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Metabolic Potential of the Adipose Tissue of Rats during Hyper- and Hypovitaminosis A[1] (42311)

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Abstract. Effect of excess feeding and depletion of vitamin A on the ability of adipose tissue to maintain plasma free fatty acid levels has been studied in rats. Both in hypervitaminosis A (fed 9 mg of retinol for 2 consecutive days) and in vitamin A deficiency (kept on a vitamin A-deficient diet for 6 weeks) the rats showed elevated levels of plasma free fatty acids. Hypervitaminosis A caused a decrease in the fatty acid release from adipose tissue on *in vitro* incubation, probably due to lowered levels of cyclic AMP. On the other hand, adipose tissue from vitamin A-deficient animals showed an increased lipolytic rate as compared to that of the controls. No change in the lipogenic ability was indicated in either of the conditions as indicated by the activities of enzymes involved in this process. We conclude that the fatty acid homeostasis can be greatly influenced by the vitamin A status of the animals.

Investigations on the manifestations of toxicity and deficiency of vitamin A have contributed significantly to the current knowledge on its physiological functions. However, a unified concept of its role in molecular terms in the multitude of its functions is yet to be put forward [see review (1)]. Extensive studies of Wolf (1) and De Luca *et al.* (2) have proposed the molecular function of retinol analogous to that of dolichols in *N*-glycosylation of proteins. Whether or not this mode of action can be related to other functions of this vitamin has not yet been established. Recently, Weber (3) has postulated a possible correlation between alterations in lipid metabolism in vitamin A deficiency and the decrease in *N*-glycosylation under this condition. The ubiquitous presence of cellular binding proteins for retinol and retinoic acid suggests the possible genomic site for the action, a mechanism similar to that of steroids (4, 5).

Our studies in the past have indicated that most of the biochemical alterations in rats fed with excess vitamin A are dependent on adrenal glands. These biochemical changes include stimulation of hepatic gluconeogenesis (6), fatty infiltration and hepatotoxicity (7), and inhibition of glycolysis (8). However, recent observations of Hillgartner *et al.* (9) on muscle protein turnover do not agree with this view. No conclusive evidence on the molecular basis of such a corticoid-dependence has yet been provided by our results.

During the course of our studies on the vitamin A-induced changes in fatty acid metabolism, we noticed that both hypervitaminosis A and hypovitaminosis A caused elevation of plasma FFA levels (10, 11). Since adipose tissue plays an important role in the storage and supply of fatty acids in coordination with the demand (12), we examined the metabolic status of this organ under the conditions of hyper- and hypovitaminosis A. Our results indicated that both excess and deficiency of vitamin A cause marked changes in the lipolytic potential of the adipose tissue, presumably due to the interplay of various biological effectors.

Materials and Methods. *Materials.* Glucose 6-phosphate, coenzyme A, ATP, NAD, NADH, NADP, malate hydrogenase (EC 1.1.1.37), glycerokinase (EC 2.7.1.30), glycerophosphate dehydrogenase (EC 1.1.1.8), and bovine serum albumin (fatty acid free) were purchased from Sigma Chemical Company. Vitamin A (prepalin) was a product of Glaxo

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Laboratories, Bombay, India. Albino rats (from Wistar Stock) were bred in the animal care facility of this Institute.

Animals and experimental protocol. Young male rats were used throughout the study. In experiments to study the effect of excess vitamin A, rats weighing 70–90 g were used. The animals were divided into two groups, "hypervitaminosis A" and "control." They were fed commercial rat chow (Hindlever diet, Hindustan Lever Ltd, India). Each rat in the hypervitaminosis A group was fed with the help of a gastric cannula, 9 mg of retinol in 0.4 ml of arachis oil once daily for 2 days, while the control group received appropriate volumes of arachis oil in lieu of retinol. Rats were sacrificed 24 hr after the second dose and the tissues excised.

