Lipid Peroxidation and Antioxidant Enzyme Status in Various Tissues of Mice with Gold-Thioglucose-Induced Obesity

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Summary To determine whether an oxidant-antioxidant imbalance exists in the non-genetic obese model, we induced obesity in 3-week-old female ICR mice by gold-thioglucose administration, and the lipid peroxide level and activities of antioxidant enzymes were measured in the plasma, liver, heart, and skeletal muscle. Two types of superoxide dismutase were assayed by radioimmunoassays. Lipid peroxide was estimated from fluorimetric measurement of thiobarbituric acid-reactive substances (TBARS). The plasma levels of both cholesterol (Chol) and triacylglycerol (TG) and liver TG content were increased in the obese mice. The superoxide dismutases were unaltered, while the activities of both glutathione peroxidase and catalase in the obese liver and heart were decreased. Lipid peroxide levels were unaltered in the obese liver and heart, decreased in the skeletal muscle, and increased in the plasma. The body weight correlated positively with the liver weight, heart weight, liver TG, liver Chol, plasma TG, plasma Chol, and plasma TBARS, and inversely with the tissue levels of some of the antioxidant enzymes and TBARS. The tissue levels of the antioxidant enzymes and TBARS had a general tendency to decrease in the obese animals. These results suggest that oxidative stress is enhanced only in the plasma of the obese mice and not in any of the tissues studied here.

Key Words: lipid peroxide, obesity, free radical, superoxide dismutase, hyperlipidemia

It has been hypothesized that superoxide generated during oxidative metabo-

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lism produces an accumulative deleterious change in mitochondrial function at a rate related to the rate of oxygen consumption, leading to the aging of the cells [1]. According to this hypothesis, food restriction decreases oxygen consumption, and, in turn, free radical production, resulting in a decreased rate of progression of degenerative disorders including lipid peroxidation [2]. We previously demonstrated that prolonged fasting suppressed mitochondrial oxidative metabolism and lipid peroxidation in certain rat tissues [3]. Conversely, increased food consumption accompanying obesity can enhance oxidative stress, thereby accelerating the degeneration process.

Obesity is known to be an independent risk factor of atherosclerosis [4], and the latter process is postulated to be accelerated by an increase in the level of circulating lipid peroxides [5]. Thus, study of the oxidant-antioxidant balance in obesity is of clinical importance. However, little is known about the oxidant-antioxidant status in obesity, and data are limited to the genetically obese ob/ob mouse [6-9]. Abnormal metabolism of trace metals, which is not directly linked to excessive weight gain, is reported to exist in the ob/ob mouse [10]. Such deviation in trace metal status *per se* may modify tissue levels of antioxidant enzymes, which contain trace metals in their active centers, independently of energy metabolism.

To determine whether an oxidant-antioxidant imbalance exists in the non-genetic model of obesity, the obese condition was induced by gold-thioglucose administration to mice, and the lipid peroxide level and activities of antioxidant enzymes were measured in various tissues.

MATERIALS AND METHODS

Animal treatment. Thirty-five 3-week-old female ICR mice (Japan SLC, Shizuoka) received intraperitoneal injection of gold-thioglucose (Sigma Chemical Co., St. Louis, MO; 250 mg/kg body weight). Eight age-matched females served as controls. They were given free access to a standard chow and water for 12 weeks. The final body weights for control mice were not more than 39 g. Seventeen injected mice, whose final body weight exceeded 39 g, were assigned to the obese group. The mice were killed under pentobarbital anesthesia (50 mg/kg), and their plasma, livers, hearts, and bilateral rectus femoris muscles were obtained. Tissues were rinsed with phosphate-buffered saline, blotted with gauze, and then stored frozen at -80°C .

Biochemical analyses. The tissues were homogenized with 20 times their volume of 10 mM potassium phosphate buffer containing 0.01% digitonin (pH 7.4) in a Potter-Elvehjem type glass-Teflon pestle homogenizer, and then sonicated on ice for 30 s. The sonicate was centrifuged at $13,000\times g$ for 5 min, then the supernatant was stored frozen in aliquots at -80°C until assayed. The radioimmunoassays for murine copper-zinc superoxide dismutase (CuZnSOD) and manganese SOD (MnSOD) have been described [9]. Glutathione peroxidase

(GPX) activity was assayed spectrophotometrically as described previously [11]. Catalase (CAT) activity in the samples of liver and heart was assayed spectrophotometrically and calculated by use of the first-order rate constant [12]. Because the CAT activity in the samples of skeletal muscle was below the detection limit of this assay, the activity was assayed polarographically [13]. Lipid peroxide both in plasma and tissues was estimated from fluorimetric measurement of thiobarbituric acid-reactive substances (TBARS), also as described previously [14]. Protein was measured by the technique of Lowry *et al.* [15].

