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REDUCTION OF ADIPOSE TISSUE LIPOPROTEIN LIPASE ACTIVITY AS A RESULT OF *IN VIVO* ADMINISTRATION OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN TO THE GUINEA PIG

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Abstract—Within 1 hr of intraperitoneal administration of 1 μg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)/kg, lipoprotein lipase (LPL) activity was reduced 38% from initial levels in the adipose tissue of the guinea pig. Maximal depression was observed after 2 days and persisted throughout the 10-day observation period. Oral administration of glucose restored LPL activity in TCDD-treated animals after 1 day but only partially after 2 and 5 days, and had no effect after 10 days of exposure. Although initial (2-day) serum insulin levels were depressed, the inability of glucose to restore LPL activity after prolonged exposure was not due to malabsorption of glucose nor to changes in serum thyroxine or insulin concentration. TCDD also inhibited the lipolytic pathway in the adipocyte, but had no effect on hormone sensitive lipase (HSL). Since HSL and LPL are reciprocally regulated, it was concluded that TCDD acts on the adipocyte to uncouple HSL–LPL reciprocity as well as to reduce LPL production.

TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) is a ubiquitous environmental contaminant having an LD_{50} in the male guinea pig of <1 μg/kg [1]. One of the most conspicuous signs of toxicity in the guinea pig after the administration of TCDD is the rapid loss of adipose tissue [2, 3]. This effect has also been observed in the rabbit and monkey and, therefore, is considered one of the major toxic expressions of TCDD [4]. The loss of adipose tissue is accompanied by serum hyperlipidemia, particularly hypertriglyceridemia [5–7].

Adipose tissue lipoprotein lipase (LPL) has a well established role in regulating serum triglyceride concentration, e.g. when LPL is inhibited serum triglyceride concentration is increased [8, 9]. Administration of TCDD to the guinea pig produces a time- and dose-dependent decrease in LPL activity which is correlated with a rise in serum triglyceride concentration. A sharp decline in activity occurs within 1 day after administration and orally administered glucose has no effect in restoring LPL activity when given 10 days after TCDD [7]. Cryer *et al.* [10] observed that the administration of glucose normally results in *de novo* protein synthesis in the adipocyte. Therefore, it was concluded that the inability of glucose to restore LPL activity after prolonged TCDD exposure was due to (a) an inhibition of glucose transport across the intestine, or (b) an alteration of pancreatic function, or (c) TCDD-induced abnormalities at the level of the adipocyte.

We have made an effort in the present study to examine these possibilities in order to elucidate the underlying mechanisms by which TCDD reduces adipose tissue LPL activity in the guinea pig.

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MATERIALS AND METHODS

Animals. Shorthair male albino guinea pigs were obtained from the Michigan Department of Health, housed in suspended stainless steel cages, and fed food (Purina Guinea Pig Chow, Ralston Purina Co., St. Louis, MO) and tap water *ad lib.* Some animals were fed the same amount of food consumed by TCDD-treated animals and were termed pair-fed controls. The animals were kept at constant temperature and humidity and were exposed to a 12-hr light 12-hr dark cycle.

To assess the effect of glucose on adipose tissue LPL activity, animals were administered 2.7 ml of a 75% glucose solution 2 hr prior to being killed with CO_2.

Chemicals. TCDD (>99% pure) was a gift from the Dow Chemical Co., Midland, MI. It was dissolved in acetone–corn oil and administered via intraperitoneal injection at a concentration of 5 ml/kg and at a dose of 1 μg/kg. Control animals were administered corn oil–acetone.

Radiolabeled triolein (glycerol tri[9,10(N)-^{3}H]oleate; sp. act. = 1 Ci/mmol) and epinephrine (DL-[7-^{3}H]adrenaline; sp. act. = 10.3 Ci/mmol) were obtained from Amersham, Arlington Heights, IL. The [^{3}H]epinephrine was used within 10 days of receipt in order to minimize decomposition. Iodinated insulin (^{125}I-insulin; sp. act. = 100 μCi/μg) was purchased from ICN Radiochemicals, Irvine, CA. Radiolabeled ATP (adenosine 5′-triphosphate, tetra(triethylammonium)salt, [γ-^{32}P]; sp. act. = 10–40 Ci/mmol), cAMP (adenosine 3′,5′-cyclic phos-

phate, ammonium salt, [2,8-^{3}H]; sp. act. = 30–50 Ci/mmol), and cGMP (guanosine 3′,5′-cyclic phosphate, ammonium salt, [8-^{3}H]; sp. act. = 5–15 Ci/mmol) were procured from New England Nuclear, Boston, MA.

