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The effect of ovariectomy of serum amino acids and cholesterol in the rat

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Summary. As steroid hormones are known to influence amino acid metabolism we tested the hypothesis that ovariectomy should lead to significant changes in this system.

We found that after ovariectomy serum alanine was significantly decreased ($p = 0.0006$) in contrast to serum glycine and branched chain amino acids (BCAA). The ratio of glycine/BCAA, a parameter for anabolism or catabolism was not changed after ovariectomy. If, however, the amino acid alanine as the link to carbohydrate and lipid metabolism was introduced the alanine/BCAA ratio was significantly altered ($p = 0.01$).

Although serum cholesterol was altered as well (increased, $p = 0.03$), no significant correlation with alanine was found. We can therefore assume that there are two independent mechanisms for lipid and amino acid changes after ovariectomy.

The most prominent finding was that estradiol replacement after ovariectomy restored increased cholesterol levels but did not restore alanine levels. Other ovarial hormones must be incriminated for the regulation of alanine metabolism. The anabolic effects of estradiol as decreasing glycine and BCAA were noticed which rules out insufficient estradiol replacement.

Keywords: Amino acids – Ovariectomy – Estradiol – Glycine – Alanine – BCAA

Introduction

The effect of ovariectomy as well as the effect of sexual steroid hormones on serum and plasma cholesterol has been reported by Seishima et al. (1991), Srivastava et al. (1992), Nozaki et al. (1993), Granfone et al. (1992), van der Mooren et al. (1992), Kameneva et al. (1992). As amino acids are not only gluconeogenetic but also lipogenic (Rosenthal et al., 1974; Scholz et al., 1977; Vernon et al., 1985; Brodin et al., Amino acids, accepted for publication), we

were interested in the evaluation of amino acid changes after ovariectomy on the one hand and the potential interactions and correlations with cholesterol metabolism on the other hand. Nothing is known to our present knowledge on the effect of ovariectomy on amino acid metabolism. In addition to serum amino acids we used amino acid ratios reflecting catabolism and anabolism (Arroyave, 1970; Lindblad et al., 1978; Holt et al., 1963), for the correlation with cholesterol.

Materials and methods

32 Wistar rats, white female, (Shaw's farm, UK), were used in the experiments. They were kept under a day night rhythm at room temperature and fed a standard rat cake (Altromin) ad libitum. 12 animals were sham operated, 9 animals were ovariectomized by the dorsal surgical approach and 11 rats were ovariectomized and supplemented with estradiol (17 beta-estradiol acetate, Sigma, E 7879) subcutaneously, 20 mg/kg body weight, 3 times per week. After a period of 8 weeks animals were sacrificed by diethylether treatment. Blood was drawn by cardiac puncture and spun down after clotting.

The animal weights at the start of the experiment did not differ significantly between the groups. At the end of the study period we measured 335g +/− 29, in the sham operated group, 381g +/− 54 in the ovariectomized group and 326g +/− 32 in the estradiol supplemented panel. The ovariectomized animals were significantly heavier than their estradiol replaced mates ($p = 0.02$).

Serum amino acid analysis was performed by a standard chromatographical technique.

A Beckman amino acid analyzer 7300/6300 was used. Buffers used were lithium citrate Beckman 338063, 338064, 338065. For ninhydrin detection Nin rx, Beckman 338069 was applied.

Serum parameters as cholesterol, triglycerides, total protein, albumin, creatinine, BUN, aspartate aminotransferase (AST) EC 2.6.1.2, alanine aminotransferase (ALT) EC2.6.1.2 were determined on a Kodak EKTACHEM Selective Autoanalyzer E700 XR.

Statistical determinations

Statistical analyses were performed following the principles of the SAS User's Guide (1985). Statistical significance was evaluated if p was less than 0.05.

Results

The results of our study are presented in Tables 1–3.

