ラット肝脂肪酸合成系酵素の誘導に対する食餌たん白質の影響

EFFECTS OF DIETARY PROTEINS ON LIPOGENIC ENZYME INDUCTION IN RAT LIVER

入谷信子・永島貴美子・福田ひとみ・桂田昭彦（帝塚山学院短期大学）
田中武彦（大阪大学医学部）

Nobuko IRITANI[1], Kimiko NAGASHIMA[1], Hitomi FUKUDA[1], Akihiko KATSURADA[1] and Takehiko TANAKA[2]

[1]Tezukayama Gakuin College, Osaka 558

[2]Osaka University Medical School, Osaka 530

ABSTRACT

When fasted rats were fed fat-free/varied protein diets for 3 days, the activities of liver glucose-6-phosphate dehydrogenase, malic enzyme, acetyl-CoA carboxylase and fatty acid synthetase were markedly lower in rats fed soybean protein or gluten than in those fed casein or fish protein. The inductions of the lipogenic enzymes appear to be suppressed with plant proteins in comparison with animal proteins. As malic enzyme mRNA activity was not low in the soybean protein-fed animals, the translation of malic enzyme appears to be suppressed by dietary soybean protein. In the soybean protein group compared with the casein group, the incorporation of tritiated water into liver fatty acids after the injection was significantly lower. The triglyceride levels in plasma and especially in liver were also lower in the soybean and gluten groups than in the casein and fish groups. In addition, when dietary soybean protein was replaced with amino acids, the lowering effects on the lipogenic enzyme inductions were reduced but still partially found. Thus, some mechanisms ascribed to the protein itself as well as to the amino acid composition seem to cause these effects. *Nutr. Sci. Soy Protein, Jpn* **6,** 54-57, 1985.

大豆たん白質のコレステロール低下作用については多くの報告があるが脂肪の代謝に対する研究はあまり行われていない。大豆たん白質の血漿や肝トリグリセリド (TG)値に対する影響についてはいくつかの報告の中に見出せるが結果は一致していない。その原因は食餌中の油の影響が大きいように思われるので今回はラットに無脂肪食を投与して主として脂肪酸合成系酵素の誘導に対する食餌たん白質の影響を研究した。

実験方法

5週令雄ラットを2日絶食させた後，18% たん白の無脂肪食で3日 refed した。たん白としてカゼイン，魚（スケソウダラ），グルテン，大豆 (Fujipro-R) を用いたが，たん白含量はいずれも 90% 前後であった。急冷凍した肝より TG, citrate, acetyl-CoA を，肝 105,000×g 上清液より脂肪酸合成系酵素の活性を測定した[1~4]。また，Chirgwin ら[5]の方法で肝 RNA を抽出して前報のように malic enzyme mRNA 活性を測定

Table 1. Effects of dietary proteins on activities of lipogenic enzymes and malic enzyme mRNA in rat livers

Dietary protein	Glucose-6-phosphate dehydrogenase[1]	Malic enzyme[1]	Acetyl-CoA carboxylase[2]	Fatty acid synthetase[2]	Malic enzyme mRNA[3]
	nmol/min/mg				$\%\times10^{-2}$
Casein	337±59.7^a	262±24.4^a	14.4±1.17^a	9.28±1.11^a	4.28±0.82^a
Fish protein	362±41.3^a	287±14.8^a	16.3±1.98^a	11.3±1.22^a	4.17±0.47^a
Gluten	178±14.4^b	133±12.7^b	8.75±1.01^b	5.56±0.78^b	—
Soybean protein	192±14.2^b	144±13.6^b	9.34±0.72^b	6.40±0.81^b	4.13±0.16^a
Fasted	36.6±1.70^c	21.5±0.30^c	1.39±0.18^c	0.79±0.10^c	0.54±0.10^b

The animals were fasted for 2 days and then refed with fat-free diets containing different protein sources for 3 days. Enzyme activities in the 105,000 x g supernatant fraction of liver homogenate are shown as nmol substrate utilized[1] or product formed[2] per min per mg protein at 37°C. [3]Relative synthesis of malic enzyme to total protein. Mean±SD (n=6). Means in the same column with different superscript letters are significantly different at $p<0.001$.

した[6,7]。

結果と考察

Refed により体重が 10～20 g 増加したが，4 群の間で有意差はなかった。また体重 100 g あたりの肝重量はカゼイン，魚の群に比べてグルテン，大豆の群でやや小さかった。

脂肪酸合成系酵素の誘導と malic enzyme mRNA 活性

Table 1 に示したように glucose-6-phosphate dehydrogenase, malic enzyme, fatty acid synthetase の誘導はグルテン，大豆群でカゼイン，魚の群の約½にとどまった。また acetyl-CoA carboxylase も 60% にとどまった。なぜこのように植物たん白で酵素の誘導が低いのかは興味ある問題で今後研究する予定である。今回は malic enzyme について無細胞たん白合成系で mRNA からの翻訳に成功しているのでこの系を用いて malic enzyme mRNA 活性を測定した。しかし，その活性は各たん白食群で差がなかったので，malic enzyme の誘導において食餌たん白質は DNA からの転写ではなく，mRNA からの翻訳の段階で関与していることが示唆された。

〔^{3}H〕H_2O よりの脂肪酸合成

ラベルした H_2O をラットの腹腔内に注射した後肝脂肪酸へのとりこみをしらべたが，大豆群ではカゼイン群より有意に低かった (Table 2)。この結果は酵素活性のほか基質や effector レベルも含めた生理的な脂肪酸の合成を示していると考えられる。

