

Weight Gain Induced by High-Fat Feeding Involves Increased Liver Oxidative Stress

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Abstract

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Objective: To assess the effects of high-fat feeding on white adipose tissue gene expression and liver oxidative stress.

Research Methods and Procedures: Male Wistar rats were fed on standard pelleted or high-fat diet to produce a diet-induced obesity model. Therefore, body composition, serum biochemical values and liver malondialdehyde (MDA) were determined after 56 days of feeding. Expression (mRNA) values of three genes were also determined by reverse transcriptase-polymerase chain reaction in white adipose tissue.

Results: Animals fed on the high-fat diet showed more body weight, higher fat deposition and total liver weight, and increased energy intake compared with those on the standard-fat diet. Serum fasting measurements (glucose, insulin, leptin) and homeostasis model assessment insulin resistance index were significantly increased by the high-fat diet consumption. As an indicator of oxidative stress, peroxide decomposition in liver was analyzed, showing an increase of MDA concentrations in rats fed on high-fat diet in comparison with control rats. Interestingly, liver MDA levels correlated positively with body weight gain, serum leptin, and homeostasis model assessment. Finally, leptin and glycerol-3-phosphate dehydrogenase mRNA levels, but not fatty acid synthase, were increased by high-fat diet in comparison with the control-fed group.

Discussion: These results show a link among increased fat depots, insulin resistance, and liver oxidative stress. Thus,

liver oxidative stress probably contributes to hepatic disorders and aggravates the metabolic syndrome, which is accompanied by a stimulation of the esterification of fatty acids as measured by glycerol-3-phosphate dehydrogenase in the adipose tissue, providing support to the hypothesis that not only calories count in the induction of weight gain or metabolic syndrome and that other factors such as oxidative stress may be involved.

Key words: cafeteria diet, inflammation, adipose, gene expression, insulin resistance

Introduction

It is generally assumed that some inflammatory processes are a consequence of obesity (1,2). Indeed, chronic inflammation in fat plays a role in the development of insulin resistance (3) and other obesity-related features, commonly recognized as metabolic syndrome manifestations (4). However, the molecular basis of the origin of this inflammatory condition is not yet well elucidated. Some reports emphasize that oxidative stress could be the cause that might trigger a proinflammatory status (5,6). Reactive oxygen species (ROS)¹ contribute to the disease state by damaging DNA, lipids, and proteins, leading to a disruption in cellular homeostasis and an accumulation of damaged molecules and, thus, promoting apoptosis, ageing, inflammatory, and degenerative dysfunction (7). It has been also suggested that hypoxia in some areas of the growing adipose tissue mass could be one of the causes of the chronic inflammatory status in fat from obese individuals (6). However, other inflammatory and oxidative stress-related factors could be implicated in the functional impairment of adipose tissue and other tissues, such as the liver, associated with an exacerbated nutrient oxidation and the type of diet. Thus, it has been reported that high glucose intake increases intra-

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¹ Nonstandard abbreviations: ROS, reactive oxygen species; PCR, polymerase chain reaction; MDA, malondialdehyde; HOMA, homeostasis model assessment; PCR, polymerase chain reaction; WAT, white adipose tissue; RT, reverse transcriptase; G3PDH, glycerol-3-phosphate dehydrogenase; FAS, fatty acid synthase; NAFLD, non-alcoholic fatty liver disease.

cellular ROS in leukocytes (8) and adipocytes (9). These data suggest that the proinflammatory state of obese individuals might be probably related to chronic excessive nutrient intake (10). However, although liver is the regulator of energy body homeostasis, oxidative stress in liver during high-fat induced obesity is not well known. After some reports of increased oxidative stress in high-fat-fed animals (11), we specifically assessed the effects of cafeteria diet intake in oxidative stress generation in the liver and the potential relationships with biomarkers associated to weight gain and the metabolic syndrome. In parallel, we measured the expression of several genes involved in key pathways of the adipocyte metabolism.