All other biochemicals were obtained from the Sigma Chemical Co., St. Louis, MO. All reagents used were of the highest purity available.

Tissue preparation and enzyme assays. Acetone–ether powders of adipose tissue were prepared according to Garfinkel and Schotz [11]. Briefly, perirenal and abdominal fat was rinsed with cold physiological saline, the large blood vessels and connective tissue were removed, and the tissue was ground in 20 vol. of cold acetone using a Sorvall Omnimix Blender (Sorvall, Newton, CT). The homogenate was filtered and the fat cake washed three times with 20 vol. of acetone followed by ether. LPL activity was determined in NH_4OH–NH_4Cl extracts of the fat powder using [^{3}H]triolein as the substrate as described by Nilsson-Ehle and Schotz [12].

Na^+–K^+-, Mg^{2+}-, and Ca^{2+}-ATPase activities were asayed in intestinal plasma membrane fractions using imidazole buffers with various amounts of cations and [γ-^{32}P]ATP as described previously for liver plasma membrane [13]. Intestinal plasma membrane was isolated by a modification of the original procedure of Miller and Crane [14]: the proximal two-thirds of the small intestine was excised and flushed with cold saline followed by homogenizing buffer (0.25 M sucrose, 0.01 M triethanolamine–HCl, 0.5 mM EDTA, pH 7.5), then everted, and the mucosal layer was removed by scraping with a glass slide. All subsequent steps were performed at 4°. The mucosa was homogenized in 50 ml buffer (25 strokes, loose-fitting glass–Teflon homogenizer, 1200 rpm), and the cellular debris was precipitated by centrifugation (2600 *g*, 15 min). The supernatant fraction was spun at 10,000 *g* for 20 min, twice, and the white fluffy layer on top of the pellet was collected each time, pooled and resuspended by homogenization (50 ml buffer, 5 strokes, 1200 rpm). The resulting homogenate was centrifuged at 20,000 *g* (10 min), and the white fluffy layer was resuspended (10 strokes 1200 rpm) and centrifuged again (20,000 *g*, 20 min). The resulting crude membrane pellet, consisting of both basolateral and brush border membrane, was resuspended in homogenizing buffer and stored at −80° until assayed for ATPase activity. Intestinal membrane preparations were monitored by electron microscopy for contamination by other subcellular organelles. Protein concentrations were determined according to the method of Lowry *et al.* [15].

Adipose tissue was prepared for the determination of cAMP and cGMP phosphodiesterase activity as follows: tissue in 5 vol. of cold homogenizing buffer (0.25 M sucrose, 25 mM Tris–HCl (pH 7.4), 0.1 mM EDTA, 5 mM $MgCl_2$:$6H_2O$, 5 mM KCl, 1 mM phenylmethylsulfonyl fluoride, and 100 units/ml aprotinin) was homogenized on ice (6 strokes, 1200 rpm) with a tight-fitting glass–Teflon homogenizer. The homogenate was then centrifuged (1000 *g*, 5 min, 4°) to separate lipids. The infranatant fraction was centrifuged at 2500 *g* for 20 min at 4°, and the supernatant fraction was frozen at −80° until use. The assay method for phosphodiesterase activity was that of Wolff *et al.* [16] modified as follows: 50 μg protein was added to 20 mM imidazole (pH 7.4) containing 100 μM $CaCl_2$ (final vol. 300 μl) and 25 μM [^{3}H]cGMP or [^{3}H]cAMP (New England Nuclear). After a 3-min incubation at 37°, the reaction was stopped by placing the tubes in boiling water for 2–3 min and 0.5 units of 5′-nucleotidase was added to hydrolyze the unreacted cyclic nucleotide. After 30 min at 37°, 1 ml AG 1-X8 ion exchange resin (Bio-Rad, Cl^- form, Richmond, CA) in a mixture of isopropanol–H_2O–resin (2:2:1) was added, the mixture was vortexed and centrifuged (10 min, 3000 rpm, IEC Clinical Benchtop Centrifuge, Intl. Equipment Co., Needham Heights, MA), and samples of the supernatant fraction were taken for liquid scintillation counting.

