The Effect of Diazoxide-induced Hormonal Secretion on Plasma Triglyceride Concentration in the Rat

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Summary. The relationship between non-esterified fatty acid (NEFA) mobilization and hepatic conversion to plasma triglycerides (TG), as modulated by diazoxide-induced effects upon endogenous catecholamine, glucagon, and insulin secretion, was examined in vivo in the rat. Thyrotropin (TSH)-induced NEFA mobilization provided the control study. – In all control experiments, TSH (1.5 IU/100 g) induced a 110% increase in NEFA availability, which was associated with a subsequent 52% increase in plasma TG concentration and a 73% increase in plasma ketone bodies. Following diazoxide administration (30 mg/kg), endogenous secretion of both catecholamines and glucagon was observed, resulting in a comparable 100% increase in NEFA availability, with the appropriate ketonaemic response. However, in contrast to the control TSH study, plasma triglyceride concentration did not increase. This suppression was secondary, at least in part, to a direct 40% inhibition of hepatic secretion of triglycerides. – Although plasma NEFA concentration is an important determinant of plasma triglyceride levels, the concurrent concentration of endogenous catecholamines, glucagon, and insulin modulate the hepatic conversion of NEFA to triglycerides in vivo.

Key words: Diazoxide, TSH, rat, triglyceride, non esterified fatty acid (NEFA), ketone bodies, glucagon, catecholamines, insulin.

glucagon, and insulin secretion upon NEFA conversion to triglycerides within the intact animal. Traditional concepts of catecholamine and glucagon influence upon lipid regulation have emphasized the role of stimulation of peripheral NEFA mobilization from adipose tissue with a secondary induction of fatty liver, hyperlipoproteinaemia [1] and hyperketonaemia [2, 3]. The possibility of other hormonal effects upon NEFA metabolism has been suggested by the demonstration in vitro in the perfused rat liver, confirmed by isotopic tracer studies in man, that fractional conversion of NEFA to triglyceride may be decreased by epinephrine and/or glucagon [4–6].

We have investigated the triglyceride and ketone body response to controlled NEFA availability in the presence and absence of excess endogenous catecholamine and glucagon secretion in the intact rat. Thyrotropin (TSH) injection was utilized to mobilize NEFA in the control studies in the absence of endogenous catecholamine and glucagon secretion. Diazoxide, a stimulus for adrenal catecholamine [7, 8] and pancreatic glucagon secretion [9], as well as for inhibition of insulin secretion [8], was utilized to mobilize NEFA in the presence of physiological increments in endogenous catecholamines and glucagon. NEFA availability during both thyrotropin stimulation and diazoxide challenge were comparable, providing an opportunity for appropriate comparisons in the presence and absence of hormone excess.

Methods

Male Sprague-Dawley rats weighing 180–200 g were used in all experiments. Animals were studied at 0800 h in the fed state having had access to Purina rat chow and water ad lib. The study

The contribution of the endogenous hormonal milieu to non-esterified fatty acid (NEFA) metabolism is not resolved. The present investigation was designed to examine the effect of endogenous catecholamine,

comprised 82 animals, divided into three paired groups, providing a control group, a thyrotropin-induced NEFA mobilization group and a diazoxide-induced catecholamine and glucagon stimulation group.

For the assay of plasma norepinephrine and epinephrine, a 1 ml sample of blood was obtained by cardiac puncture in lightly ether-anaesthetized animals. The blood was placed in an heparinized tube containing reduced glutathione at a final concentration of 5 mM, immediately centrifuged, and the plasma frozen at -20°C until assay within ten days. Plasma epinephrine and norepinephrine were assayed with a sensitivity of 0.5 ng/ml and a precision of $\pm 10\%$ according to the radioenzymatic method of Peuler and Johnson [10], with the thin layer chromatography modifications of DaPrada and Zurcher [11].