Discussion

As shown in the results and illustrated in a sketch in Fig. 1, significantly lower serum alanine was found after ovariectomy. This effect could not be reversed by estradiol replacement indicating the influence of either other ovarial hormones or intermediary metabolism. Glycine and branched chain amino acids (sum of valine, leucine, isoleucine) were unaffected by ovariectomy. Glycine and branched chain amino acids (BCAA) were significantly reduced by estradiol replacement after ovariectomy. Changes in the glycine/BCAA ratio would express protein catabolism, anabolism or reflect decreased or increased protein intake. As no effect was observed in the ovariectomized rats we may draw the conclusion that amino acid and protein metabolism per se remain unaffected by this procedure.

Table 1a. Sham operated rats
Tabular presentation showing means, standard deviation and range of parameters

	Mean	Std Dev	Range	UNITS
Glycine	225,5	44,6	177-313	µM/L
Alanine	607,5	84	472-756	µM/L
BCAA	608,4	84,8	422-726	µM/L
Gly/BCAA	0,34	0,1	0,06-0,46	
Ala/BCAA	1	0,1	0,88-1,18	
Cholesterol	86,1	14,29	65-108	mg/dL
Triglycerides	146,5	48,5	61-237	mg/dL
Total Protein	8,1	1,5	6,06-12,0	g/dL
Albumin	4,5	0,8	3,1-6,2	g/dL
AST	88	14,2	71,0-121,0	U/L
ALT	25	3,28	18,0-31,0	U/L
Creatinine	0,85	0,1	0,71-1,04	mg/dL
BUN	21,3	3,29	17,5-27,5	mg/dL
Body weight	334,5	29,4	305,0-383,0	g

Table 1b. Ovariectomized rats
Tabular presentation showing means, standard deviation and range of parameters

	Mean	Std Dev	Range	UNITS
Glycine	231,4	45,2	165,0-330,0	µM/L
Alanine	472,2	45,1	441,0-584,0	µM/L
BCAA	568,5	116,4	443,0-821,0	µM/L
Gly/BCAA	0,35	0,11	0,05-0,46	
Ala/BCAA	0,77	0,28	0,07-1,01	
Cholesterol	101,8	16,5	72,0-134,0	mg/dL
Triglycerides	159,4	67,6	70,0-249,0	mg/dL
Total Protein	7,31	0,59	6,7-8,5	g/dL
Albumin	4,03	0,43	3,6-4,9	g/dL
AST	101,2	40,4	58,0-174,0	U/L
ALT	22,2	6,9	14,0-38,0	U/L
Creatinine	9,73	26,7	0,68-81,0	mg/dL
BUN	19,4	1,46	16,9-21,4	mg/dL
Body weight	381	54,3	300,0-460,0	g

Table 1c. Ovariectomized rats with estradiol replacement
Tabular presentation showing means, standard deviation and range of parameters

	Mean	Std Dev	Range	UNITS
Glycine	169	29,6	130,0-221,0	µM/L
Alanine	451,8	114	273,0-633,0	µM/L
BCAA	460,4	91,4	318,0-605,0	µM/L
Gly/BCAA	0,37	0,06	0,3-0,5	
Ala/BCAA	0,98	0,2	0,72-1,44	
Cholesterol	85,5	14,9	62,0-110,0	mg/dL
Triglycerides	121,8	66,1	43,0-258,0	mg/dL
Total Protein	7,8	0,87	6,05-8,97	g/dL
Albumin	4,4	0,63	3,03-5,2	g/dL
AST	77,2	12,7	61,0-101,0	U/L
ALT	23,8	3,09	19,0-29,0	U/L
Creatinine	0,82	0,07	0,74-0,99	mg/dL
BUN	18,7	2,2	14,8-21,2	mg/dL
Body weight	325,6	32,13	264,0-367,0	g

Table 2. Tabular presentation of the results of Wilcoxon analysis comparing the groups

