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Influence of different dietary proteins on plasma growth hormone in rats

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Summary: Dietary casein, compared to vegetable protein, causes hypercholesterolemia in some animal species. This may be associated with a change of hormonal status. Among others, GH has an important impact on cholesterol metabolism; GH deficiency results in hypercholesterolemia.
This paper shows that the rhythmic variation of GH levels in rats is differently affected by different dietary proteins. Within a 4-h observation period overall mean values and integrated areas under the GH levels plotted against time are higher with casein as compared to soy protein. Secretory GH peak values are lower than reported before for chow-fed rats. These observations support the idea that different dietary proteins cause a different endocrine response.
As GH levels are higher with casein, while lower levels would be expected to be associated with hypercholesterolemia, the observed differences are obviously of less relevance for the expression of casein-induced hypercholesterolemia.

Zusammenfassung: Casein, im Gegensatz zu pflanzlichen Proteinen, führt bei einigen Tierspezies zu Hypercholesterinämie. Dies könnte mit einer Änderung des Hormonstatus einhergehen. Neben anderen Hormonen hat das Wachstumshormon (GH) eine wichtige Rolle in der Regulation des Cholesterinstoffwechsels. Ein GH-Mangel führt zu Hypercholesterinämie.
Diese Untersuchung zeigt, daß die zyklischen Schwankungen der GH-Spiegel durch die Art des Nahrungsproteins beeinflußt werden. Die GH-Mittelwerte und die errechnete Fläche unter den GH-Spiegeln über 4 Stunden sind bei Casein-gefütterten Tieren höher als bei Sojaprotein-gefütterten Tieren. Höhere GH-Spiegel in Casein-gefütterten im Vergleich zu Gluten-gefütterten Tieren wurden bereits beobachtet. Unsere Daten unterstützen also die These, daß verschiedene Nahrungsproteine zu unterschiedlichen endokrinen Antworten führen.
Da aber niedrige GH-Spiegel die Voraussetzung für die Ausbildung einer Hypercholesterinämie sind, können die beobachteten Unterschiede nicht die Basis für die Ausprägung einer Casein-induzierten Hypercholesterinämie sein.

Key words: growth hormone, casein, soy protein, endocrine response, hypercholesterolemia

Schlüsselwörter: Wachstumshormon, Casein, Sojaprotein, endokrine Reaktion, Hypercholesterinämie

Introduction

Casein in a semisynthetic diet has a hypercholesterolemic effect in some animal species as compared to soy protein or other vegetable proteins. This is not the case in healthy man (1). The biochemical mechanism for this effect is not yet fully understood, though several gastrointestinal and metabolic parameters are influenced by dietary proteins and have been proposed as the main cause (2).

It might also be that hormones are involved. Casein in comparison to soy protein (3–6) or gluten (7) has been reported to raise plasma insulin (3, 4) and GH concentrations (7) and to lower glucagon (4) and thyroid hormone concentrations (5, 6). All these hormones affect lipid metabolism and plasma lipids. Hypophysectomy causes hypercholesterolemia in the rat (8, 9). Growth hormone deficiency in man is also associated with hypercholesterolemia (10–13). In some (10, 11) – but not all – cases plasma cholesterol concentrations were lowered following physiological doses of GH. One study showed that supraphysiological doses of GH lowered plasma cholesterol in subjects with normal GH concentrations (14). Several changes in lipid metabolism have been observed which may contribute to this effect: GH stimulates (15) and GH deficiency decreases cholesterol synthesis (9). Application of GH reduces lipoprotein lipase and hepatic lipase activities (11) and inhibits lipogenic enzymes (16). On the other side GH stimulates bile acid synthesis (12) and enhances hepatic cholesterol 7α-monoxygenase activity (17).

GH effect might as well be indirect. GH induces both glucagon and insulin release (18). T_3 and GH act synergistically in lowering plasma cholesterol of either hypophysectomized or thyroidectomized rats (8, 9). The combined action of T_3 and GH is necessary for maintaining normal levels of a subset of T_3-responsive m-RNA species (19).