All other chemical determinations were performed on plasma samples obtained by decapitation of the lightly ether-anaesthetized animals, with collection of the blood in heparinized test tubes at 5°C containing benzamidine, as previously reported [12]. All samples were assayed for insulin [13], glucagon [12], thyroxine [14], NEFA [15], betahydroxybutyrate (BOH) and acetoacetate (AcAc) [16], glucose [17], and triglycerides [18]. The ketone bodies were assayed on the day the experiments were performed.

Thyrotropin (TSH) Stimulation

To stimulate endogenous NEFA mobilization without stimulating catecholamine or glucagon secretion, TSH (Thyropar, Armour) was diluted in 4 ml of normal saline, and animals were given 1.5 IU/100 g body weight under light ether anaesthesia by intraperitoneal injection. Ten animals were sacrificed at time zero and six animals thereafter at 30, 45, 60, and 90 minutes after TSH injection.

Diazoxide Stimulation

To stimulate endogenous NEFA mobilization in association with enhanced endogenous catecholamine and glucagon secretion, diazoxide (Hyperstat, Shering, Bloomfield, NJ) (30 mg/kg body weight) was administered by stomach tube. The animals receiving diazoxide were studied in parallel with those receiving TSH to eliminate variables in diet and environment. The ten animals sacrificed at time zero served as basal controls for the TSH, diazoxide and saline control studies. Thereafter, six additional animals were sacrificed at 30, 45, 60, and 90 min after diazoxide administration.

Saline Control Studies

A separate saline control group was performed in parallel with the TSH and diazoxide groups. Following the administration of 4 ml of 0.154 mol/l saline, six animals were sacrificed at 30, 45, 60, and 90 min., and the plasma assayed as above.

Triglyceride Production In Vivo

In separate experiments, triglyceride production rates were determined using the Triton method described by Robertson et al. [19]. Triton WR-139 (polymeric-p-iso-octyl polyoxyethylene phenol) was injected via a foot vein at a concentration of 300 mg/ml, under light ether anaesthesia. Animals weighing approximately 200 g received a total dose of 135 mg, which results in maximal inhibition of triglyceride removal from the blood [19]. Serial retro-orbital blood samples (0.5 ml) were obtained prior to Triton injection and at 15 min intervals thereafter, and immediately placed in heparinized plasma tubes at 5°C . Four animals were studied

following the intragastric administration of diazoxide or intraperitoneal injection of TSH as described above. Triglyceride secretion rate (TGSR) was calculated from the plasma volume (PV_e) determined by Evans Blue Dye injection in Sprague-Dawley rats according to the relationship: $\text{TGSR} = \text{slope} \times PV_e$. In our dye injection studies, $PV_e = 0.03 \times \text{wt.} + 4.01$.

The diazoxide data were compared to the data from the TSH treated rats, and differences in study means analyzed by Student's "t" test. The TG slopes were compared utilizing an analysis of variance as applied to regression [20].

Results

Control Saline Study

To exclude substrate or hormonal change unrelated to either TSH or diazoxide exposure, control studies were performed in 30 rats. In rats given saline, there was no consistent alteration in plasma glucagon (127 ± 13 pg/ml), glucose (181 ± 24 mg/dl), NEFA (0.51 ± 0.03 mmol/l), total ketone bodies (0.29 ± 0.5 mmol/l), or triglyceride concentration (125 ± 8 mg/dl). A slight reduction in circulating levels of insulin (from 16 ± 4 to 13 ± 4 $\mu\text{U/ml}$) and of catecholamines (from 2.44 ± 0.22 to 1.28 ± 0.14 ng/ml) was observed.

Effect of TSH

on Non-esterified Fatty Acid Mobilization and Plasma Ketone Body and Triglyceride Levels

Hormonal Response (Table 1). Basal concentrations of total plasma catecholamines (2.37 ± 0.45 ng/ml) were not significantly altered by TSH injection. Similarly, plasma glucagon concentration (180 ± 11 pg/ml pre-TSH) was unaltered by TSH injection, while plasma insulin concentration rose from basal levels of 16 ± 2 $\mu\text{U/ml}$ to 24 ± 3 $\mu\text{U/ml}$ ($p < 0.05$) 60 minutes after TSH. Plasma thyroxine levels (5 ± 3 $\mu\text{g/dl}$) were unaltered by acute TSH injection.