Research Methods and Procedures

Animals and Body Composition

Male Wistar rats supplied by the Center of Applied Pharmacology (CIFA, University of Navarra, Pamplona, Spain), weighing ~265 grams, were housed at 21 °C to 23 °C with a 12-hour light cycle (8 AM to 8 PM) and distributed in different groups. A group of animals (control, $n = 8$) were fed a standard pelleted diet (Harlan Iberica, Barcelona, Spain) containing 16.6% of energy as protein, 73.1% of energy as carbohydrate and 10.3% of energy as lipid by dry weight. The remaining animals (cafeteria group, $n = 8$) were fed a high-fat diet to generate a diet-induced obesity model. High-fat diet components were pate, bacon, chips, cookies, chocolate, and chow with proportions 2:1:1:1:1:1, which was given to each rat daily as published elsewhere (12). The composition of the cafeteria diet was 9.3% of energy as protein, 31.5% of energy as carbohydrates, and 59.2% of energy as lipids by dry weight. At the end of the experimental period (56 days), rats were anesthetized in the fasted state with ketamine (50 mg/kg intraperitoneally; Parke-Davis, Madrid, Spain) and medetomidine (0.025 mg/kg intraperitoneally; Pfizer S.A., Madrid, Spain) for the analysis of the body composition by using a non-invasive electromagnetic apparatus specifically devised for rodents (EM-SCAN model SA-2; EM-SCAN, Inc., Springfield, IL) (12). Afterwards, animals were sacrificed by decapitation, and serum and plasma were collected. All of the procedures were performed according to national and institutional guidelines of the Animal Care and Use. This experiment was approved by the Ethical Committee at the University of Navarra.

Serum and Hepatic Measurements

Serum triacylglycerides were determined with the Randox kit for in vitro triacylglyceride diagnostics (Randox Laboratories Ltd., Crumlin, United Kingdom). Glucose was measured with the HK-CP kit (ABX Diagnostic, Montpellier, France). Both measurements were adapted for a COBAS MIRA equipment (Roche Diagnostics, Basel, Swit-

zerland). Serum leptin and insulin were assayed by radioimmunoassay as described by the supplier (Linco Research, St. Charles, MO). Hepatic malondialdehyde (MDA) was determined using a colorimetric assay for lipid peroxidation (Bioxytech, Portland, OR), and total protein was analyzed using Total Protein 250 kit (ABX Diagnostic, Geneva, Switzerland) adapted again for a COBAS MIRA as described by the kit instructions. Insulin resistance was calculated according to the homeostasis model assessment (HOMA) calculation: insulin (microunits per milliliter) \times glucose (millimolar)/22.5.

Real-Time Polymerase Chain Reaction (PCR)

Total RNA from retroperitoneal white adipose tissue (WAT) of eight control and eight cafeteria-fed rats was isolated using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The purified total RNA was used as a template to generate first strand cDNA synthesis using M-MLV reverse transcriptase (RT; Invitrogen) as described by the manufacturer. Quantitative real-time PCR was performed using an ABI PRISM 7900 HT Sequence Detection System as described by the provider (Applied Biosystems, Foster City, CA). Taqman probes for rat glycerol-3-phosphate dehydrogenase (G3PDH), leptin, fatty acid synthase (FAS), and 18S rRNA as internal control were also supplied by Applied Biosystems.

Statistical Analysis

All results are expressed as mean \pm standard error. Data were evaluated by *U*-Mann Whitney and Spearman tests. The SPSS 12.0 package for Windows (SPSS Inc., Chicago, IL) was used for the statistical analysis. The level of probability was set at $p < 0.05$ as statistically significant.