	(sham operated vs. ovariectomized rats)		(sham operated vs. ovariectomized rats&E2		(ovariectomized vs. ovariectomized rats&E2)	
	I - II		I -III		II -III	
	p=	t=	p=	t=	p=	t=
Bodyweight	0,07	1,76	0,54	0,6	0,02*	2,24
Glycine	0,59	0,53	0,005*	2,8	0,001*	3,15
Alanine	0,0006*	3,44	0,003*	2,92	0,87	0,15
BCAA	0,21	1,24	0,001*	3,1	0,04*	2,05
Glycine/BCAA	0,64	0,46	0,82	0,21	0,49	0,68
Alanine/BCAA	0,01*	2,38	0,53	0,58	0,08	1,74
Cholesterol	0,03*	2,09	0,96	0,04	0,13	1,48
Triglycerides	0,54	0,6	0,37	0,88	0,2	1,27
Total Protein	0,21	1,24	0,58	0,54	0,28	1,05
Albumin	0,16	1,38	0,39	0,84	0,39	0,84
AST	0,88	0,14	0,39	0,84	0,39	0,84
ALT	0,07	1,75	0,41	0,81	0,22	1,22
Creatinine	0,91	0,1	0,61	0,5	0,87	0,15
BUN	0,25	1,13	0,09	1,64	0,39	0,84

Probabilities lower than 0,05 are labeled with an asteriks

In order to rule out gluconeogenetic effects of ovariectomy on amino acid metabolism we paid attention to the key role of alanine by calculating the alanine/BCAA ratio (Felig et al., 1969). Alanine/BCAA was significantly decreased in ovariectomized rats, indicating the involvement of carbohydrate or lipid intermediary metabolism (Rosenthal et al., 1974). In congruence with observations of other authors we found elevated serum cholesterol after ovariectomy. There was, however, no correlation between cholesterol and the parameters of amino acid metabolism determined. This finding would mean that there is no association between amino acid metabolism and cholesterol metabolism and that ovariectomy affects both systems independently.

In the group of ovariectomy and estradiol replacement which was used to illustrate pharmacological effects of estradiol we found significantly lower levels of glycine, alanine and BCAA as compared to sham operated controls. An effect compatible with the anabolic activity of sexual steroid hormones. Neither the glycine/BCAA ratio nor the alanine/BCAA ratio was influenced by pharmacological doses of estradiol. We therefore learn from this experiment that estradiol although restoring cholesterol and phenylalanine-tyrosine levels (unpublished observation) did not restore alanine levels and the alanine/BCAA quotient, a parameter for gluconeogenesis. Alanine could form the link between amino acid, carbohydrate and lipid metabolism. Alanine and leucine are known to be lipogenic (Rosenthal et al., 1974). There was, however, no correlation between cholesterol and alanine in any of the groups examined. Insufficient estradiol replacement can be ruled out as cholesterol elevated after ovariectomy and the impaired phenylalanine tyrosine ratio (data not shown) was corrected by estradiol replacement.

Based upon these observations we conclude that ovariectomy affects cholesterol metabolism without any significant link to amino acid metabolism per se.

Table 3a. Sham operated rats
Tabular presentation of correlations presenting the correlation coefficients (*r*) and probabilities (*p*)