Lipid and Metabolite Response (Fig. 1). A brisk lipolytic response was observed which was maximal at 60 minutes post TSH (pre-TSH NEFA level, 0.49 ± 0.03 mmol/l; maximal post-TSH; 1.18 ± 0.140 mmol/l, $p < 0.001$). Plasma total ketone body concentration rose promptly in parallel with the rise in NEFA, from basal levels of 0.26 ± 0.02 mmol/l to a maximum of 0.45 ± 0.08 mmol/l ($p < 0.001$) at 60 minutes. Both 3-hydroxybutyrate and acetoacetate responded in a comparable fashion to this ketogenic response (data not shown). Plasma triglyceride concentration rose from basal levels of 142 ± 10 mg/dl to a maximum of 217 ± 20 mg/dl ($p < 0.001$), also at 60 minutes after TSH.

Table 1. Hormonal responses to TSH and diazoxide

Minutes	Glucagon (pg/ml)	Catecholamine (ng/ml)	Insulin (μ U/ml)
Response to TSH			
0 n = 10	180 $\pm$ 11	2.37 $\pm$ 0.45	16 $\pm$ 2
30 n = 6	177 $\pm$ 13	2.35 $\pm$ 0.39	19 $\pm$ 4
60 n = 6	213 $\pm$ 19	1.95 $\pm$ 0.30	22 $\pm$ 3 ^a
90 n = 6	176 $\pm$ 13	2.15 $\pm$ 0.43	21 $\pm$ 2 ^a
Response to diazoxide			
0 n = 10	180 $\pm$ 11	2.37 $\pm$ 0.45	16 $\pm$ 2
30 n = 6	387 $\pm$ 33 ^a	3.59 $\pm$ 0.48	19 $\pm$ 4
60 n = 6	256 $\pm$ 55 ^a	6.17 $\pm$ 0.96 ^a	5 $\pm$ 3 ^a
90 n = 6	155 $\pm$ 26	5.27 $\pm$ 0.90 ^a	10 $\pm$ 2

^ap < 0.01 **Fig. 1.** Comparison of the effects of TSH and diazoxide administration upon the concentration of plasma NEFA (basal concentration = 0.48 ± 0.03 mmol/l), total ketone bodies (basal concentration = 0.26 ± 0.02 mmol/l), and triglycerides (basal concentration = 142 ± 10 mg/dl) in the rat. Plasma from the same ten animals as in Table I was analyzed at all time points. Data are presented as the mean $\pm$ SEM change from baseline **Fig. 2.** Effect of TSH (Δ -) and diazoxide ($\bullet$ -) administration upon the Triton induced rise in plasma TG concentration from basal levels of 120 ± 16 mg/dl, as an estimate of hepatic TG production. The diazoxide-TG production rate determined from the slope ($Y = 3.86X + 64$) was significantly less than the TSH-TG production rate (slope: $Y = 4.36X + 80$ ($p < 0.01$)) as given in Results

No change in plasma glucose concentration was observed during the 90 minute period of observation (pre-TSH, 192 ± 5 mg/dl; maximum post-TSH, 208 ± 3 mg/dl, $p < 0.05$).

Effect of Diazoxide on Fatty Acid Mobilization

Hormonal Response (Table 1). The oral administration of diazoxide resulted in a rise in plasma catecholamine concentration from basal levels of 2.37 ± 0.45 ng/ml to a maximum of 6.17 ± 0.96 ng/ml at 60 minutes ($p < 0.001$). Epinephrine and norepinephrine both showed proportionate increases in concentration. Simultaneously, plasma glucagon concentration rose from pre-diazoxide levels of 180 ± 11 pg/ml to a maximum of 387 ± 33 pg/ml ($p < 0.001$) 30 minutes postdiazoxide, while plasma insulin concentration fell from 16 ± 1 μ U/ml to 5 ± 3 μ U/ml 60 minutes post-diazoxide ($p < 0.001$).