Vitamin A deficiency was induced in rats as follows. Weanling rats weighing 40–50 g were divided into two groups "hypovitaminosis A" and "pair-fed control." Rats of both groups were maintained on a vitamin A-free diet formulated as described earlier (13). Controls were pair-fed with the same vitamin A-free diet. In addition, they were given 0.6 mg of retinol per week. Animals were sacrificed in the mild vitamin A-deficient state itself as indicated by the plateau stage of their body weights (13). No vitamin A was detectable in the livers of hypovitaminotic rats at the time of sacrifice.

All the animals used in this study were fasted overnight before they were sacrificed. Blood was collected from the jugular vein, in heparinized tubes for the isolation of plasma prior to sacrifice. Both liver and epididymal fat pads were excised and immediately used for the various measurements.

Plasma free fatty acid measurement. Plasma was carefully separated and transferred to 15-ml tubes. Free fatty acids from the plasma were extracted by the method of Dole and Meinertz (14). An aliquot of the extracted fatty acids was titrated against 0.018 *N* NaOH solution using thymol blue as an indicator (14).

Lipid extraction. Livers of rats were minced and ground in chloroform:methanol (2:1, v/v) and the lipids were extracted by the method of Folch *et al.* (15). Triglycerides were separated by thin-layer chromatography on silica gel G plates using a solvent system consisting of hexane:ether (1:1, v/v). The spots were scraped and eluted with the same solvent system. Suitable aliquots were used for the determination of glyceride glycerol by the method of Van Handel and Zilversmit (16).

Assay of lipolysis in vitro. Freshly excised epididymal fat pads (80–100 mg wet wt) were placed in 3 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) made up with half the recommended (17) amount of calcium. To each flask 105 mg of fatty acid-free bovine serum albumin was added. As indicated some of the incubations also contained 10 m*M* theophylline. Incubations at 37°C were carried out for 2 hr in a Dubnoff metabolic shaker. A mixture of CO_2:O_2 (5:95) served as the gas phase. The incubations were stopped by quickly chilling and removing the tissue. A portion (usually 2 ml) of the assay medium was deproteinized and the protein was sedimented. The supernatant was then divided into two parts. One part was used for the extraction of free fatty acids and the other part was used for the assay of glycerol by the method of Wieland (18).

Enzyme assays. A 20% homogenate of the adipose tissue was prepared in 0.25 *M* sucrose solution. The homogenates were centrifuged at 400*g* for 10 min to remove the fatty material which floated on the top. This was carefully removed and discarded. All the enzymes were assayed using the homogenates without further purification. Adenylate cyclase activity was measured by the method of Krishna *et al.* (19) using [^{3}H]ATP and the cyclic [^{3}H]AMP was separated by paper chromatography as described by Laraia and Reddy (20). Cyclic AMP phosphodiesterase was assayed by the method of Butcher and Sutherland (21). Acetyl CoA carboxylase was assayed by CO_2 fixation in the presence of citrate (22). The spectrophotometric method of Srere (23) was employed in the assay of ATP citrate lyase activity. Malate dehydrogenase activity was measured spectrophotometrically based on the method of Ochoa (24). Combined activity of hexose monophosphate shunt dehydrogenases was assayed according to Glock and McLean (25).

Results. Table I shows the levels of plasma-free fatty acids in hyper- and hypovitaminotic A animals. The data clearly show that plasma free fatty acid levels were significantly high under both conditions as compared to the respective controls.