Lipid content of liver was determined as follows: The liver was homogenized with 25 times its volume of distilled water, and then the lipid was extracted twice with the equal volume of chloroform/methanol mixture (2:1, v/v). The chloroform layer was obtained and pooled. A part of this solution was evaporated under a gentle stream of nitrogen, and then dissolved in 70% isopropanol. Cholesterol (Chol) and triacylglycerol (TG) in this solution and in the plasma were measured enzymatically by use of commercial kits.

Statistical analyses. The data are presented as the mean $\pm$ SEM. Statistic methods used were the unpaired *t*-test and the least-square linear regression analysis.

RESULTS

Body weight, organ weights, and lipid contents

Mean body weight of the obese mice was 142% of that of the controls (Table 1). Obese mice had larger livers than controls, while the weights of their hearts and skeletal muscles were similar to those of the controls. Each organ weight relative to body weight was lower in the obese mice than in controls. Plasma levels of both Chol and TG were higher in the obese than in the control mice. Similarly, liver

Table 1. Body weight, organ weights, organ weights relative to body weight, and lipid contents in liver and plasma for control and obese mice.

	Controls (<i>n</i> =8)	Obese (<i>n</i> =17)	<i>p</i> -value
Body weight (g)	35.1 $\pm$ 1.3	49.8 $\pm$ 2.3	<i>p</i> < 0.001
Liver weight (mg)	1.49 $\pm$ 0.06	1.82 $\pm$ 0.07	<i>p</i> < 0.01
Liver/body weight (mg/g)	42.4 $\pm$ 1.1	37.1 $\pm$ 1.1	<i>p</i> < 0.01
Heart weight (mg)	144 $\pm$ 3.7	151 $\pm$ 3.4	ns
Heart/body weight (mg/g)	4.12 $\pm$ 0.08	3.10 $\pm$ 0.11	<i>p</i> < 0.001
Muscle* weight (mg)	476 $\pm$ 26	492 $\pm$ 13	ns
Muscle*/body weight (mg/g)	13.3 $\pm$ 0.3	10.3 $\pm$ 0.6	<i>p</i> < 0.001
Plasma triacylglycerol (mg/dl)	70.0 $\pm$ 6.0	97.5 $\pm$ 9.0	<i>p</i> < 0.02
Plasma cholesterol (mg/dl)	61.0 $\pm$ 5.1	82.6 $\pm$ 3.6	<i>p</i> < 0.01
Liver triacylglycerol (mg/g)	11.8 $\pm$ 1.0	24.7 $\pm$ 2.9	<i>p</i> < 0.001
Liver cholesterol (mg/g)	2.63 $\pm$ 0.11	2.75 $\pm$ 0.05	ns

Data are means $\pm$ SEM. Statistical significance was evaluated by the unpaired *t*-test. ns, Not significant. *Bilateral rectus femoris muscles were combined and weighed.

Table 2. Levels of antioxidant enzymes in various tissues of control and obese mice.

	CuZnSOD	MnSOD	GPX	CAT
Liver				
Controls	3.44±0.24	3.19±0.19	1.27±0.04	204±7.2
Obese	3.14±0.09	3.53±0.12	1.17±0.02	160±7.5
<i>p</i> -value	ns	ns	<i>p</i> <0.05	<i>p</i> <0.01
Heart				
Controls	0.71±0.04	1.58±0.10	41.0±4.1	9.28±0.60
Obese	0.64±0.06	1.49±0.08	29.2±1.1	7.52±0.50
<i>p</i> -value	ns	ns	<i>p</i> <0.05	<i>p</i> <0.05
Muscle				
Controls	0.84±0.02	2.32±0.10	19.5±0.9	526±61
Obese	0.82±0.02	2.36±0.10	18.7±0.9	473±23
<i>p</i> -value	ns	ns	ns	ns

CuZnSOD, Copper-zinc superoxide dismutase; MnSOD, manganese superoxide dismutase; GPX, glutathione peroxidase; CAT, catalase. Values are $\mu\text{g}/\text{mg}$ protein for CuZnSOD and MnSOD, $\mu\text{mol}/\text{min}/\text{mg}$ protein for liver GPX, $\text{nmol}/\text{min}/\text{mg}$ protein for GPX in heart and muscle, Unit $\text{K}(\text{s}^{-1})/\text{g}$ protein for CAT in liver and heart, and $\text{nmol}/\text{min}/\text{mg}$ protein for muscle CAT.

Table 3. Thiobarbituric acid-reactive substances in tissues and plasma of control and obese mice.