This work presents plasma GH data obtained from casein- and soy protein-fed rats by repetitive blood sampling over 4 h in free-moving, unanesthetized animals. Repetitive determinations are essential as rat plasma GH concentrations change in a rhythmic pattern (20).

Methods

Animals and diets

Seventeen male Wistar rats (4 weeks old, mean body weight 41 g) were housed in wire-mesh stainless steel cages under constant temperature (21 °C) and humidity (52 %) and a 12 h light-dark cycle. Light period was from 0600 to 1800 hours. They were kept in groups of four during the first four weeks and then one per cage for a further five–seven weeks. They were fed ad libitum a semisynthetic diet as detailed in Table 1 and as used before by Sugano et al. (3, 4) during the whole 9–11 weeks period. Dietary protein was either casein or soy protein isolate. Food consumption and weight gain were identical for the two groups. Body weights at the end of the feeding period were 334.0 ± 6.2 g (casein) and 328.8 ± 5.1 g (soy protein).

Experimental design

In the morning, 2 h before beginning the blood sampling, a silicon catheter was inserted into the vena jugularis of two to three animals under light ether anesthesia (21). Blood sampling was performed in the animal house between 1035 and 1445

Table 1. Diet composition.

Ingredient	g/100 g
Sucrose	69
Protein[1]	20
Corn oil	1
Cellulose	2
Vitamins[2]	2
Minerals[3]	6

[1] Protein source was either casein (acid-precipitated, Serolact P 65 with 15 % N of dry weight) from Bayerische Milchversorgungsbetriebe, Nürnberg, FRG, or soybean protein isolate (Purina No 610 with 14.8 % N of dry weight) from Ralston Purina, St. Louis, Mo., USA

[2, 3] Vitamin and mineral mix was prepared by Altromin GmbH, Lage, FRG. Vitamin mix was supplemented with 0.35 % methionine.

hours in non-fasted, unanesthetized animals with free access to food and water. Care was taken not to disturb the animals. Every 15 min a 0.2 ml heparinized plasma sample was drawn from the catheter and was centrifuged immediately. Plasma samples were first kept on ice and then stored at −70 °C. Erythrocytes were resuspended in saline and reinjected after the following blood withdrawal.

Prolactin and GH levels were determined in plasma samples using RIA tests kindly provided by the NIAMDD. From the first and last blood sample an aliquot

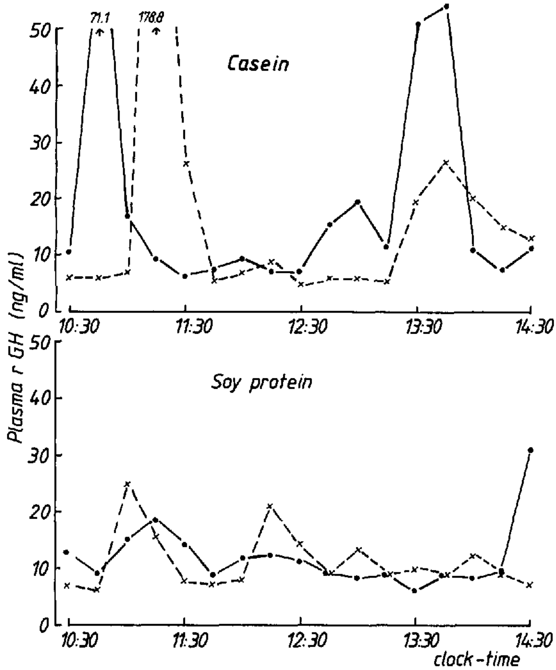

Fig. 1. Circadian rhythm of GH levels in casein-fed (● – ● No. 5, × – – × No. 9) and soy protein-fed rats (● – ● No. 6, × – – × No. 4) over 4 h.

was taken for hematocrit. Statistical calculations were done using Student's *t*-test. Means ± SEM are given throughout.

Results

Erythrocytes were reinjected during the blood sampling period in order to prevent anemia and hemodilution. It was, however, not possible to avoid blood loss completely. The hematocrit averaged 42.5 ± 0.7 % at the beginning and 38.3 ± 0.9 % in the end of the blood sampling period.