		Glycine	Alanine	BCAA	Gly/BCAA	Ala/BCAA	Cholesterol	Triglycerides	Total Protein	Albumin	GOT	GPT	Creatinine	BUN
Glycine	r	1	0,73	0,56	0,58	0,17	0,53	0,42	0,17	0,26	-0,47	0,33	0,25	0,24
	p	0	0,007 *	0,05	0,04 *	0,59	0,07	0,17	0,59	0,4	0,11	0,28	0,42	0,44
Alanine	r	0,73	1	0,76	0,48	0,24	0,54	0,53	0,41	0,47	-0,67	0,36	0,32	0,4
	p	0,007 *	0	0,003 *	0,1	0,43	0,06	0,07	0,18	0,12	0,01 *	0,24	0,29	0,19
BCAA	r	0,56	0,76	1	0,15	-0,42	0,49	0,42	0,64	0,75	-0,67	0,4	0,23	0,48
	p	0,054	0,003 *	0	0,63	0,16	0,1	0,17	0,02 *	0,004 *	0,015 *	0,18	0,46	0,1
Glyc/BCAA	r	0,58	0,48	0,15	1	0,51	0,45	0,52	-0,002	0,051	-0,55	0,57	0,41	0,18
	p	0,044 *	0,1	0,63	0	0,084	0,14	0,07	0,99	0,87	0,058	0,04 *	0,18	0,57
Ala/BCAA	r	0,17	0,24	-0,42	0,51	1	0,07	0,15	-0,36	-0,44	0,03	-0,02	0,16	-0,14
	p	0,59	0,43	0,16	0,08	0	0,82	0,63	0,23	0,14	0,9	0,9	0,59	0,65
Cholesterol	r	0,53	0,54	0,49	0,45	0,073	1	0,72	0,68	0,7	-0,34	0,57	0,59	0,68
	p	0,07	0,06	0,1	0,14	0,82	0	0,008 *	0,01 *	0,01*	0,27	0,05	0,04 *	0,01 *
Triglycerides	r	0,42	0,53	0,42	0,52	0,15	0,72	1	0,38	0,47	-0,3	0,6	0,1	0,32
	p	0,17	0,07	0,17	0,07	0,63	0,008 *	0 *	0,22	0,11	0,34	0,03 *	0,73	0,3
Total Protein	r	0,17	0,41	0,64	-0,002	-0,36	0,68	0,38	1	0,95	-0,48	0,36	0,62	0,66
	p	0,59	0,18	0,02 *	0,99	0,23	0,01 *	0,22	0	0,0001 *	0,1	0,24	0,02 *	0,01 *
Albumine	r	0,26	0,47	0,75	0,05	-0,44	0,7	0,47	0,95	1	-0,51	0,44	0,52	0,72
	p	0,4	0,12	0,004 *	0,87	0,14	0,01 *	0,11	0,001 *	0	0,08	0,14	0,07	0,007 *
AST	r	-0,47	-0,67	-0,67	-0,55	0,03	-0,34	-0,3	-0,48	-0,51	1	-0,48	-0,46	-0,26
	p	0,11	0,015 *	0,015 *	0,05	0,9	0,27	0,34	0,1	0,08	0	0,1	0,12	0,4
ALT	r	0,33	0,36	0,4	0,57	-0,021	0,57	0,6	0,36	0,44	-0,48	1	0,48	0,37
	p	0,28	0,24	0,18	0,04 *	0,94	0,05	0,03 *	0,24	0,14	0,1	0	0,1	0,22
Creatinine	r	0,25	0,32	0,23	0,41	0,16	0,59	0,1	0,62	0,52	-0,46	0,48	1	0,66
	p	0,42	0,29	0,46	0,18	0,59	0,04 *	0,73	0,02 *	0,07	0,12	0,1	0	0,01*
BUN	r	0,24	0,4	0,48	0,18	-0,14	0,68	0,32	0,66	0,72	-0,26	0,37	0,66	1
	p	0,44	0,19	0,1	0,57	0,65	0,013 *	0,3	0,01 *	0,007 *	0,4	0,22	0,01 *	0 *

Probabilities < 0,05 are labeled with an asteriks

Table 3b. Ovariectomized rats
Tabular presentation of correlations presenting the correlation coefficients (*r*) and probabilities (*p*)

