77

BARD

FINAL REPORT

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**An *in vitro* and *in vivo* Investigation of the Diverse
Biological Activities of Bovine Placental Lactogen**

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An in vitro and in vivo investigation of the diverse biological activities of
bovine placental lactogen

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Table of Contents

Cover Page.....	i
Table of Contents.....	ii
Abstract.....	1
Introduction and General Objectives.....	2
Hebrew University of Jerusalem.....	2
University of Missouri (Rodent Studies).....	12
University of Missouri (Rodent Studies) Figures.....	38
University of Missouri (Bovine Studies).....	77
University of Missouri (Bovine Studies) Figures.....	86
Description of Cooperation.....	111
Evaluation of Research Achievements and Benefits to Agriculture.....	111
Publications.....	112
Abstracts.....	112
Departmental Reports.....	113
Thesis Extract.....	114

ABSTRACT

In order to understand the structure-function relationship of bovine placental lactogen (bPL) and initiate production of material for *in vivo* testing, 28 different bPL analogues were prepared by either truncation or site-directed mutagenesis. The effect of these mutations was determined by measuring binding capacity, ability to homodimerize extracellular domains (ECDs) of several lactogenic and somatogenic receptors, and by *in vitro* bioassays. Two analogues were prepared in large amounts for *in vivo* studies. These studies (a) identified the residues responsible for the somatogenic activity of bPL (K73, G133, T188) and for both lactogenic and somatogenic activity (N-terminus, K185, Y190); (b) allowed preparation of bPL analogues with selectively abolished or reduced somatogenic activity; and (c) provided a tool to understand the kinetic difference between lactogenic and somatogenic receptors. *In vivo* studies using rodent and dairy models showed that bovine growth hormone (bGH) is superior to bPL in stimulating growth and lactation. Likewise, bGH has greater somatogenic activity in different age groups and thermal environments. Initial studies of bPL analog T188 suggest that its lactogenic potential is superior to bGH. Effective experimental models have now been developed and tested for analysis of new bPL analogs.

FINAL SCIENTIFIC REPORT

AN *IN VITRO* AND *IN VIVO* INVESTIGATION OF THE DIVERSE BIOLOGICAL ACTIVITIES OF BOVINE PLACENTAL LACTOGEN

(BARD Project No. US-2109-92R)

I INTRODUCTION AND GENERAL OBJECTIVES

The primary objectives of this study, as stated in the original proposal, were 1) to elucidate the biological activities of bovine placental lactogen (bPL) using both *in vitro* bioassays and *in vivo* studies, and 2) investigate the structure-function relationship of the molecule to determine which domains may be responsible for the diverse activities mediated through these different receptor types. The structure-function studies were to allow for production of bPL variants with more specific actions that target particular biological functions. At the same time, it was necessary to identify the physiological actions of bPL and its analogs, and determine how these actions overlap with bovine growth hormone (bGH) and bovine prolactin (bPRL) using *in vivo* comparison studies.

Research during the first year of this study, as defined in the proposal, was conducted entirely by Dr. Arie Gertler and his colleagues at the Hebrew University of Jerusalem. Studies in the University of Missouri laboratory began during the second year and continued through the final year of the project.

The accomplishments of both laboratories and the evaluation of the specific objectives are described in detail in Sections II (Hebrew University of Jerusalem) and III and IV (University of Missouri).

II. HEBREW UNIVERSITY OF JERUSALEM

Results of research (Rehovot)

Final Report of the Israeli Lab (Prof. Arie Gertler)

a. Specific Objectives

The main objective of this study which was planned to be carried-out in the Israeli lab was to investigate the structure-function relationship of the bPL molecule to determine which domains may be responsible for the diverse activities mediated through three different receptor types. This investigation was envisioned to let us prepare novel bPL variants with more specific actions that can be targeted at a particular biological activity.