	Controls	Obese	<i>p</i> -value
Liver (nmol/mg protein)	0.92±0.07	0.80±0.06	ns
Heart (nmol/mg protein)	6.33±0.50	5.64±0.27	ns
Muscle (nmol/mg protein)	6.30±0.28	4.96±0.37	<i>p</i> <0.05
Plasma (nmol/ml)	3.05±0.19	4.13±0.19	<i>p</i> <0.001

TG content was increased in the obese mice, whereas the liver Chol level was not increased significantly.

Antioxidant enzymes and lipid peroxide

Table 2 lists the levels of antioxidant enzymes in the liver, heart, and skeletal muscle. The levels of both CuZnSOD and MnSOD in the obese liver, heart, and skeletal muscle were similar to the respective control levels. The GPX activities in the obese liver and heart were lower than those in controls, while the activity in the skeletal muscle was unaltered. Similarly, CAT activity was lower in the obese liver and heart than in the respective controls, and was unchanged in the skeletal muscle. The TBARS levels in the obese liver and heart were similar to those in controls (Table 3), while muscle TBARS level was slightly lower in the obese than in the control mice. In contrast, the plasma TBARS level was higher in the obese than in the control mice.

Table 4. Correlations between body weight or organ weight and other variables.

Variables		<i>r</i> -value	<i>p</i> -value
Body weight	vs. Liver weight	0.847	$p < 0.005$
	vs. Heart weight	0.601	$p < 0.005$
	vs. Liver GPX	-0.480	$p < 0.025$
	vs. Heart CuZnSOD	-0.539	$p < 0.01$
	vs. Heart GPX	-0.574	$p < 0.005$
	vs. Heart CAT	-0.479	$p < 0.025$
	vs. Muscle GPX	-0.426	$p < 0.05$
	vs. Liver TG*	0.860	$p < 0.005$
	vs. Liver Chol**	0.507	$p < 0.01$
	vs. Liver TBARS***	-0.597	$p < 0.005$
	vs. Plasma TG	0.640	$p < 0.005$
	vs. Plasma Chol	0.610	$p < 0.005$
	vs. Plasma TBARS	0.445	$p < 0.05$
Liver weight	vs. Liver TG	0.660	$p < 0.005$
	vs. Liver Chol	0.520	$p < 0.01$
	vs. Liver TBARS	-0.583	$p < 0.005$
	vs. Plasma Chol	0.703	$p < 0.005$
Heart weight	vs. Plasma Chol	0.444	$p < 0.005$
Muscle weight	vs. Muscle CAT	-0.605	$p < 0.005$
	vs. Muscle TBARS	-0.417	$p < 0.05$

Data on both control and obese mice are combined ($n=25$). *TG, Triacylglycerol; **Chol, cholesterol; ***TBARS, thiobarbituric acid-reactive substances.

Table 5. Correlations between antioxidant enzymes, lipids, and thiobarbituric acid-reactive substances.

Variables		<i>r</i> -value	<i>p</i> -value
Liver MnSOD	vs. Muscle MnSOD	0.420	$p < 0.05$
Liver CAT	vs. Muscle CAT	0.422	$p < 0.05$
Heart CuZnSOD	vs. Heart MnSOD	0.551	$p < 0.005$
	vs. Heart GPX	0.561	$p < 0.005$
Heart GPX	vs. Heart CAT	0.558	$p < 0.005$
	vs. Heart TBARS	0.413	$p < 0.05$
Liver TG	vs. Liver Chol	0.515	$p < 0.01$
	vs. Plasma Chol	0.498	$p < 0.025$
Liver TBARS	vs. Plasma TG	-0.603	$p < 0.005$
Plasma TBARS	vs. Liver TG	0.434	$p < 0.05$
	vs. Heart TBARS	-0.415	$p < 0.05$
	vs. Plasma TG	0.410	$p < 0.05$
	vs. Plasma Chol	0.496	$p < 0.025$

Linear regression analysis

When the data on control and obese mice were combined, significant correlations were obtained between body weight and other variables (Table 4). The body

weight correlated positively with the liver weight, heart weight, liver TG, liver Chol, plasma TG, plasma Chol, and plasma TBARS. On the other hand, it correlated inversely with the liver GPX, heart CuZnSOD, heart GPX, heart CAT, muscle GPX, and liver TBARS. Next, the correlation between each organ weight and the variables in either the same organ or plasma was evaluated. The liver weight correlated positively with TG and Chol in the liver and the plasma Chol, but inversely with the liver TBARS. There was a positive correlation between the heart weight and plasma Chol, and inverse correlations between the muscle weight and either the muscle CAT or muscle TBARS.