Table II shows the levels of triglycerides in the livers of these rats. Intake of excess vitamin

TABLE I. EFFECT OF HYPER- AND HYPOVITAMINOSIS A ON PLASMA FREE FATTY ACID LEVELS

Group	Plasma FFA (μeq/100 ml plasma)	P
Control (6)	71.9 ± 0.78	
Hypervitaminosis A (6)	131.7 ± 3.36	<0.001
Pair-fed control A (6)	95.2 ± 3.71	
Hypovitaminosis A (5)	144.1 ± 3.80	<0.01

Note. Acute hypervitaminosis A was induced by orally feeding 9 mg of vitamin A once daily for 2 consecutive days to young male rats. Control animals received appropriate volumes of arachis oil in lieu of the vitamin. Rats were made vitamin A-deficient by maintaiing them on a vitamin A-deficient diet for a period of 6 weeks. Control animals were pair-fed and received 0.6 mg retinol once a week. At the time they were used for the experiment, the body weights of the vitamin A-deficient animals had reached a plateau and there was no detectable vitamin A in the liver. All rats were fasted for 18 hr before they were sacrificed. Blood was collected from the jugular vein in heparinized tubes and the plasma was separated by centrifugation. Free fatty acids were extracted and estimated as detailed in the text. Values in parentheses indicate the number of rats used. Each value is the mean ± SEM.

A caused a marked increase in the triglyceride content. On the other hand, deficiency of vitamin A caused no effect on the hepatic triglyceride levels. Data given in Tables I and II show that the control rats of the hypervitaminosis A group contain low plasma free fatty acid and hepatic triglyceride levels compared to the levels in the control rats of the vitamin A-deficient group. This disparity may be due to the difference in the age of the rats at the time they were sacrificed. Dietary composition could also have contributed to the effect.

We further explored the possible factors conducive to these changes under the extreme vitamin A status. It is well documented that hepatic triglycerides are affected by plasma FFA levels, which, in turn, are regulated by adipose tissue. We therefore examined the changes occurring in this tissue. We also reasoned that since adipose is sensitive to a variety of biological effectors, the metabolic potential of this tissue can be influenced by the fluctuations in the endocrine functions of the animals under the experimental condition. We therefore examined this possibility.

The lipolytic rates of adipose tissue from control and hypervitaminotic rats are presented in Table III. As evident, no difference in either fatty acid release or glycerol release was noticed between the two groups. The basal level of fatty acid release was increased significantly when theophylline was present in the assay. The response to theophylline in inducing fatty acid release was approximately threefold higher in the adipose tissue from control rats compared to those in the hypervitaminotic group.

A comparison of the lipolytic rate in the adipose tissue of hypovitaminotic rats with that of the controls is given in Table IV. Vitamin A deficiency caused a marked stimulation in lipolysis as measured by both fatty acid and glycerol release. The ratio of the fatty acid to glycerol release was 3 indicating that the reesterification was very little. A similar ratio was also noticed in hypervitaminotic tissue as well (Table III).

Owing to the dynamic rate by which the adipose responds to various hormones and biological signals (26, 27) for the release and storage of fatty acids, we examined the influence of vitamin A status on lipid turnover. Initially we injected precursors such as [2-^{14}C]acetate and [U-^{14}C]glucose to experimental animals to follow the incorporation of these precursors into the adipose tissue lipid components. No conclusion could be derived from these studies because of the individual variation (data not given). The next best possible way to assess the metabolic status of this tissue was to examine the key enzymes involved in the major pathways. Since DNA content of adipose tissue was very stable under extreme conditions, all the enzyme activities were expressed on the basis of DNA content. A substantial amount of DNA of fat pad would have been derived from stromal-vascular cells.

TABLE II. HEPATIC TRIGLYCERIDE LEVELS IN HYPER- AND HYPOVITAMINOSIS A

Group	mg/g fresh liver	mg/mg DNA
Control	7.78 ± 0.15	2.99 ± 0.06
Hypervitaminosis A	19.18 ± 0.30[a]	7.37 ± 0.11[a]
Pair-fed control	14.04 ± 0.56	5.85 ± 0.23
Hypovitaminosis A	13.63 ± 0.59	5.48 ± 0.28

Note. Treatment of the animals was the same as described in the legend to Table I and under Materials and Methods. A portion of the minced liver was extracted with chloroform:methanol (2:1 v/v) for the determination of the triglycerides as described in the text. Values given in the table are means ± SEM of six rats.

[a] Denotes significantly different from controls at $P < 0.05$.