Total cellular and plasma membrane bound cAMP-dependent and cAMP-independent adipose tissue protein kinase activity was ascertained with [γ^{32}P]ATP using the method of Corbin and Reiman [17] as previously modified [13]. Cellular protein kinase activity was determined in extracts of acetone–ether fat powders as follows: 50 mg of powder was homogenized on ice with 1 ml of 50 mM Tris–HCl buffer (pH 6.8) using a 1-ml glass–glass homogenizer. The homogenate was centrifuged (30 min, 2500 rpm, 4°, IEC Clinical Benchtop Centrifuge), and the supernatant fraction was used for determination of protein kinase activity with histone as a substrate.

Fat cell plasma membrane, for determination of protein kinase activity and insulin and epinephrine binding, was isolated according to the method of Jarett [18] utilizing a 9% and 15% discontinuous Ficoll gradient in 0.25 M sucrose. Briefly, perirenal and abdominal fat tissue was homogenized in 3.5 vol. of membrane isolation buffer (10 mM Tris–HCl, 1 mM EDTA, 0.25 M sucrose, pH 7.4) using a Thomas "B" (Thomas Scientific, Swedesboro, NJ) homogenizer (10 strokes, 1800–2600 rpm). After centrifugation (16,000 *g*, 15 min, 4°), the resulting pellet was resuspended in 4 ml buffer (6 strokes, 1000–1250 rpm, Thomas "A" homogenizer) and centrifuged over the Ficoll gradient for 15–18 hr. The topmost band was collected, precipitated with 10 mM Tris–HCl (pH 7.4), 1 mM EDTA (10,000 *g*, 20 min, 4°), resuspended in membrane isolation buffer or 50 mM phosphate buffer, and frozen at −80°. Adipose tissue plasma membrane preparations were monitored by electron microscopy for contamination by other subcellular organelles.

Adipose tissue hormone sensitive lipase (HSL) was assayed according to the procedures of Khoo *et al.* [19, 20] with the following modifications: 100 μg protein in 50 μl of 10 mM Tris–HCl, 1 mM EDTA (pH 7.4) was added to 50 μl of 10 mM Mg^{2+} acetate, 1 mM Tris–ATP, 0.02 mM cAMP, and 200 μg/ml cAMP-dependent protein kinase and incubated for 5 min at 30° in order to activate the enzyme. The addition of 0.1 ml of a [^{3}H]triolein substrate composed of 1 vol. of the concentrated substrate used for the LPL assay [12] and 5 vol. of 125 mM phosphate buffer, containing 2.5 M NaCl and 3% bovine serum albumin, was added followed by a second incubation at 30° for 30 min. The reaction was stopped

with the addition of methanol–chloroform–heptane (1.43:1.25:1.0). K_2CO_3–$K_2B_4O_7$ (0.05 M, pH 10.5, 1.05 ml) was added, and the aqueous and organic layers were separated by centrifugation at 3000 rpm (IEC Clinical Centrifuge) for 15 min. Aliquots of the aqueous (top) layer were sampled for labeled hydrolyzed oleic acid.

Binding assays. Epinephrine binding to adipose tissue plasma membrane was studied as follows: to 50 μg protein in 0.25 M sucrose, 10 mM Tris (pH 7.4) was added reaction buffer (50 mM Tris, 1% bovine serum albumin, pH 7.4) to a final vol. of 500 μl. After a 10-min preincubation at 30°, [^{3}H]epinephrine (New England Nuclear) was added (final concentration 10^{-7} M) and the tubes were incubated for an additional 20 min. Cold reaction (3 ml) buffer was added to stop the reaction. The mixture was quickly filtered through a 0.45 μm cellulose-nitrate membrane filter (HAWP, Millipore, Bedford, MA) and washed with two 5-ml aliquots of chilled buffer. The filters were allowed to air dry and were added to Aquasol Scintillation fluid (New England Nuclear) and quantified by liquid scintillation spectroscopy. Nonspecific binding was measured by the addition of 10 μl of cold epinephrine (final concentration 10^{-4} M) to alternate tubes prior to preincubation, and specific binding was calculated by subtracting the nonspecific binding from the total amount bound.