Specific objectives of the Israeli lab included:

- (a) Identification of the amino acids in the N-terminal region of bPL molecule, that affect the ability of the hormone to bind and to exert biological activity to bPL, somatogenic and lactogenic receptors.
- (b) Elucidation of the role of the C-terminal domain in determining the ability of bPL to bind and activate the distinct receptors.
- (c) Elucidation of whether the somatogenic, lactogenic and specific PL activities of bPL are located in the same or different areas of the molecule.
- (d) from the endometrium and liver of pregnant cow and from fetal liver.
- (e) Development of an in vitro endometrium culture in which the effect of bPL will be tested.
- (f) Production of sufficient amount of selected recombinant bPL analogues to conduct in vivo studies.

b. Preparation and characterization of bPL analogues (objectives a - c)

Within the frame of this project 28 different bPL analogues were prepared. The target for mutations were chosen mainly on the base of the information that origins from the structure-function studies of hGH according to the rational detailed in the original proposal and on the experience acquired in the course of this study. Mutational (1-5) and structural (6,7) studies of hGH's interaction with soluble hGHR-ECD and hPRLR-ECD raised a paradigm of hormone-dependent sequential dimerization of the receptor as an initial event subsequent to the binding. These studies also allowed identification of sites I and II in hGH, which sequentially interact with two receptors. As parallel information for other somatogenic and lactogenic hormones and in particular for bPL does not yet exist, only an indirect comparison can be made. For this purpose an aligned sequence of several related hormones have been presented in Table I and the relevant regions in hGH have been outlined. This comparison has to be treated with caution since the alignment may be erroneous. Two interesting features may be however emphasized: (a) out of over 30 amino acids involved in the interaction of hGH's site I with the receptor, 8 residues contribute ~85% of total binding energy, 6 residues contribute ~15% and others have nil contribution only (2); (b) interaction of hGH's site I with hPRLR-ECD indicates that vast majority of amino acids involved in this interaction is also involved in a corresponding interaction with hGHR-ECD (6,7). Relevant information concerning site II is not yet available as hGH formed only 1:1 stable complex with hPRLR-ECD (7). The numbering in Table 1 is based on that of hGH whereas the bPL analogues prepared within the frame of this project are numbered according to bPL sequence. Therefore in order to avoid confusion the comparative information is given in the legend to Table I.

The main results of the effect of these mutations on the binding capacity, ability to

homodimerize the hGHR-ECD and on the in vitro bioactivity of the analogues is summarized in Table II. More detailed analysis is given in the four enclosed publications and in the manuscript which summarizes the most recent results of the bPL(G133K) and bPL(G133R). In the course of this we have adapted a novel more accurate in vitro bioassay which is based on proliferation of PDC-P1 myeloid cells stable-transfected with either human or rabbit GH receptor, which replaced the former bioassay based on measuring the somatogenic receptor-mediated differentiation of 3T3-F442A preadipocytes.

Table 1. Amino acid sequence of several somatogenic and lactogenic hormones

Helix 1									
	1		*	*	*		**	**47	
hGH	FPTiPLs	RlFdnAMlra	HrLHQLAFDT	YQEFEEAYIP	KEQKYSFLQ				
bGH	AFPAMSLS	GLFANAVLRA	QHLHQLAADT	FKEFERTYIP	EGQRYs-IQN				
bPL	AEDYAPYCKN	QPGNCRIPLG	SLFERATLVA	SNNYRLAREM	FNEFNKQFGE	GKNTFS----			
bPRL	TPVCPN	GPGNCQVSLR	DLFDRAVMVS	HYIHDLSSEM	FNEFDKRYAQ	GKGFITT---			
Helix 2									
	48*		****					107	
hGH	PQTSLCFSES	IPTPSNREET	QOKSNLELLR	ISLLLIQSWL	EPVQFLRSVF	ANSLvyGAsD			
bGH	TQVAFCFSET	IPAPTgKNEA	QOKSDLELLR	ISLLLIQSWL	GPLQFLSRVF	TNSLVFGTSD			
bPL	KVINSCHTEF	MTTPNNKEAA	ANTEDEALLR	LVISLLHSWD	EPLHQAVTEL	LHR--NGASP			
bPRL	MALNSCHTSS	LTPPEDKEQA	QQTHHEVLMS	LILGLLRSWN	DPLYHLVTEV	RGM--KGAPD			
Helix 3									
	108							160	
hGH	SNvYDlLKdL	EegIQtLMGR	LEDGSPRT--	GQIFKQTYSK	FDTNS--HND	D---ALLKNY			
bGH	R-VYEKLKDL	EEGILALMRE	LEDGTPRA--	GQILKQTYDK	FDTNM--RSD	D---ALLKNY			
bPL	D-ILARAKEI	EDKTKVLLEG	VEMIQKRVHP	GEKKNEPYPV	WSEKSSLTAD	DEDVRQTAFY			
bPRL	A-ILSRAIEI	EEENKRLLEG	MEMIFGQVIP	GAKETEPYPV	WSGLPSLQTK	DEDARTSAFY			
Helix 4									
	161	*	*	*	*	*	*	*191	
hGH	GLLYCFRKDM	DKVETFLRIV	QCRS-VEGSC	GF					
bGH	GLLSCFRKDL	HKTETYLRVM	KCRRFGEASC	AF					
bPL	RMFCHLRDS	SKISTYINLL	KCR---FTPC	--					
bPRL	NLLHCLRRDS	SKIDTYLKLL	NCRIIYNMNC	--					