血漿，肝 TG レベル

Table 3 に示したように血漿 TG はグルテン，大豆の群で，カゼイン，魚の群に比べて有意に低かったが，特に肝 TG は植物たん白群で動物たん白群のわずか⅓にとどまった。一方，脂肪酸合成の key enzyme である acetyl-CoA carboxylase の活性化因子である

Table 2. Effects of dietary proteins on incorporation of tritiated water into fatty acids in livers

Dietary protein	Incorporation of tritiated water into fatty acids	
	0900-1000 hr	2100-2200 hr
	cpm$\times10^{-3}$/hr/g liver	
Casein	3.52±0.93^a	3.20±0.50^a
Soybean protein	2.16±0.54^b	2.27±028^b
Fasted	0.28±0.02^c	

Rats were fasted for 2 days and refed with fat-free diets containing different protein sources for 3 days. Then the animals were intraperitoneally injected with 0.05 mCi tritiated water at 0900 hr or 2100 hr and killed 1 hr after the injection. Mean±SD (n=6). Means in the same column with different superscript letters are signficantly different at $p<0.05$ at least.

Table3. Effects of dietary proteins on concentrations of liver citrate, acetyl-CoA and triglycerides, and plasma triglycerides

Dietary protein	Liver			Plasma triglycerides
	Citrate	Acetyl-CoA	Triglycerides	
	nmol/g		mg/g	mg/ml
Casein	235±24.3[a]	49.3±3.28[a]	62.5±4.70[a]	1.28±0.11[a]
Fish protein	247±39.7[a]	51.3±2.31[a]	58.1±6.98[a]	1.32±0.16[a]
Gluten	150±15.5[b]	49.6±8.45[a]	21.2±3.81[b]	1.00±0.12[b]
Soybean protein	150±19.0[b]	49.0±3.28[a]	20.2±2.09[b]	0.83±0.04[b]
Fasted	67.5±15.3[c]	33.8±1.04[b]	6.55±2.13[a]	0.82±0.06[b]

The animals were fasted for 2 days and then refed fat-free diets containing different protein sources for 3 days. Mean±SD (n=6). Means in the same column with different superscript letters are significantly different at $p<0.001$ for liver citrate, acety-CoA and triglycerides; at $p<0.01$ for plasma triglycerides.

Table4. Effects of dietary amino acids simulating casein and soybean proteins on lipogenic enzyme activities, and triglyceride and citrate concentrations in rat livers

Regimen	Enzyme activities				Liver citrate	Liver triglycerides	Plasma triglycerides
	Glucose-6-phosphate dehydrogenase[1]	Malic enzyme[1]	Acetyl-CoA carboxylase[2]	Fatty acid synthetase[2]			
	nmol/min/mg				nmol/g	mg/g	mg/ml
Casein	375±21.6	321±31.3	13.4±2.65	7.80±0.79	238±50.5	64.2±12.4	1.31±0.13
Soybean	251±30.1[b]	218±7.85[b]	8.47±0.43[a]	5.00±0.50[b]	167±19.2[a]	30.2±11.1[a]	0.92±0.07[b]
Casein amino acids[3]	242±21.9[d]	163±12.9[d]	8.94±2.71	6.02±0.78[c]	100±21.6[d]	28.1±4.59[d]	1.05±0.01[d]
Soybean amino acids[3]	184±37.0[ac]	137±9.80[ad]	8.80±1.91	5.42±0.98	92.7±18.6[d]	20.3±6.65	1.05±0.11

Rats were fasted for 2 days and refed fat-free diets containing protein or amino acids for 3 days. Body weights were 125±2.53 and 123±3.31 g in the animals fed casein and soybean protein, respectively, and 121±3.52 and 120±4.21 g in those fed amino acid mixtures simulating casein and soybean, respectively. The food intake was 10.3±0.34 and 10.4±0.39 g in the protein groups, and 9.99±0.49 and 9.77±0.52 g in the amino acid groups, respectively. Enzyme activities in the supernatant fraction of liver homogenate are shown as nmol substrate utilized[1] or product formed[2] per min per mg protein at 37°C. Mean±SD (n=6). Significantly different from casein amino acids, a: $p<0.01$, b: $p<0.001$; from the corresponding protein, c: $p<0.01$, d: $p<0.001$.

citrate もまたグルテン，大豆の群で著明に低かった。

アミノ酸混合物による影響

大豆とカゼインをアミノ酸混合物でおきかえた時の結果を Table4 に示した。アミノ酸混合物でおきかえると酵素の誘導が全体に減少した。glucose-6-phosphate dehydrogenase と malic enzyme ではカゼインアミノ酸群に比べて大豆アミノ酸群で有意な酵素誘導の抑制がみられたが，たん白の効果よりはるかに小さかった。そして acetyl-CoA carboxylase と fatty acid synthetase では大豆アミノ酸による誘導の抑制は消失した。

以上の結果は，2週間飼育した時にも再現された。一方，糞中 N 消化率は大豆群でカゼイン群より 2% 高かったが N 吸収率が 90% 以上であるので大豆たん白の消化吸収率は問題にならないであろう。即ち，大豆たん白による脂肪酸合成系酵素誘導の抑制はアミノ酸組成にも幾分起因するもののたん白が消化されるまでの作用による方が大きいように思われる。

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