TABLE III. EFFECT OF HYPERVITAMINOSIS A ON ADIPOSE TISSUE LIPOLYSIS *IN VITRO*

Group	Fatty acids released (μeq/hr/mg DNA)	Glycerol released (μmole/hr/mg DNA)	Theophylline-induced FA release
Control	5.17 ± 0.59	1.72 ± 0.26	8.93 ± 0.42
Control + theophylline	14.10 ± 0.60[a]	4.82 ± 0.78[a]	
Hypervitaminosis A	4.67 ± 0.41	1.49 ± 0.09	3.16 ± 0.32
Hypervitaminosis A + theophylline	7.83 ± 0.29[a]	2.63 ± 0.22[a]	

Note. Paired epididymal fat pads from control and vitamin A-fed rats were incubated for 2 hr in Krebs–Ringer bicarbonate buffer (pH 7.4) containing albumin (3.5 g/100 ml) in an atmosphere of O_2:CO_2 (95:5) in the presence or absence of 10 m*M* theophylline. At the end of incubation, the tissue was removed and the released fatty acids and glycerol were assayed as described in the text. The results are means ± SEM of four separate experiments.

[a] Significantly different from the controls at $P < 0.005$.

However, no difference in DNA content per wet weight of the tissue was noticed.

Since cyclic AMP is involved in the regulation of lipolysis, we examined the activities of the enzymes that determine its intracellular level at a given time. Adenylate cyclase activity was reduced significantly in tissues from hypervitaminotic rats (Table V). It was also noticed that cyclic AMP phosphodiesterase activity was increased in these animals as compared to that in controls. These results are suggestive of low cyclic AMP levels in hypervitaminotic tissue. This result together with the decreased response to theophylline (Table III) suggests that cyclic AMP level in adipose tissue of hypervitaminotic rats should be lower. Vitamin A-deficient animals, on the other hand, did not seem to show any change in cyclic AMP levels as indicated by the activities of these enzymes (Table V).

Activities of the lipogenic enzymes were measured in both hyper- and hypovitaminosis A (Table V). Hypervitaminosis A caused an increase in the activity of the combined hexose monophosphate shunt enzymes while acetyl CoA carboxylase, ATP citrate lyase, and malate dehydrogenase (decarboxylating) remained unchanged. Vitamin A deficiency showed no significant change in the enzyme activities except for the ATP citrate lyase which exhibited lower activity under this condition.

Discussion. Results presented in this paper show that (a) in both hypervitaminosis A and hypovitaminosis A plasma free fatty acid levels were significantly elevated; (b) hypervitaminosis A results in accumulation of triglycerides in liver while vitamin A deficiency does not affect the liver lipids significantly; (c) the *in vitro* lipolysis in adipose tissue from vitamin A-fed animals showed a tendency to decrease while it was accelerated in vitamin A deficiency.

The apparent elevation of plasma free fatty acids both in excess and deficiency of vitamin A is the reflection of the complex nature of the manifestations. As suggested earlier (6–8, 28), vitamin A can mimic the biological effects of glucocorticoids in stimulating metabolic pathways such as gluconeogenesis, hepatic fatty acid uptake and utilization, and amino acid catabolism. On the other hand, results from the *in vitro* experiments presented here provide a different picture. The reasoning for our earlier hypothesis originated from the fact that most of the biochemical changes caused by excess vitamin A could be abolished by bilateral adrenalectomy (7). Vitamin A-fed rats

TABLE IV. EFFECT OF HYPOVITAMINOSIS A ON ADIPOSE TISSUE LYPOLYSIS *IN VITRO*

Measurement	Pair fed control	Hypovitaminosis A	*P*
Fatty acid released (μeq/hr/mg DNA)	6.20 ± 0.56	8.33 ± 0.38	<0.01
Glycerol released (μmole/hr/mg DNA)	1.84 ± 0.08	2.54 ± 0.10	<0.01

Note. Paired epididymal fat pads from control and vitamin A-deficient rats were incubated as described in the legend to Table III. Fatty acid and glycerol released were measured as detailed in the text. The values are means ± SEM of five separate experiments.