		Glycine	Alanine	BCAA	Gly/BCAA	Ala/BCAA	Cholesterol	Triglycerides	Total Protein	Albumin	GOT	GPT	Creatinine	BUN
Glycine	r	1	0,8	0,78	-0,02	-0,22	0,12	-0,42	-0,05	-0,008	-0,14	0,27	-0,01	-0,09
	p	0	0,009 *	0,01 *	0,94	0,55	0,74	0,25	0,89	0,98	0,71	0,47	0,96	0,81
Alanine	r	0,8	1	0,85	0,25	-0,18	0,008	-0,41	0,1	0,02	-0,02	0,4	-0,17	-0,08
	p	0,009 *	0	0,003 *	0,5	0,63	0,98	0,26	0,78	0,95	0,95	0,27	0,65	0,81
BCAA	r	0,78	0,85	1	0,34	-0,5	-0,29	-0,58	-0,08	-0,11	-0,27	0,15	-0,02	-0,27
	p	0,01 *	0,003 *	0	0,36	0,16	0,43	0,09	0,82	0,76	0,48	0,69	0,94	0,46
Glyc/BCAA	r	-0,02	0,25	0,34	1	-0,18	-0,64	-0,53	0,55	0,42	0,46	-0,13	0,16	-0,51
	p	0,94	0,5	0,36	0	0,63	0,05	0,13	0,11	0,25	0,2	0,73	0,66	0,15
Ala/BCAA	r	-0,22	-0,18	-0,5	-0,18	1	0,04	0,61	0,36	0,38	0,3	0,16	0,04	0,66
	p	0,55	0,63	0,16	0,63	0	0,91	0,07	0,33	0,31	0,41	0,67	0,91	0,05
Cholesterol	r	0,12	-0,008	-0,29	-0,64	0,04	1	0,06	-0,19	-0,1	-0,06	0,02	-0,04	0,07
	p	0,74	0,98	0,43	0,05	0,91	0	0,86	0,6	0,79	0,86	0,95	0,91	0,85
Triglycerides	r	-0,24	-0,41	-0,58	-0,53	0,61	0,06	1	-0,33	-0,37	0,06	0,46	-0,4	0,61
	p	0,25	0,26	0,09	0,13	0,07	0,86	0	0,38	0,31	0,86	0,2	0,28	0,07
Total Protein	r	-0,05	0,1	-0,08	0,55	0,36	-0,19	-0,33	1	0,95	0,48	-0,17	0,21	0,08
	p	0,89	0,78	0,82	0,11	0,33	0,6	0,38	0	0,001 *	0,18	0,64	0,58	0,98
Albumine	r	-0,008	0,024	-0,11	0,42	0,38	-0,1	-0,37	0,95	1	0,31	-0,38	0,29	-0,01
	p	0,98	0,95	0,76	0,25	0,31	0,79	0,31	0,001 *	0	0,41	0,3	0,44	0,96
AST	r	-0,14	-0,02	-0,27	0,46	0,3	-0,06	0,06	0,48	0,31	1	0,41	0,15	0,008
	p	0,71	0,95	0,48	0,2	0,41	0,86	0,86	0,18	0,41	0	0,26	0,68	0,98
ALT	r	0,27	0,4	0,15	-0,13	0,16	0,02	0,46	-0,17	-0,38	0,41	1	-0,44	0,39
	p	0,47	0,27	0,69	0,73	0,67	0,95	0,2	0,64	0,3	0,26	0	0,23	0,29
Creatinine	r	-0,01	-0,17	-0,02	0,16	0,042	-0,04	-0,4	0,21	0,29	0,15	-0,44	1	0,13
	p	0,96	0,65	0,94	0,66	0,91	0,91	0,28	0,58	0,44	0,68	0,23	0	0,71
BUN	r	-0,09	-0,08	-0,27	-0,51	0,66	0,07	0,61	0,008	-0,01	0,008	0,39	0,13	1
	p	0,81	0,81	0,46	0,15	0,05	0,85	0,07	0,98	0,96	0,98	0,29	0,71	0

Probabilities < 0,05 are labeled with an asteriks

Table 3c. Ovariectomized rats with E2-replacement
Tabular presentation of correlations presenting the correlation coefficients (*r*) and probabilities (*p*)