Binding of ^{125}I-insulin to adipose plasma membrane was examined using the method of O'Keefe *et al.* [21]. Plasma membrane (50 μg protein in Krebs–Ringer bicarbonate buffer) was incubated with or without native insulin before the addition of ^{125}I-insulin. Separation of bound and unbound ligand was then accomplished by filtration as described for the epinephrine binding.

Determination of hormone and cAMP concentrations. Blood was obtained via cardiac puncture, allowed to clot (20–25 min, 24°) and then centrifuged for 20 min at 2000 rpm (IEC Benchtop Clinical Centrifuge). Serum was aspirated and frozen at −80° until used. Serum glucose concentrations were measured using an enzymatic ultraviolet analysis procedure (Sigma Technical Bulletin No. 15—UV, Sigma Chemical Co.). Serum insulin and serum thyroxine concentrations were estimated via radioimmunoassay (Cambridge Medical Diagnostics Inc., Billerica, MA). The pellet formed during the final step of the radioimmunoassay procedures was dissolved in 0.5 ml NaOH (0.2 N) and sampled for radioactivity.

Pancreatic tissue was removed from the animals, weighed, and frozen in physiological saline at −80°. Insulin was extracted by homogenization in hot (82°) 2 N acetic acid followed by centrifugation and lyophilization as described by Potter *et al.* [22]. Quantitative was by radioimmunoassay as above.

Intracellular adipose tissue concentrations of cAMP were measured by extracting fat samples as follows: tissue mixed with 9 vol. of cold 10% trichloracetic acid was homogenized at 4° with a Sorvall Omnimix at maximum speed for 45 sec. After centrifugation (10 min, 2000 rpm, IEC Clinical Centrifuge), the supernatant fraction was extracted three times with 5 vol. of H_2O saturated ether. After heating to 45° to remove all traces of ether, the extracts were frozen at −80° until assayed for cAMP concentration using the radioimmunoassay procedure obtained from Biomedical Technologies Inc., Cambridge, MA.

Statistical analysis. Comparisons between pair-fed control animals and TCDD-treated animals were made using Student's paired *t*-test with $P < 0.05$ or with Tukey's test for unconfounded comparisons ($P = 0.01$) after factorial ANOVA.

RESULTS

Body weight and clinical toxicology. There was a slight decrease in body weight gain 2 days after TCDD treatment compared to *ad lib.* control guinea pigs (Table 1). After 10 days this difference became more apparent. There was little difference between the TCDD-treated and pair-fed control groups after either 2 or 10 days.

Clinical observations revealed no difference between the TCDD-treated and control groups 2 days after treatment. Piloerection, hair loss, abnormal posture, vasodilation of the extremities, and salivation was apparent in ~50% of the TCDD-treated animals 10 days after administration, but no animals died within this 10-day exposure period. Previous experiments indicated that death does not occur until 14–18 days after exposure to 1 μg TCDD/kg, i.p. [23].

Glucose reversal of LPL suppression. LPL activity in TCDD-treated animals underwent a time-dependent decrease in activity as soon as 1 hr after treatment (Fig. 1). Glucose given 1 day after TCDD

