]. Subjects with glucose intolerance showed an intermediate situation [51]. Therefore, substituting SFA with unsaturated fatty acids may produce some health benefits, these benefits include; improved insulin sensitivity, a reduction in the abdominal subcutaneous fat area and a higher membrane fluidity [52-54]. In our study, olive oil and maize oil representing MUFA and PUFA respectively had the same effect on HOMA-IR, in the maize starch-fed group, and both showed better insulin sensitivity compared to sheep tallow (representing SFA).

The biochemical and molecular processes linking dietary fat to insulin resistance remain unresolved. In this study we examined the effect of dietary fat type on DNA binding activity of the transcription factor PPARγ. Many fatty acids and their derivatives are ligands of PPARγ [26, 27]. Results regarding the potency of various fatty acids in activating PPARγ are inconsistent [11, 21, 29], but

it has been suggested that the activation of PPARγ by fatty acids increases with chain length and degree of unsaturation of the fatty acid [55]. Therefore, SFA may be considered poor ligands of PPARγ, and because PPARγ activation results in the up-regulation of insulin sensitization [25], a decreased activation of PPARγ by SFA may lead to reduced insulin sensitivity. Nevertheless, if this was the case in our study, differences in PPARγ activation between different dietary fat types, in the maize starch-fed group, would have been observed. The comparable effects of the different dietary fat types used in this study suggest that the deleterious effect of sheep tallow was independent of PPARγ activation. Conversely, the comparable effects of the different dietary fat types could be due to the inability to isolate the effect of particular fatty acids when the effect of diets containing olive oil, maize oil or sheep tallow, which are blend of different fatty acids, is studied.

In the maize starch-fed group, dietary fat type did not affect leptin concentration which is consistent with several studies [22, 40] and contrast others [39,

41]. Indeed, the percentage of dietary fat used, the duration of each study, animal vs. human studies and the complex regulation of leptin may have contributed to the inconsistency produced by different studies. As a matter of fact, dietary fat can directly or indirectly regulate leptin. ob gene expression, adiposity and insulin can be involved in the dietary fat regulation of leptin. In this study, ob gene expression was not examined, but the activation of PPARγ, a transcription factor that represses ob gene expression [28] and thus may contribute to a lower leptin concentration, was. We have found that PPARγ activation, in the maize starch-fed group, was not affected by dietary fat type; in a similar behaviour, there were no changes in leptin concentration.

It has been demonstrated that dietary fat type can have different regulatory effects on body-fat accumulation, adipogenesis and fat oxidation [41]; besides, circulating leptin is generally positively correlated with adiposity in humans and rodents [41]. We have found that dietary fat type, in the maize starch-fed group, did not significantly influence body weight or epididymal fat weight which is a better predictor of circulating leptin than either retroperitoneal or peritoneal depots [40]; therefore, this may have contributed to the comparable leptin concentrations observed in our study.

The role of hormones in leptin regulation is important. A prime candidate for such regulation is insulin. Insulin can stimulate ob gene expression and leptin production [56, 57]. However, changes in insulin status produced by dietary fat type, in the maize starch-fed group, did not influence leptin concentration in our study. It can be speculated that the effect of insulin to stimulate glucose uptake and metabolism is more important than the direct effect of insulin per se in regulating leptin secretion. Otherwise, it can be suggested that the presence of frank hyperinsulinaemia is required for an observed shift in circulating leptin. In addition, it is important to point out that serum analyses were carried out after an overnight fast; it is well known that fasting results in fall in circulating leptin [58]. Therefore, this fasting-induced effect should be taken into consideration.

Finally, we have shown that, in the maize starch-fed group, dietary fat type altered insulin resistance by mechanisms independent of affecting PPARγ activation or leptin concentration with sheep tallow showing a deleterious effect.

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