		Glycine	Alanine	BCAA	Gly/BCAA	Ala/BCAA	Cholesterol	Triglycerides	Total Protein	Albumin	GOT	GPT	Creatinine	BUN
Glycine	r	1	0,84	0,71	0,19	0,35	-0,11	0,23	-0,0007	0,02	0,33	0,15	-0,05	0,3
	p	0	0,001 *	0,01 *	0,56	0,28	0,73	0,48	0,99	0,94	0,31	0,65	0,86	0,36
Alanine	r	0,84	1	0,72	0,02	0,57	0,2	0,4	0,23	0,23	0,29	-0,09	-0,03	0,44
	p	0,001 *	0	0,01 *	0,94	0,06	0,54	0,21	0,48	0,48	0,37	0,7	0,91	0,16
BCAA	r	0,71	0,72	1	-0,53	-0,14	-0,22	0,23	-0,01	0,03	0,13	-0,11	-0,23	0,31
	p	0,01 *	0,01 *	0	0,08	0,67	0,5	0,48	0,96	0,91	0,69	0,74	0,48	0,34
Glyc/BCAA	r	0,19	0,02	-0,53	1	0,66	0,21	-0,01	0,07	0,04	0,1	0,22	0,25	-0,02
	p	0,56	0,94	0,08	0	0,02 *	0,52	0,96	0,82	0,89	0,76	0,51	0,44	0,93
Ala/BCAA	r	0,35	0,57	-0,14	0,66	1	0,58	0,33	0,39	0,35	0,13	-0,07	0,23	0,34
	p	0,28	0,06	0,67	0,02 *	0	0,05	0,3	0,22	0,28	0,68	0,83	0,49	0,3
Cholesterol	r	-0,11	0,2	-0,22	0,21	0,58	1	0,45	0,9	0,84	0,26	0,05	0,39	0,51
	p	0,73	0,54	0,5	0,52	0,05	0	0,15	0,0001 *	0,001 *	0,42	0,86	0,22	0,1
Triglycerides	r	0,23	0,4	0,23	-0,01	0,33	0,45	1	0,43	0,41	0,17	-0,06	-0,01	0,6
	p	0,48	0,21	0,48	0,96	0,3	0,15	0	0,18	0,19	0,61	0,84	0,96	0,04 *
Total Protein	r	0,0007	0,23	-0,01	0,07	0,39	0,9	0,43	1	0,97	0,14	0,01 *	0,44	0,63
	p	0,99	0,48	0,96	0,82	0,22	0,0001 *	0,18	0	0,0001 *	0,67	0,95	0,17	0,03 *
Albumin	r	0,02	0,23	0,03	0,04	0,35	0,84	0,41	0,97	1	0,03	-0,02	0,41	0,69
	p	0,94	0,48	0,91	0,89	0,28	0,001 *	0,19	0,0001 *	0	0,91	0,94	0,2	0,01 *
AST	r	0,33	0,29	0,13	0,1	0,13	0,26	0,17	0,14	0,03	1	0,68	0,23	0,15
	p	0,31	0,37	0,69	0,76	0,68	0,42	0,61	0,67	0,91	0	0.019*	0,48	0,65
ALT	r	0,15	-0,09	-0,11	0,22	-0,07	0,05	-0,06	0,01	-0,02	0,68	1	0,58	0,23
	p	0,65	0,77	0,74	0,51	0,83	0,86	0,84	0,95	0,94	0,01 *	0	0,05	0,48
Creatinine	r	-0,05	-0,03	-0,23	0,25	0,23	0,39	-0,013	0,44	0,41	0,23	0,58	1	0,56
	p	0,86	0,91	0,48	0,44	0,49	0,22	0,96	0,17	0,2	0,48	0,05	0	0,06
BUN	r	0,3	0,44	0,31	-0,02	0,34	0,51	0,6	0,63	0,69	0,15	0,23	0,56	1
	p	0,36	0,16	0,34	0,93	0,3	0,1	0,04 *	0,03 *	0,01 *	0,65	0,48	0,06	0
Probabilities < 0,05 are labeled with an asteriks														