TABLE V. REGULATORY ENZYMES OF ADIPOSE TISSUE DYNAMICS IN HYPER- AND HYPOVITAMINOSIS A

Enzyme	Control	Hypervitaminosis A	Pair fed control	Hypovitaminoisis A
Adenylate cyclase[a]	11.11 ± 1.94	4.08 ± 0.98*	5.27 ± 1.14	4.01 ± 0.76
Cyclic AMP phosphodiesterase[b]	2.63 ± 0.16	6.94 ± 0.45**	4.86 ± 7.22	9.74 ± 2.62
Acetyl CoA carboxylase[c]	71.0 ± 6.0	76.0 ± 4.0	N.D.	N.D.
ATP citrate lyase[d]	1.21 ± 0.15	1.01 ± 0.20	1.71 ± 0.21	0.99 ± 0.16**
Malate dehydrogenase[e] (decarboxylating)	1.20 ± 0.25	0.80 ± 0.10	0.92 ± 0.29	1.26 ± 0.11
Combined hexose monophosphate shunt dehydrogenase[f]	0.55 ± 0.11	1.48 ± 0.11**	1.13 ± 0.30	1.61 ± 0.05

Note. Epididymal adipose tissue from three rats were pooled for the enyzme preparation. The method of centrifugation and the assay procedures are given in detail in the text. The values are means ± SE of four separate experiments.

[a] cpm × 10^{-6}/min/mg DNA.

[b] μmole of P_i formed/hr/mg DNA.

[c] nmole of $^{14}CO_2$ fixed/min/mg DNA.

[d] μmole of NADH oxidized/min/mg DNA.

[e] μmole of NADP reduced/min/mg DNA.

[f] μmole of NADP reduced/min/mg DNA.

* Significantly different from control ($P < 0.05$).

** Significantly different from control ($P < 0.005$).

were also found to have elevated levels of circulating glucocorticoids (8). It may therefore be argued that the manifestations due to hypervitaminosis A could be attributed to the high levels of plasma glucocorticoids.

A close examination of our current and previous data (29) shows that excess vitamin A administration causes a stimulation of the hepatic uptake, utilization and storage of circulating fatty acids. This causes an increased demand for fatty acids, the primary source of which is the adipose. On the contrary, vitamin A deficiency causes a decrease in the capacity for the liver to take up fatty acids, presumably due to the low activity of fatty acyl coenzyme A synthetase (25% of controls, data not given). If such a lesion occurs in the liver through whatever possible mechanism, adipose has to regulate its function accordingly. The degree of utilization of fatty acids by extrahepatic tissues was not measured in this study. If excess vitamin A causes any impairment in the extrahepatic utilization of fatty acids, it can also contribute to the elevated levels of plasma FFA. On the other hand, in vitamin A deficiency the elevated levels of plasma free fatty acids might be the result of increased lipolysis as well as decreased hepatic utilization of the fatty acids.

It is not clear at this point what is the physiological significance of the opposing effects of adrenal hormones and vitamin A. Recent studies of Roberts *et al.* (30) have shown the antagonistic actions of retinoic acid and dexamethasone in the anchorage-independent cell growth. The ubiquitous presence of cellular retinol and retinoic acid binding proteins and the similarity between the mechanism of action of steroids and retinoids has been a subject of intense investigation in recent years (1). Recently Ramachandran *et al.* (31) and Ramachandran and Melnykovych (32) have shown that glucocorticoids stimulate dolichol-linked glycosylation; a mechanism similar to that believed to be one of the molecular functions of retinoids. Some of the recent observations by one of us (C.K.R.) suggest that glucocorticoids and retinol act synergistically in inducing certain membrane bound enzymes. Although fragmentary, the possibility of a causal relationship and antagonism between the steroids and retinol is worth exploration.

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