Residues of hGH that interact with the hGHR-ECD and are buried to varying degrees at the site I (capital letters) and site II (small letters) are marked in bold. Out of 32 residues contributing to site I, eight residues that contribute over 85% of the binding energy are double underlined, the six residues that contribute less than 15% of the binding energy are marked in bold only, whereas all other residues are marked with capital bold italics (2, 6). Residues that have been identified as buried to varying degrees in the interaction of hGH (site I) with hPRLR-ECD are marked with * (7). Residues of bPL that are marked bold letters were targets for mutagenesis. These include according to bPL numbering: truncated analogues des-13 to des 27 (hGH numbering -13 to +14), mutations in G20, T27 and L28 (hGH numbering 7, 14, 15), K73 and G133 (hGH numbering 64 and 127), mutations in R168, H180, R181, S184, K185, S187, T188, Y199 and I190 (hGH numbering 155, 167, 168, 171, 172, 174, 175, 176 and 177).

Table 2. Bovine PL analogues - comparison of binding, dimerization and *in vitro* activity (48,49,50)

Analogue (bPL numbering)	Binding assays:				Ability to form dimers with hGHR-ECD (SR)	<i>In vitro</i> bioassays ^{a,b} :		
	IC ₅₀ bPLH/IC ₅₀ analogue in:					Relative activity (bPL=100)		
	hGHR -ECD (SR)	IM19 cells (SR)	bPRLR ECD (LR)	Nb ₂ cells (LR)		3T3-F442A preadipocytes or PDC cells ^c (SR)	Nb ₂ cells (LR)	HC11-TS cells (LR)
bPL	1.00	1.00	1.00	1.00	Very good	100	100	100
des-13	1.00	1.00	0	0.11	Partial	202	40	NT
des-13(L28F)	1.00	1.00	0	0.11	Very good	211	45	NT
des-17	0.13	0.29	0	0.02	Partial	23	7	NT
des-17(L28F)	1.00	0.29	0	0.02	Very good	13	16	NT
des-20	0.06	0.08	0	0	None	2	1	NT
des-27	0	0	0	0	None	0	0	NT
G20F	NT	NT	NT	NT	NT	100°	67	83
T27F/L28F	NT	NT	NT	NT	NT	200°	100	110
G20F/T27F/ L28F	NT	NT	NT	NT	NT	110°	63	80
K73A	0.04	0.15	0.58	NT	Very good	3°	81	106
K73D	0	0	0.23	NT	Partial	0°	125	77
K73F	0.06	0.15	0.20	NT	Very good	4°	156	77
G133K	0.01	0.01	1.00	1.05	Very good ^d	0°	155	NT
GT33R	0.01	0.01	1.00	1.05	Very good ^d	0°	155	NT
H180A	0.52	0.77	0.15	NT	NT	65°	76	NT
H180F	0.22	0.25	0.06	NT	NT	25°	59	NT
R181A	0.15	0.15	0.04	NT	NT	38°	87	NT
R181F	0.26	0.20	0.04	NT	NT	32°	41	NT
R181F/K185E	0	0.01	0.01	NT	NT	0°	0	NT
S184H	0.01	0.01	0.65	1.02	Very good	110	52	32
S187A	1.00	0.80	0.80	1.19	Very good	120	160	121
S184F	1.00	0.65	0.65	1.50	Very good	80	160	93
T188F	0.01	0.01	1.00	1.05	None	8	155	38
T188D	0	NT	1.00	NT	NT	0.3°	200	125
T188W	0	NT	1.00	NT	NT	0.7°	170	159
Y189F	0.30	NT	1.00	NT	NT	53	59	125
T188F/190F	0	0	0.01	0.06	No binding	1	6	4
T188F/Y189F/ R168H	0	NT	0.01	NT	NT	1	33	42

^a the biological activity in FDCP1-9D11 and in Nb₂-11C was determined as hGH-dependent proliferation and in HC11-TS cells as hGH-dependent β -casein synthesis. The FDCP1-9D11 cells were transfected with hGHRs and HC11-TS cells by rbPRLRs.