Prolactin levels were measured in order to evaluate potential stress of the animals. Values were below the 18.3 ± 0.3 ng/ml reported in another study (21). There was no significant difference between dietary groups with 9.75 ± 0.77 (range 7.13–15.11) for casein-fed and 8.00 ± 0.33 (range 5.95–14.9) ng/ml for soy protein-fed animals.

There was a different response of the rhythmic GH secretory pattern following dietary casein or soy protein. Figure 1 shows the GH pattern in two casein- and soy protein-fed rats. GH secretory peak values over 200 ng/ml have been reported for chow-fed rats (20). In the present study secretory peak values exceeding 100 ng/ml were observed in the casein group only. In a few cases, in animals of both groups, pulsatile GH secretion was hardly observed (data not shown).

Table 2. Mean GH levels over 4 h, GH maxima and integrated areas of GH levels plotted against time in casein- and soy protein-fed rats.

Dietary	group	GH Area	Mean GH	GH Maxima
		ng/ml × 4 h	ng/ml	ng/ml
Casein	1	101.1	6.2	10.6
	2	141.4	8.3	14.6
	3	397.3	24.5	72.2
	4	108.0	6.8	7.9
	5	317.1	19.3	71.7
	6	359.1	22.4	89.6
	7	328.7	19.9	201.4
	8	273.9	16.9	34.8
	9	**353.5**	**21.4**	**178.8**
	x̄	264.4[a]	16.2[b]	75.7
	SEM	± 38.7	± 2.4	± 23.8
Soy	1	140.2	8.5	22.3
	2	130.4	8.1	13.7
	3	168.9	10.5	13.8
	4	183.8	11.3	24.8
	5	204.4	13.2	26.1
	6	183.4	12.1	30.8
	7	130.6	8.1	9.7
	8	**130.8**	**13.2**	**58.2**
	x̄	159.0[a]	10.6[b]	28.3
	SEM	± 10.5	± 0.8	± 8.5

Mean values sharing common superscripts are significantly different at $p < 0.05$ (a, b).

Typically, the length of a secretory cycle, as marked by two sequential secretory peaks, amounted to 2 h in both groups. As, however, one of the two sequential secretory GH peaks was often much lower than the other, one might wonder whether they did really mark the beginning of a new cycle or whether they were just weak secretory pulses within a cycle lasting much longer than 2 h.

For statistical evaluation of protein-dependent differences of GH levels the integrated area under the GH levels over 4 h was calculated. Also the mean values and the maximum GH peak values of casein- and soy protein-fed animals were compared (Table 2). Mean GH values and GH areas of the time-concentration curve were significantly higher in casein-fed as compared to soy protein-fed rats. Due to the high variability of GH secretory episodes in both groups GH maxima were, however, not significantly different.

Discussion

As cholesterol levels are positively correlated with the risk of atherosclerosis and coronary heart disease, attention has been paid to the hypercholesterolemic effect of casein observed in some animal species. Hormonal status may be one of the relevant factors by which the protein effect is exerted.

Chow-fed rats show a rhythmic GH secretory pattern with periods of 3.3 h and with very high pulsatile GH secretory values (20). In our study the interval between secretory episodes was about 2 h and pulsatile GH secretory values were lower, particularly in the soy protein group.

The data reported herein show that time-dependent variation of GH levels is differently affected by dietary casein and soy protein and that values are higher in casein-fed animals. Higher GH levels have also been reported before for casein-fed as compared to gluten-fed rats (7). Thus our data support further the idea that different dietary proteins cause a different endocrine response.

The observation of higher GH levels in casein-fed rats is difficult to reconcile with the fact that, though hypercholesterolemia is associated with low GH levels (8–14), casein is under certain presuppositions hypercholesterolemic as compared to soy protein. However, only hypophysectomy but not GH deficiency lead to a pronounced hypercholesterolemia and the application of physiological doses of GH has only a limited effect on plasma cholesterol, if there is any at all. This may explain why differences of GH levels like those in our study are of no relevance for plasma cholesterol.

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