Results

Animals fed on the high-fat diet (cafeteria group) for 8 weeks gained more weight (23%, $p < 0.01$) than those animals (control group) fed on the standard-fat diet (Table 1). The assessment of the body composition by using the EM-SCAN showed a higher proportion of total fat in overweight rats (86%, $p < 0.01$) than in lean animals. In addition, energy intake (expressed in kilocalories per day) was significantly increased (86%, $p < 0.01$) in rats fed on cafeteria diet as compared with controls. Moreover, total liver weight was increased (36%, $p < 0.05$) in cafeteria-fed rats in comparison with control group. Serum fasting measurements (glucose, insulin, leptin) and HOMA as an insulin resistance index were significantly increased by the high-fat diet (Table 1). Serum triglycerides, however, were not significantly affected by high-fat diet.

To study the possible effect of cafeteria diet on adipose gene expression pattern, leptin and two lipogenic genes

Table 1. Body composition, energy intake, serum measurements, and liver malondialdehyde levels in experimental groups (control and cafeteria)

	Control	Cafeteria	U test
Body weight gain (g)	144 ± 7	233 ± 22	**
Fat content (g)	58.6 ± 3.5	109.0 ± 12.6	**
Liver weight (g)	9.0 ± 0.4	12.2 ± 1.2	*
Energy intake (kcal/d)	84 ± 1	155 ± 11	**
Glucose (mg/dL)	215 ± 14	290 ± 21	*
Insulin (ng/mL)	0.16 ± 0.04	0.49 ± 0.12	**
Leptin (ng/mL)	3.36 ± 1.01	14.26 ± 2.01	**
HOMA	2.26 ± 0.68	8.64 ± 2.52	**
Triglycerides (mg/dL)	101 ± 14	121 ± 18	NS
Liver MDA (μmol MDA/g protein)	0.31 ± 0.03	0.54 ± 0.06	*

HOMA, homeostasis model assessment; NS, not significant; MDA, malondialdehyde. Data are given as mean ± standard error. Control vs. cafeteria: * $p < 0.05$; ** $p < 0.01$.

(*G3PDH* and *FAS*) were analyzed by RT-PCR. Leptin and *G3PDH* mRNA expression resulted significantly increased (125%, $p < 0.01$ and 95%, $p < 0.05$, respectively) by high-fat diet in comparison with control group, although *FAS* mRNA was not apparently affected (Figure 1).

High-fat intake induced an increase (77%, $p < 0.05$) of hepatic MDA (micromoles per milligram of protein) in comparison with the control group (Table 1). Interestingly, liver MDA levels correlated positively (Figure 2) with important markers defining this dietary obesity model: body weight gain, serum leptin, and HOMA (Spearman correlation coefficients 0.70, $p < 0.01$; 0.64, $p < 0.01$; and 0.63, $p < 0.01$ respectively), which suggests that more than 40% to 49% (r^2) of MDA variability is explained by these markers.

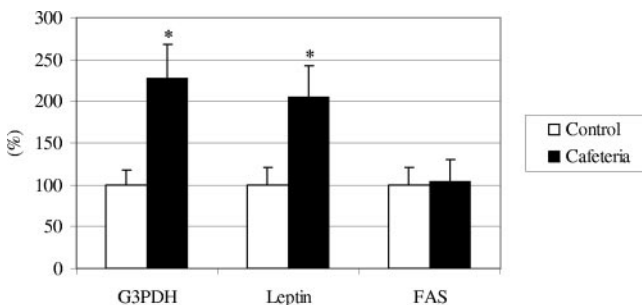


Figure 1: *G3PDH*, *FAS*, and leptin mRNA levels measured by RT-PCR in experiment, expressed as percentage of control group gene expression, with expression in control group = 100. Control, $n = 8$; cafeteria, $n = 8$ (control vs. cafeteria: * $p < 0.05$; ** $p < 0.01$).

Discussion

Increased food intake and decreased energy expenditure associated to modern lifestyles have contributed to the current rising prevalence of obesity and type 2 diabetes (2,6). In this sense, cafeteria diet feeding has been broadly applied in animal studies due to its similarities with human obesity (13). This model shares common Western diet features (high-fat intake and hyperphagia), which are thought to lead to hyperinsulinemia, type 2 diabetes, and metabolic syndrome conditions (14).