^b determined as differentiation of 3T3-F442A preadipocytes

^c determined by proliferation of FDCP1-9D11 cells (see above).

^d forms only 1:1 complex and are unable to homodimerize the receptor.

^e acts as antagonist in both FDCP1-9D11 or FDCP1-9B cells.

NT, not tested; S, somatogenic receptors; LR, lactogenic receptors.

The present results (see details in the enclosed publications and the highlights in Table 2) show that the like in hGH the amino acid residues located at the C-terminal part of the molecule, namely in the fourth (-helix, (K185 and T188) and the residue K73 located in the intervening sequence between the first and the second (-helix are extremely important for the somatogenic activity of bPL. The residue K185 and I190 are also important for the lactogenic activity of bPL and the residues located in the N-terminal part of the hormone (the first (-helix) are critical for both activities. Some of these results are quite surprising since the results of binding and gel-filtration experiments which show (particularly in that the analogues modified at K73 and G133) exhibited remarkable decrease in their ability to form a homodimeric complex with both somatogenic and even more with lactogenic receptors. We could thus anticipate corresponding losses in biological activity mediated through both type of receptors. However we have clearly shown that in spite of the reduced affinity of analogues site 2 toward both somatogenic and lactogenic receptors, dramatic reduction in biological activity and even conversion of the agonist to antagonist was observed in events mediated through somatogenic receptors only. In contrast in events mediated through lactogenic receptors, in which the effect of mutation on the binding to lactogenic receptors or their ability to homodimerize the PRLR-ECDs was strongly impaired, the lactogenic receptors-mediated biological activity was barely or not at all affected. This happened particularly in bPL(G133R) and bPL(G133K) even the interaction of both analogues with rPRLR-ECD was much more affected than the interaction with rbPRLR-ECD or hGHR-ECD, indicating that in the former case the interaction with site 1 was also reduced as also shown in the binding experiments. Furthermore these two analogues became antagonists toward somatogenic receptors.

Although the three-dimensional structure of bPL is not known, we have recently found that the related hormone oPL has 2 binding sites and forms a 1:2 complex with rPRLR-ECD (unpublished data) suggesting that a similar complex may be also formed with bPL. Structural analysis of the complex suggests that G133 is located close to W112 (Devos unpublished data) which is homologous to W104 in hGHR and is conserved in all GH and PRL receptors. Replacement of G by a large, positively charged side chain interferes with formation a homodimeric complex and in the case of hGH (8) or bGH (9) this mutation led to appearance of antagonistic activity. We could therefore expect that similar mutation in bPL, namely G133K/R would lead to an appearance of antagonistic activity toward lactogenic receptors as well. As shown above at least in the two tested models (rabbit acini culture and Nb2 lymphoma cells) this did not happen. One possible explanation would be that dimerization of lactogenic receptors is not obligatory for initiation of transduced signal. Such suggestion seems extremely unlikely in view of both direct and indirect evidence which shows the opposite.

An alternative explanation may be however proposed considering our recent suggestion that transient dimerization of PRLRs, lasting a few seconds or less, is sufficient to elicit full biological response (10). This suggestion is supported by the finding that after the homodimeric complex is formed, receptor-associated JAK2 or other kinases are instantly activated by mutual transphosphorylation, forming docking sites for other downstream proteins (11,12). Once this occurs, the receptor dimers are no longer needed. If this hypothesis is true for biological events induced through both

lactogenic and somatogenic receptors, our present experiments indicate that the minimal duration of the homodimeric complex required for the initiation of the biological signal mediated by lactogenic is much shorter than for somatogenic receptors. A possible reason for this difference is the recent finding that JAK2 kinase which serves as a mediator of both receptors is already preassociated with lactogenic receptors prior to hormone binding-induced receptor dimerization, whereas in somatogenic receptors the JAK2 - receptor association occurs subsequently to receptor dimerization

c. Purification and characterization of bPL receptors (objective d)