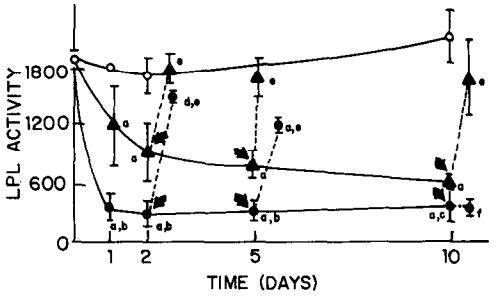

Fig. 1. Time–course effect of adipose tissue LPL activity in TCDD-treated (1 μg/kg, i.p.) (●), pair-fed control (▲), or *ad lib.* control (○) guinea pigs (mean ± SD, N = 5, solid lines). Glucose (2 g) was orally administered 2 hr prior to sacrifice to an additional four pair-fed or TCDD animals, 2, 5, or 10 days after treatment (arrows). The glucose-induced reversal of LPL suppression is depicted by the dotted lines. LPL activity in control guinea pigs or in animals 1 hr after TCDD treatment was 1976 ± 113 and 1221 ± 395 nmol free fatty acid/mg extracted fat powder/hr respectively (N = 5). Data was analyzed with Tukey's test for unconfounded comparisons ($P = 0.01$) after ANOVA. Key: (a) significantly different from *ad lib.* control; (b) significantly different from pair-fed control; (c) not significantly different from pair-fed control; (d) not significantly different from pair-fed control supplemented with glucose; (e) significantly different from respective treatment without glucose supplement; and (f) not significantly different from 10-day TCDD without glucose supplement.

Table 1. Change in the body weight of *ad lib.* control, pair-fed control, or TCDD-treated (1 μg/kg) guinea pigs 2 or 10 days after exposure

Time after treatment	Treatment	Initial weight (g)	Termination weight (g)	% of Initial weight
2 days	*Ad lib.* control	259 ± 18	271 ± 18	105 ± 2
	Pair-fed control	256 ± 28	253 ± 24	99 ± 4*
	TCDD	255 ± 36	250 ± 30	98 ± 2*
10 days	*Ad lib.* control	239 ± 13	329 ± 30	138 ± 9
	Pair-fed control	216 ± 31	252 ± 31	117 ± 11*
	TCDD	214 ± 37	243 ± 37	115 ± 17*

Values are means ± SD of six to eight animals.
* Significantly different from *ad lib.* control as determined by ANOVA and Tukey's test for unconfounded comparisons (P = 0.01).

administration fully restored LPL activity in both the pair-fed control and TCDD-treated guinea pigs to the same extent and were no different from *ad lib.* control guinea pigs without glucose (data not shown). After 2 days, glucose reversed LPL activity of TCDD-treated animals to only 89% of pair-fed controls. After 5 days orally administered glucose reversed LPL activity of TCDD-treated animals to 83% of pair-fed controls, and after 10 days to only 23% of pair-fed controls. In all cases, the pair-fed control animals given glucose had levels of activity approximating that of the *ad lib.* control guinea pigs. TCDD, therefore, produced a time-dependent inability to provide active LPL upon adequate nutritional stimulation.

TCDD had no effect on circulating glucose levels 2 or 10 days after administration (Table 2). Serum glucose concentrations in both TCDD and pair-fed control animals, orally intubated with glucose 2 hr prior to being killed, were increased significantly relative to those without glucose but were no different from each other. TCDD also had no effect on any of the intestinal transport enzymes tested (Table 3).

Serum insulin and thyroxine levels. Serum insulin concentrations were approximately 35% lower in the TCDD-treated guinea pigs as compared to the pair-fed controls 2 days after treatment (Table 4). Serum insulin levels 10 days after treatment were increased significantly in the TCDD-treated animals. Serum concentrations of thyroxine from animals dosed with TCDD were not significantly different from pair-fed control animals either 2 or 10 days after treatment (Table 4).

Pancreatic insulin levels. The synthetic capability of the pancreas to produce insulin, in response to orally administered glucose, was reduced by TCDD 2 days after treatment. The insulin concentration of extracted pancreatic protein was 6.6-fold lower in animals previously treated with TCDD as compared to control animals (7.8 ± 4.8 and 51.5 ± 28.2 μU/dl, TCDD and pair-fed control respectively). Pancreatic weight of pair-fed control animals compared to TCDD-treated animals was 0.75 ± 0.06 and 0.84 ± 0.2 g respectively (mean ± SD, N = 3).

Effects of TCDD on the adipocyte. TCDD increased specific ^{125}I-insulin binding ~3.4-fold in adipocyte plasma membrane preparations from TCDD-treated animals relative to pair-fed controls (Table 5).