High-fat feeding-induced obesity led to lipid accumulation in visceral adipose tissue, as fat content data indicate, which could be associated with enhanced *G3PDH* and leptin gene expression, thus confirming previous data (13). *FAS* adipose tissue gene expression is not significantly influenced by the diet. However, it does not mean that changes in *FAS* activity could not affect fatty acid synthesis from glucose. Anyway, this finding concerning triglyceride accumulation in adipocytes seems to be a major source of oxidative stress in WAT and further adipocytokine dysregulation driving to metabolic syndrome (6).

In recent years, obesity has been associated with an inflammatory status (1,2). On the contrary, insulin has shown to be anti-inflammatory and to suppress ROS generation and NADPH oxidase (15). Therefore, in this way, the present cafeteria diet model coursing with obesity and insulin resistance could be characterized by oxidative and inflammatory stress and may be useful to study the role of inflammation in the genesis and development of obesity. Inflammatory status related to obesity may be also originated by oxidative stress, which induces cell injury and could be able to dysregulate adipocytokine production and

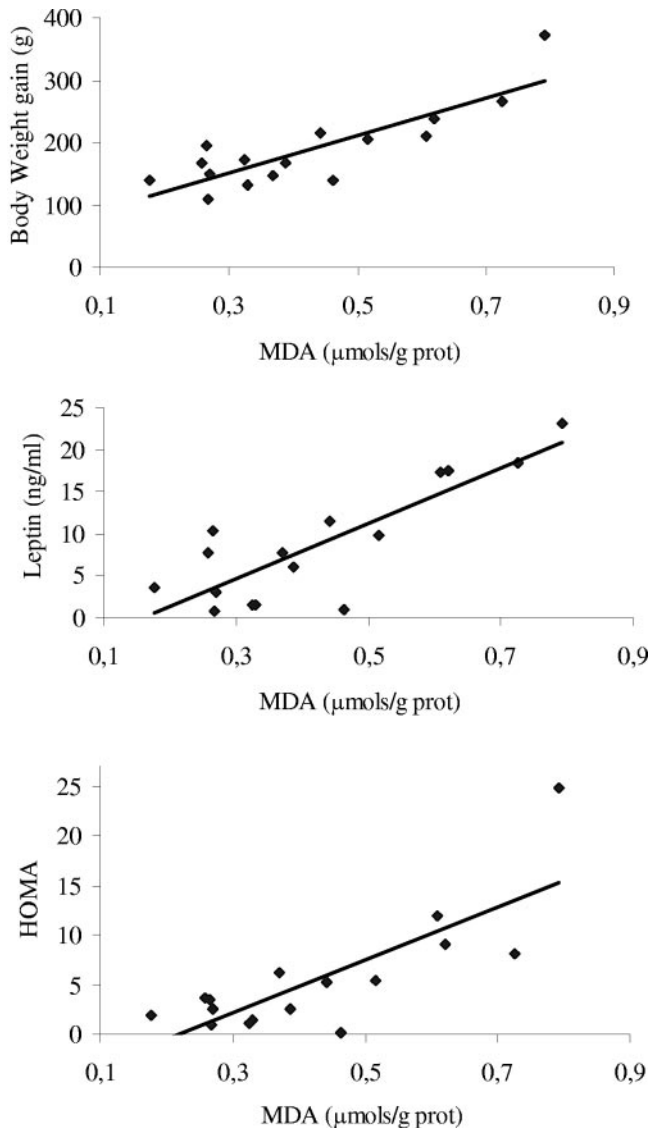


Figure 2: Correlation between liver MDA levels and body weight gain, serum leptin, and HOMA insulin resistance index ($r =$ Spearman correlation coefficient; control vs. cafeteria: $* p < 0.05$).