Lipid and Metabolite Response (Fig.1). In response to diazoxide administration, plasma NEFA concentration rose from 0.49 ± 0.03 mmol/l to a maximum of 1.01 ± 0.11 mmol/l ($p < 0.001$) 60 minutes post-diazoxide, comparable to the rise seen with TSH stimulation in the absence of excess catecholamine. Plasma ketone body concentration also rose from basal levels of 0.26 ± 0.02 mmol/l to a maximum of

0.52 ± 0.05 mmol/l ($p < 0.001$) at 60 minutes. The rise in endogenous catecholamine and glucagon secretion, with the associated reduction in insulin secretion, was accompanied by hyperglycaemia, with basal glucose levels rising from 192 ± 4 mg/dl to a maximum of 323 ± 39 mg/dl at 60 minutes. In contrast to the TSH study, there was no comparable rise in plasma triglyceride concentration (Fig. 1).

Triglyceride Production Studies. To investigate the mechanism of the diazoxide-induced inhibition of TG elevation in spite of increased NEFA availability (Fig. 2), TG production rates were examined in TSH versus diazoxide treated animals. Triton 1339 injection into TSH treated rats resulted in a brisk rise in plasma triglyceride concentration to maximum levels of 582 ± 21 mg/dl (Fig. 2). In contrast, Triton 1339 injection into diazoxide treated rats resulted in a significantly smaller rise of plasma TG, with the maximum at 90 minutes being 311 ± 26 mg/dl ($p < 0.001$). The TGSr in TSH treated rats was 0.69 ± 0.07 mg/min, and was reduced by 40% to 0.41 ± 0.09 mg/min ($p < 0.01$) in diazoxide treated rats.

Discussion

These investigations examined the quantitative role of regulatory hormones upon the conversion of NEFA into circulating triglycerides. The effects of physiological changes in the concentration of epinephrine, norepinephrine, and glucagon suggested that these hormones exerted a direct modulation of lipid production rates, such that the expected hyperlipaemia in response to NEFA mobilization was not observed.

The observations of Heimberg et al. [21, 5], which have been confirmed by other investigators in the perfused liver [22, 23, 24], demonstrated the dependence of lipogenesis and of ketogenesis upon the concentration of NEFA entering the liver. In this setting, the addition of pharmacological amounts of catecholamines to the perfusate resulted in a reciprocal augmentation of ketone body generation and a simultaneous depression of triglyceride output from the liver [33]. Our data extend to the in vivo situation confirmation of a dependence of ketone and triglyceride production upon plasma NEFA concentration, demonstrated following TSH injection. They also demonstrate a regulatory role of catecholamines in concert with glucagon in limiting TG production following diazoxide administration. These data provide a potential hormonal mechanism for the previously reported TG lowering response to diazoxide therapy in man [40].

The present in vivo observations defining the quantitative TSH response relative to lipolytic, ketonaemic and lipaemic changes, extend observations obtained with this peptide since the 1950's. Previous studies of TSH examined its potency among pituitary peptides in promoting mobilization of NEFA in association with ketosis. The lipolytic response has been carefully defined by the studies of Freinkel [25] in rat adipose tissue in vitro, and the ketonaemic response described by Peterson et al. in 1955 [26] and by Engel et al. [27] in 1957. Our data extend these observations to quantitate the simultaneous elevation in plasma triglyceride concentration which occurs following TSH injection. While our data do not resolve the mechanism of this TG rise, it seems likely to represent the response to fatty acid mobilization which provides excess NEFA substrate to the liver for metabolism. The small elevation in plasma insulin concentration which was associated with TSH injection may also have contributed to the rise in TG concentration, since insulin has been proposed from indirect evidence in man [28] and by direct replacement in the diabetic rat [29] to enhance hepatic TG production.