TCDD reduced binding of [^{3}H]epinephrine to the adipocyte membrane by ~55% after 2 days of exposure (Table 5). Cellular cAMP concentration in TCDD animals was 160% (100% in controls), and levels of cAMP phosphodiesterase activity in TCDD animals was 158% (100% in controls). No change was observed in cGMP phosphodiesterase activity, total cellular cAMP-independent protein kinase, or in total cellular cAMP-dependent protein kinase activity. Plasma membrane associated cAMP inde-

Table 2. Serum glucose concentrations 2 or 10 days after treatment with 1 μg TCDD/kg (i.p.) or acetone–corn oil (and pair-fed) with and without glucose supplement

	Serum glucose concentrations (mg/dl)			
Treatment	2 days	2 days + glucose	10 days	10 days + glucose
Pair-fed control*	156 ± 18 (6)	457 ± 36† (3)	164 ± 33 (4)	500 ± 35† † (3)
TCDD	164 ± 18‡ (6)	435 ± 11†, ‡ (3)	191 ± 62‡ (4)	588 ± 15†, ‡ (3)

Values are means ± SD (the number of animals is given in parentheses).
* Pair-fed control guinea pigs were fed the same amount of food consumed by the TCDD-treated animals.
† Significantly different from respective treatment without glucose (P = 0.01).
‡ Not significantly different from pair-fed control with Tukey's test for unconfounded comparisons after factorial ANOVA.

Table 3. Na^+-K^+-, Mg^{2+}-, and Ca^{2+}-ATPase activities in isolated guinea pig intestinal plasma membrane 10 days after treatment with either 1 μg TCDD/kg or vehicle alone and pair-fed to the TCDD-treated animals

Treatment	Na^+-K^+-ATPase	Mg^{2+}-ATPase	Ca^{2+}-ATPase
	(nmol ATP hydrolyzed/mg protein/hr)		
Pair-fed control	1189.6 ± 257.9	1300.3 ± 225.6	825.9 ± 197.3
TCDD	1196.3 ± 137.6	1330.6 ± 66.3	729.4 ± 81.6

Values are means ± SD, N = 3. No significant differences were noted with Student's paired *t*-test.

Table 4. Serum insulin and thyroxine concentrations in guinea pigs 2 and 10 days after administration of 1 μg TCDD/kg, or acetone–corn oil and pair-fed

Treatment	Serum insulin (μU/ml)		Serum thyroxine (μg/dl)	
	2 days	10 days	2 days	10 days
Pair-fed control	58 ± 21 (8)	17 ± 1 (3)	1.1 ± 0.3 (6)	1.4 ± 0.5 (3)
TCDD	39 ± 19*, † (8)	47 ± 16* (3)	1.5 ± 0.5 (6)	1.7 ± 0.6 (3)

Values are means ± SD, N = number of animals.
* Significantly different from pair-fed control with Student's paired *t*-test ($P < 0.05$).
† Serum insulin concentration was 5–56% that of pair-fed control.

Table 5. Effect of TCDD on various lipogenic and lipolytic variables in the adipose tissue of guinea pigs 2 days after treatment with either 1 μg TCDD/kg or acetone–corn oil

Variable	Pair-fed control	TCDD
Insulin binding*	45.6 ± 8.0	154.2 ± 90.0†
Epinephrine binding*	665.2 ± 560.1	301.8 ± 164.2†
Cellular cAMP‡	329 ± 80	525 ± 69†
Cellular cAMP phosphodiesterase§	226.1 ± 42.9	357.5 ± 17.6†
Cellular cGMP phosphodiesterase§	1679.7 ± 333.4	1688.9 ± 273.5
Protein kinase		
Plasma membrane‖		
cAMP-dependent	111.5 ± 16.5	76.5 ± 13.3†
cAMP-independent	210.7 ± 25.2	188.1 ± 23.2
Cellular¶		
cAMP-dependent	1756.1 ± 58.2	1709.6 ± 183.1
cAMP-independent	312.6 ± 61.5	302.9 ± 58.5
Cellular hormone sensitive lipase**	43.4 ± 1.6	45.5 ± 3.3

Values are means ± SD for three to four pairs of animals.
* Expressed as pg ^{125}I-insulin or [^{3}H]epinephrine specifically bound/mg adipocyte membrane/20 min.
† Significantly different from pair-fed control with paired *t*-test, $P < 0.05$.
‡ Expressed as pmol cAMP/g adipose tissue.
§ Expressed as pmol cAMP or cGMP hydrolyzed/mg protein/3 min.
‖ Expressed as pmol P_i incorporated/mg plasma membrane protein/10 min.
¶ Expressed as pmol P_i incorporated/mg acetone–ether powder/10 min.
** Expressed as nmol oleic acid/mg protein/hr.

pendent kinase was reduced 11% by TCDD. Membrane bound cAMP-dependent protein kinase was depressed significantly by 31% relative to control animals. Cellular cAMP-dependent kinase activity was 15 to 20-fold higher than that found in the plasma membrane portion (Table 5). TCDD did not produce any change in hormone sensitive lipase activity.