We also evaluated the correlations between antioxidant enzymes and TBARS within each organ, between lipids or TBARS in each organ and plasma, and between each antioxidant enzyme in different organs. The significant correlations obtained are listed in Table 5. For MnSOD and CAT, there was a positive correlation between the liver and muscle enzymes. In the heart, there were correlations between CuZnSOD and MnSOD or GPX, and between GPX and CAT or TBARS. The liver TG correlated with Chol in the liver and plasma, while the liver TBARS correlated inversely with the plasma TG. The plasma TBARS correlated positively with the liver TG, plasma TG and plasma Chol, and inversely with the heart TBARS.

DISCUSSION

Obesity is known to shorten the life span because of accompanying degenerative disorders (e.g., atherosclerosis and fatty liver). In recent years, such degenerative changes have been postulated to be, at least in part, attributable to oxidative injury mediated by reactive oxygen species [16, 17]. However, until now, there had been no systematic study in this regard. Therefore, we decided to measure the activities of four antioxidant enzymes and the lipid peroxide level in plasma and various tissues of obese animals. For the induction of obesity, we chose the gold-thioglucose method. This sugar is transported, in particular, into cells of the ventromedial hypothalamus area where it produces necrotic lesion responsible for the subsequent development of hyperphagia and hyperinsulinemia, thereby causing hypothalamic-type obesity [18].

The GPX and CAT activities were decreased in the liver and heart, but not in the skeletal muscle, of the present obese mice. Similarly, liver GPX was reported to be decreased in a previous study on ob/ob mice [7, 8], but the CAT activity was not determined in those studies. The CuZnSOD was unaltered in the present obese liver. This result is inconsistent with that obtained with ob/ob mice, in which the liver CuZnSOD, assayed for either the biological activity [8] or the immunoreactivity [9], was found to be decreased. Lack of the decrease in liver CuZnSOD in the present obese model may have been due to the fact that the present model was less severely obese than the ob/ob mice in those reports [8, 9]. On the other hand, the liver immunoreactive MnSOD was unchanged both in the present obese

mice and in the previous ob/ob mice [9]. The consistency of the liver MnSOD levels in the two obese models and their respective control animals excludes the possibility that the discrepant results of the liver CuZnSOD between the present obese and the previous ob/ob mice has derived from such technical errors as nonspecific reaction in the SOD assay and the data expression on the basis of protein content. Metabolic derangement not directly related to obesity (e.g., abnormal trace metal status) may contribute to the decrease in the liver CuZnSOD in the ob/ob mice.

Lipid content was increased in both the plasma and liver of the present obese mice. Their plasma TBARS was also increased, but the tissue levels of TBARS were decreased or tended to be decreased, suggesting that the oxidative stress was enhanced only in their plasma. Although the possibility remains that the lipid peroxide detected in the plasma was derived from other organs, the present results do not support the existence of enhanced oxidative stress and subsequent oxidative injury to the organs studied. The observed significant correlations between plasma lipids and plasma TBARS were found previously in other clinical models [19]. A larger amount of lipid substrate provided by hyperlipidemic plasma may increase *de novo* lipid peroxidation during incubation in the assay system, thereby resulting in an apparent elevation in the plasma TBARS levels. However, such was not the case in the present obese liver, in which the lipid content was increased but the TBARS was not.

A question arises whether the changes in TBARS and antioxidant enzymes were induced by direct action of gold-thioglucose rather than by obesity *per se*. However, significant correlations between the body weight and many variables observed in the present study indicate that the weight gain has a profound effect on the oxidant-antioxidant status. The weight gain appeared to be associated with the increase in the organ weights, lipid content in both the liver and plasma, and plasma TBARS. On the other hand, such variables by no means correlated positively with the tissue levels of either antioxidant enzymes or TBARS. In fact, the significant correlations observed in these combinations of the variables were invariably inverse. Coordinate changes in some of the tissue antioxidant enzymes and TBARS were also found. Thus, the tissue levels of antioxidant enzymes and TBARS appeared to have a general tendency to decrease in obesity, although an exact cause-effect relationship needs to be investigated further.

Hyperlipidemia as a complication of obesity, which also occurred in the present obese mice, is known to accelerate atherosclerosis [20]. Likewise, accumulating evidence suggests the atherogenic nature of lipid peroxides. Such peroxides were recently reported to injure vascular endothelial cells [5], and to inhibit prostacyclin biosynthesis in arteries [21]. Furthermore, biological oxidation of low density lipoprotein facilitates its uptake by macrophages, and this is postulated to lead to foam cell formation [22]. The oxidation of lipoproteins is assumed to be mediated by the reactive oxygen species arising from either activated neutrophils, platelets, vascular endothelial cells, or smooth muscle cells [23]. The

present results support the contention that the lipid peroxide in plasma is produced by the peroxidation of circulating lipoproteins rather than being released from tissues.

In conclusion, oxidative stress was enhanced in the plasma of the obese mice, whereas the profiles of the levels of antioxidant enzymes and TBARS did not suggest the increase in oxidative stress in the tissues studied.

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