In contrast to the TSH response, the augmented NEFA availability occurring with diazoxide-induced endogenous catecholamine and glucagon excess, resulted in a significant elevation in ketone body concentration without a simultaneous rise in plasma triglyceride levels. We have demonstrated that reduced hepatic production of TG contributed to the failure of plasma TG concentration to rise during endogenous catecholamine and glucagon mobilization of NEFA. Thus, our data support the concept that these endogenous hormones have the potential to reduce TG production even during periods of availability of excess NEFA, extending observations in the perfused liver.

It is difficult to separate direct effects of catecholamines from those of increased glucagon and reduced insulin secretion [30]. The secretion of catecholamines documented in our studies may be expected to influence NEFA mobilization, and to directly stimulate the secretion of glucagon [31]. Traditional concepts of catecholamine influence upon lipid regulation have emphasized this role of stimulation of peripheral NEFA mobilization from adipose tissue with a secondary, substrate-induced fatty liver [1] and hyperketonaemia [32, 3]. The possibility of other catecholamine effects upon fatty acid metabolism has been suggested by the demonstration in vitro in the perfused rat liver [33], confirmed by isotopic tracer studies in both man and the rat [4, 34], that fractional conversion of NEFA to TG may be reduced by epinephrine. Acute stress in the Sand Rat

has been reported to induce an immediate decrease in endogenous triglyceride secretion and circulating triglyceride levels, which could be partially reproduced in vivo with epinephrine injection [35]. Moreover, in both the dog and the rat, with the persistent lipolytic response to chronically administered epinephrine, plasma TG levels remain normal while cholesterol levels rise [36, 37]. Our data extend these studies of exogenous catecholamine injection to confirm a limitation of NEFA conversion to plasma TG by endogenous catecholamine, with an appropriate control study of NEFA mobilization in the absence of excess catecholamine.

The limitation of endogenous insulin secretion imposed by catecholamine secretion, as well as by diazoxide, may be expected to participate in the physiological responses to catecholamines and glucagon in vivo. This would permit peripheral lipolysis to evolve readily in response to lipolytic hormones, and would allow oxidation of fatty acid to ketones to be more readily expressed [22]. The concept of such a restraining role for insulin has recently been emphasized [38]. In this setting, it is attractive to relate the reduced TG production to the reduced ambient insulin levels, consistent with the suggestions by many investigators relating hyperinsulinemia with hepatic overproduction of lipids [19, 39, 40].

Participation of endogenously secreted glucagon in the limitation of NEFA conversion to TG has been previously proposed from observations in several species. In the duck, glucagon in pharmacological dosages has been reported to have a marked triglyceride lowering effect, which is dependent upon the presence of the liver [41]. In this bird, immediately upon cessation of glucagon administration, a marked rebound hypertriglyceridaemia occurs, which parallels the sustained NEFA elevation resulting from the earlier lipolytic response to glucagon. In the rat, glucagon excess has been reported to inhibit apolipoprotein protein formation in the isolated liver slice [42] and depress hepatic triglyceride synthesis [43]. Moreover, direct injection of glucagon in man and in the rat has been reported to result in a reduction in plasma lipid concentration [44, 45].

Interpretation of the in vivo responses to diazoxide must be made with caution, since unrecognized or undefined effects of the drug may influence the physiological response. Thus, alterations in regional blood flow as well as a general hypotensive response may participate in the metabolic effects, in addition to a contribution by catecholamine, glucagon, insulin, and of hyperglycaemia. The mechanisms of the physiological effects have been the subject of a recent symposium [46], but the total metabolic response has remained controversial [47]. Moreover, all of our

data have been collected in the anaesthetized state, which may have also influenced hormone levels. In spite of these reservations, our data are consistent with in vivo rapid regulation of triglyceride production by catecholamines, glucagon, and insulin, which results in significant changes of circulatory levels of plasma triglycerides. Thus, these in vivo studies with parallel TSH and diazoxide stimulation of NEFA mobilization have provided an experimental approach to further define the hormonal regulation of lipid metabolism in which altered hepatic regulation of NEFA conversion to triglycerides may be observed.

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