DISCUSSION

The current results indicate that reduction of adipose tissue LPL began as soon as 1 hr after TCDD administration, attained maximum depression after 2 days, and remained at that level throughout the 10-day observation period. This rapid decline was accompanied by two important biochemical changes evoked by TCDD: a decline in serum insulin concentration probably due to functional changes in the pancreas beyond that observed in pair-fed control animals, and time-dependent changes in the adipocytes to respond to insulin. TCDD-induced depression in adipose tissue LPL could be reversed completely by glucose intubation on day 1, reversed only partially on days 2 and 5, and not affected on

day 10. Oral doses of glucose are known to reverse the starvation-induced depression of this enzyme [10, 24] and are correlated with rising serum concentrations of insulin. The insulin-evoked synthesis and/or activation of LPL, therefore, seemed normal at an early stage of poisoning, but the affected adipocytes lost this ability at a later stage. This loss does not seem to be due to malabsorption of glucose into the blood or from a change in blood thyroxine levels. One of the causes for the decline of LPL activity could be related to insulin, since on day 2 both serum and pancreatic insulin levels were depressed. However, reversal of LPL activity after prolonged TCDD exposure seemed to be independent of serum insulin concentrations and due to an inability of the LPL synthesizing system to respond to insulin. While there are a number of changes observed to occur in the biochemical variables associated with the epinephrine-related lipolytic pathway, the lack of TCDD's effect on HSL, confirming the observations of Swift *et al.* [5], clearly indicated that the effect of TCDD on LPL is not mediated through activation of this pathway. Therefore, it is concluded that TCDD acts on the adipocyte to uncouple the HSL–LPL reciprocity [25].

While it is not possible to screen all factors known to affect adipose tissue LPL, the mere fact that such an effect was observable as early as 1 hr after TCDD treatment indicates the nature of the causative effect to be a quick-acting system. Because of the reversibility of the phenomenon with the excess glucose administration, the entire insulin signal transduction and subsequent LPL synthetic process appear to be unaffected. The most logical explanation may be that TCDD causes an intracellular change in the rate of LPL synthesis in the adipocyte. The rate of LPL turnover is known to be fast and can accommodate such a possibility; however, more work is needed to clarify this point.

Simultaneous declines occurred in both adipocyte LPL and serum insulin, but whether these two events are causally related is not known at this time. Rat adipocyte LPL is not affected significantly by 25 μg TCDD/kg, 10 days after administration (control LPL = 1694 ± 388, TCDD = 1626 ± 231 nmol free fatty acid/mg extracted fat powder/hr) [26], and yet a report by Potter *et al.* [22] indicated that both pancreatic and serum insulin levels were lowered by TCDD treatment in this species. In the rabbit (unpublished observations), TCDD significantly lowered adipose tissue LPL 10 days after treatment and slightly increased serum insulin concentrations. Taken together these findings indicate that TCDD affects these two tissues independently. The fact that hypertriglyceridemia is observed in the guinea pig and the rabbit, but not in the rat indicates that hypoinsulinemia alone cannot induce hypertriglyceridemia in all animal species and that suppression of adipose LPL activity is necessary for the final expression of hypertriglyceridemia. Considerable evidence exists for adipose LPL being the critical regularity step in controlling serum triglyceride concentration [9, 27–30].

The TCDD-evoked reduction of LPL appears to be a specific event in the adipose tissue. Adipocytes are very specialized cells with LPL and HSL playing prominent roles in their lipogenic and lipolytic functions. Because of their extreme sensitivities to TCDD and other related halogenated aromatic hydrocarbons, our current finding provides a promising clue to future research concerning the biochemical basis of the wasting syndrome and the hyperlipidemia produced by these environmental pollutants.

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