Competitive binding experiments using iodinated bPL, bGH and hGH as a ligands (Fig 1) show that the ability of uniodinated bGH and hGH to compete with 125I-bPL is only 1% as compared to bPL in membranes prepared from both pregnant cow and young calfs livers. In contrast when 125I-bGH or hGH were used as a ligands all three hormones displayed similar competitive ability. This would indicate that bPL is capable to bind to homologous somatogenic receptors but specific receptors for bPL exist as well. In order to identify the molecular mass a cross-linking experiments were carried out. SDS-PAGE electrophoresis carried out on both reducing and non reducing conditions. Under reducing conditions two specific bands with respective molecular mass 127 kDa and 74 kDa were identified (Fig 2). Excess of bGH but not oPRL could compete with the ligand but obviously the affinity for bPL was remarkable higher than for bGH (Fig 3). Two interpretations of these data may be suggested: (a) the bPL receptor has a molecular mass of ~104 kDa and the 74 kDa band is a covalent complex of bPL (23 kDa) and partially proteolized receptor (51 kDa) or (2) bPL homodimerize a receptor of molecular mass of 51-52 kDa. At present we can not decide which hypothesis is true since further experiments to partially purify the receptor (not shown) were inconclusive, despite many efforts.

d. Development of an in vitro endometrium culture (objective e)

Large number of experiments were carried out to established bovine endometrial primary culture of both epithelial and fibroblast cells. Separation of the two cell types as achieved and short term (up to 10 days) cultures were established. Binding experiments have indicated that both cell lines have specific bPL receptors with binding characteristics similar to that shown in liver (see above) no specific bPL effect on neither protein synthesis (determined by incorporation of labeled methionine) or progesterone synthesis (determined by RIA) was found. Therefore the goal of establishing specific bioassay for bPL was not achieved.

e. Production of sufficient amount of selected recombinant bPL analogues (objective f)

Two bPL analogues (bPL-T188F and bPL T188D) were prepared in 1 to 1.5 g and sent to the US collaborator (for details of their use see the the report of the US collaborator).

f. References

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g. Legends to Figures

1. Competition of unlabeled bPL (full circles), hGH (empty circles), bGH (full triangles) and oPRL (empty triangles) with labeled bPL, hGH and bGH for binding to membranes prepared from the liver of pregnant cows or young calves. The experiment was carried-out in 250 for 16 h. The specific binding (B_0) for iodinated bPL, hGH and bGH in the membranes prepared from the pregnant cow liver was respectively 40, 27 and 5.5%. The corresponding values for the membranes prepared from the liver of young calves were 38, 20 and 5.5%.
2. Affinity labeling of bPL receptor in the membranes prepared from the liver of pregnant cows. The labelling by ^{125}I -bPL was performed in absence or presence of excess of non-labeled bPL, bGH or oPRL. The separation was carried-out on SDS-PAGE (7.5%) gel in presence (+) or absence (-) of reducing agent (DDT). Molecular mass markers are shown on the left side of the figure.
3. Affinity labeling of bPL receptor in the membranes prepared from the liver of pregnant cows. The labelling by ^{125}I -bPL was performed in absence or presence of different concentrations of non-labeled bPL (A) or bGH (B). The separation was carried-out on SDS-PAGE (7.5%) gel in presence of reducing agent (DDT). Molecular mass markers are shown on the left side of the figure.

Fig 1

Fig 2

Fig 3

III. UNIVERSITY OF MISSOURI (Rodent Studies)

Results of research (Univ. of Missouri)

Final Report of the Missouri Lab (Assoc. Prof. Donald Spiers)

a. Specific Objectives

- a) Determination of acute dose-related effects of bGH, bPRL, bPL and PL analogs on thermoregulatory ability of female rats at different stages of growth.
- b) Identification of dose-related changes in growth rate, food consumption, and body temperature regulation of female rats following repeated injections of bGH, bPRL, bPL, and PL analogs at different ages.

The original proposal submitted to BARD stated that rats would only be tested under thermoneutral conditions. We decided in the first year of our study to use both thermoneutral and heat-challenge conditions, in order to increase the scope of the database used for analysis. Although this decision resulted in a doubling of our effort, it was worthwhile because it allowed us to isolate ambient temperature differences in the response to the growth promotants. At the same time, it was decided to reduce the number of test doses from 1, 3, 8, and 24 mg/kg BW to 10 and 20 mg/kg BW, in order to allow time for the additional temperature measurements.