insulin sensitivity (16,17). Furthermore, oxidative stress in obesity may be generated by hypoxia in adipocytes (6) or by exacerbated nutrient oxidation, as it has been reported after glucose uptake (9). In any case, oxidative stress has been correlated with BMI in obese subjects (18). Furthermore, it has been reported that obesity per se may induce systemic oxidative stress. However, contradictory results regarding intracellular redox state in adipocytes as compared with other tissues have been recently found (16,19). Moreover, conflicting results have been observed regarding liver peroxidation. In a recent report (20), an increase in liver MDA in a genetic model of obesity fed a high-fat diet was reported when compared with *fa/fa* rats fed the control

diet. However, in other animal models of obesity (16), lipid peroxidation is not altered in liver. Thus, the role of obesity-induced oxidative stress in the liver, a chief organ regulating body metabolism, remains to be clarified.

This trial evidenced a correlation between liver MDA, a good estimate of lipid peroxidation, and different variables accompanying the common features of the cafeteria obesity model in rats (increased body weight, hyperleptinemia, and hyperinsulinemia). These results suggest that obesity induced by high-fat diets enhances oxidative stress not only in WAT (16) or in plasma (11), but also in liver, which probably contributes to hepatic steatosis and other disorders.

A possible role of increased hepatic ROS and lipid peroxidation in causing inflammation and non-alcoholic fatty liver disease (NAFLD) is an important clinically relevant issue. NAFLD is a major cause of liver-related morbidity and has been related to obesity in human patients in epidemiological studies (21), but it also has been associated with other characteristics of the metabolic syndrome, such as impaired glucose tolerance and hyperinsulinemia (22). A two-hit theory best describes the progression from simple steatosis to NAFLD, fibrosis, or cirrhosis (23). These two hits consist of the accumulation of excessive hepatic fat primarily because of insulin resistance and oxidative stress because of ROS occurrence.

As described earlier, one of the main causes of NAFLD is the accumulation of excessive hepatic fat because of insulin resistance and the exacerbated lipid mobilization and oxidation that it is usually found in the liver of obese subjects (24). The cafeteria diet is a good model of insulin resistance and lipid accumulation in the liver, with increased HOMA index and liver weight and enhanced TG hepatic content (data not shown). Furthermore, excess accumulation of free fatty acids in liver and other non-adipose tissues commonly led also to cell dysfunction and lipotoxicity or lipid-induced cell death (25).

The elevated levels of MDA in rats fed on a cafeteria diet, even the *fa/fa* rats (20), suggest increased lipid peroxidation in fat deposits that could be released and have detrimental effects on hepatocytes and other hepatic cells. In hepatocytes, ROS and lipid peroxidation products further impair the respiratory chain, either directly or indirectly through oxidative damage to the mitochondrial genome. These features, in turn, lead to the generation of more ROS, and a vicious cycle ensues. Mitochondrial dysfunction can also lead to apoptosis or necrosis depending on the energy status of the cell. Finally, ROS and lipid peroxidation products also activate stellate cells, thus resulting in fibrosis (26).

On the other hand, adipokines have been implicated in the pathogenesis of type 2 diabetes and NAFLD, through their metabolic and pro-/antiinflammatory activity (27). We present a correlation between MDA and serum leptin levels, showing a possible link between adipose tissue and liver.

Leptin promotes insulin resistance and hepatic disease in cell cultures and animal models through activation of the transforming growth factor β axis and stellate cells (28), and recent reports have shown that serum leptin correlates with liver steatosis, but not fibrosis, in non-alcoholic steatohepatitis (29).

In summary, the data presented in this paper show that the cafeteria diet is characterized not only by lipid accumulation in adipose tissue but also by an increase in liver oxidative stress. Hepatic free radical production probably contributes to additional liver disorders, aggravating metabolic syndrome features, as suggested by the fact that almost 50% of the weight gain is associated to liver MDA changes, as pointed out by the r^2 values in the correlation analysis. Lately, a reduced redox state has been linked to a proadipogenic status in adipocytes, so our data give new insights to the concept that obesity development depends not only on energy intake, but also associated oxidative stress changes may be related.

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