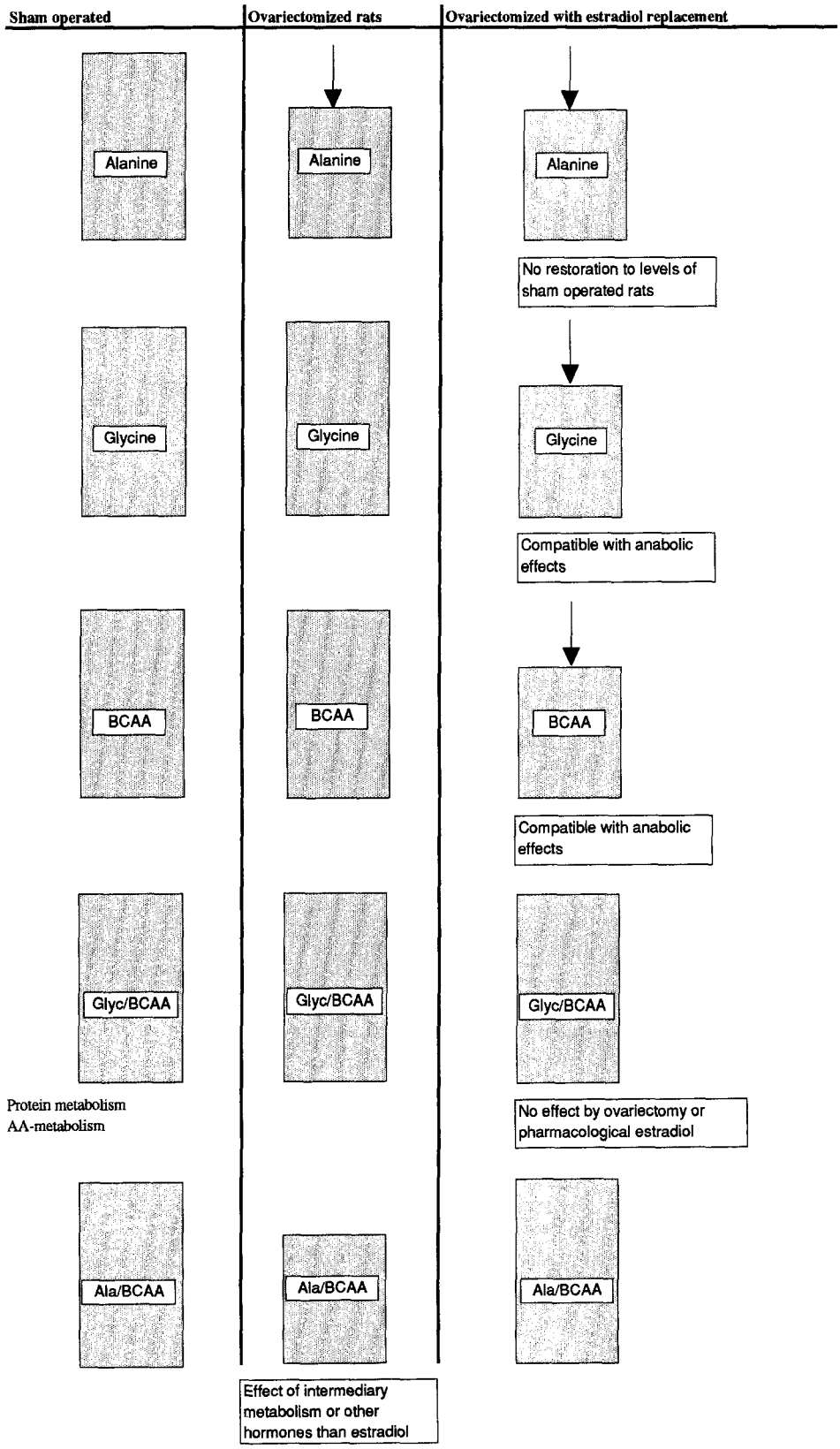

Fig. 1. Sketch diagram of our findings

Alanine would have been the candidate as an excellent link but failed to correlate with cholesterol.

We also realize that alanine is decreased by ovariectomy but not restored (in contrast to cholesterol and phenylalanine tyrosine ratio) by pharmacological substitution with estradiol, indicating that other hormones must be involved.

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