A number of other tests were added to the study that were not a part of the original proposal. All rats were tested at the end of a treatment period to determine thermoregulatory responses to both thermoneutral and heat stress ambient temperatures. Originally, they were only to be tested at thermoneutrality. Once again, this allowed us to determine if responses to these ambient temperatures had been altered as a result of chronic treatment with growth promotants. Additional tests added to this portion of the study included determinations of body composition (i.e., fat free mass, total body water, selected organ weights).

Tests of PL analogs were originally scheduled to be the same as tests using the other growth promotants. There are several reasons why this did not take place. It was learned early in the third year that the analogs were designed to be lactogenic with very little somatogenic potential. This was a concern, since none of our earlier tests were designed to evaluate lactogenic potential. Therefore, we designed new dam/pup tests, described later in this report, to make this determination. Because we were unable to receive adequate quantities of analogs for both somatogenic and lactogenic tests, we decided to concentrate on the lactogenic studies. Tests were also conducted of bGH ability to stimulate lactogenesis, to serve as a comparison with the analogs.

b. Introduction

Repeat-injection of adult mammals with growth hormone often results in

increases in somatogenesis. Treatment of lactating dairy cows with bovine growth hormone (bGH) during heat challenge can result in increases in production rates of milk and metabolic heat (Manalu et al., 1991; West, 1994), without necessarily producing a shift in whole-body thermal balance (Johnson et al., 1991). Adult female rats maintained at thermoneutrality and administered bGH at 24 mg/kg body weight for 10 days exhibit elevations in both food intake and growth rates above control level (Byatt et al., 1993). There has been no acute comparison study of the effect of growth promotants, such as bGH, bPL, or bPRL, on thermoregulatory ability. Likewise, there are no chronic comparison studies of these compounds administered during a critical period of growth in the rat maintained in different thermal environments. Such comparisons of physical and physiological responses to these promotants allow for the determination of improved performance and/or identify any reduction in maintenance of homeostasis below control level that could occur under stress conditions.

c. Acute experiment of growth promotant activity

c.1. Design

Female Sprague-Dawley rats, either 50 or 100 days of age, were randomly assigned to treatment groups receiving either bGH (n=33), bPL (n=32) or bPRL (n=33) and tested at either thermoneutral or hot ambient temperatures (approx. eight rats/group). Each rat was conditioned for one week to partial restraint in Plexiglas stocks and used in only one test. Rats were housed at 21°C, with lights on from 0700 to 1900 daily and provided ad libitum access to chow and water between conditioning and test sessions.

Each rat was weighed to .1g before testing. Rat set-up began with insertion of a sterilized polyethylene cannula (PE 10, .28 mm i.d., .61 mm o.d., Becton Dickinson, Rutherford, NJ) into the peritoneal cavity using a 19-gauge syringe needle inserted into the abdominal wall as a slide tube. After cannula insertion into the cavity, the needle was withdrawn and the cannula attached to the body wall using cyanoacrylate glue. Thermocouple wires (40-gauge copper-constantan), with a reference junction in a melting ice bath, were inserted 20 to 30 mm beyond the anal sphincter to measure rectal temperature (T_{re}) and attached to the mid-dorsal surface of the tail base to measure tail-skin temperature (T_{tail}). A thin film of flexible collodion was used to attach thermocouples to the skin. All tests were conducted using a water-jacketed Plexiglas metabolic chamber, with horizontal cylinders for individual animal tests. Cylinder wall and air temperatures were controlled independently of each other throughout each test. These temperatures were averaged to derive an estimate of ambient test temperature. Ambient temperature for thermoneutral tests was maintained at 21°C for the test duration. Heat exposure tests were performed at 31°C.

Chamber ventilation was by an open air flow system. Dried room air was pumped through the cylinder at a controlled rate of 560 mL/min. Oxygen content of influent and effluent air was measured with a calibrated paramagnetic oxygen analyzer (Model 755, Rosemount Analytical, Eden Prairie, MN). All oxygen content values were adjusted to

STPD conditions and used to estimate metabolic heat production (MR) assuming a respiratory quotient of .83. Rate of heat production was expressed relative to whole body weight (W/kg). Each rat was allowed a 1-h equilibration to test conditions before injection of either bGH or bPL (20 mg /kg body weight) or bPRL (2mg/kg body weight). No rat was tested before body temperatures or MR had reached resting value. All measurements were recorded 5 min before injection and for 120 min after injection using a computerized data acquisition system (Acro Systems 900, Analogic Corp., Peabody, MA).

c.2. Acute experimental results

bGH Results:

Acute exposure of rats at 50 days of age to 21 and 31°C (Figure 1) did not result in a significant change in T_{re} ($P < .39$). A 6.2°C increase in T_{tail} occurred at 31°C from the 21°C level ($P < .0001$). Likewise, a 1.34 W/kg decrease in MR ($P < .0093$) occurred from 21 to 31°C. These results were expected since the animals were actively thermoregulating to maintain a constant T_{re} . An increase in MR would occur with exposure to a cooler T_a . Injection of 20 mg BST/kg bw at either T_a resulted in no significant change in T_{re} , T_{tail} , or MR.

Similar results were noted for the 100 day-old rats (Figure 2). There were no significant differences in T_{re} at 21 and 31°C prior to injection ($P < .80$), with a 5.6°C increase in T_{tail} ($P < .0001$) and a 2.55 W/kg decrease in MR ($P < .0001$). Once again, there were no significant effects of bGH injection on T_{re} , T_{tail} , or MR.

bPL Results:

Acute exposure of 50 day-old rats to 21 and 31°C had no effect on T_{re} ($P < .59$), with a 5.73°C increase ($P < .0001$) in T_{tail} and a 2.1 W/kg decrease ($P < .0013$) in MR occurring from 21 to 31°C (Figure 3). Injection of bPL at 21°C resulted in a significant decrease ($P < .05$) in T_{re} below the starting level, at 10 to 70 minutes postinjection, with no accompanying changes in T_{tail} or MR to indicate shifts in heat loss or heat production, respectively. Peak decrease in T_{re} was 0.38°C at 30 minutes postinjection. A significant increase ($P < .05$) in MR above starting level occurred at 70 minutes postinjection. This was likely a compensatory response to the hypothermia, and an attempt to return body heat content to normal level. There was no acute effect of bPL on the 50 day-old rat at 31°C.

Initial exposure of 100 day-old rats to 21 and 31°C had no effect on T_{re} ($P < .13$), but increased T_{tail} by 6.1°C ($P < .0001$) and decreased MR by 2.7 W/kg ($P < .0001$) at 31°C (Figure 4). As at 50 days of age, there was no effect of bPL on thermoregulatory ability at 31°C. An even greater hypothermic effect of bPL occurred in the 100 day-old rat

(Figure 4). A 0.6°C reduction in T_{re} at 30 minutes postinjection ($P < .05$) was accompanied by a reduction in T_{tail} ($P < .05$) and MR ($P < .05$) over the same period. The 1.7 W/kg reduction in MR preceded the decrease in T_{re} , and was the most likely explanation for the experienced hypothermia.

bPRL Results:

Exposure of 50 day-old rats to 21 and 31°C prior to injection of bPRL resulted in an increase of 0.95°C ($P < .0001$) in T_{re} , a 6.71°C increase in T_{tail} ($P < .0001$), and a 1.4 W/kg decrease in MR ($P < .05$) at 31°C (Figure 5). As noted previously for bGH and bPL, there was no bPRL effect at 31°C . The significant effect ($P < .05$) of bPRL on the 50 day-old rat at 21°C was a continuous increase in MR to 1.9 W/kg above initial level at 120 minutes postinjection.

Acute exposure of 100 day-old rats to 21 and 31°C resulted in no significant difference ($P < .67$) in T_{re} prior to bPRL injection (Figure 6). Tail-skin temperature was increased by 5.4°C ($P < .0001$), and MR decreased by 2.0 W/kg ($P < .0004$) from 21 to 31°C . Only MR was altered by bPRL treatment, as evidenced by a 1.86 W/kg increase from 0 to 60 minutes postinjection. Both 50 and 100 day-old rats exhibited significant increases in MR at 21°C with injection of bPRL.

Implication

Injection of bGH at 21 or 31°C has no acute effect on T_{re} , T_{tail} , or MR of 50 or 100 day-old rats. Effects of bPL and bPRL are different from bGH, but only at 21°C . For bPL, there was a hypothermic effect in both age groups at 21°C that was associated with a decrease in MR in the older age group. Injection of bPRL resulted in an increase in MR in both age groups, with not change in the other thermoregulatory endpoints.

d. Chronic growth experiments

d.1. Design

Female Sprague-Dawley rats were assigned at either 40-43 ($n = 35$) or 90 - 93 days of age ($n = 48$) to either control or experimental treatment groups maintained at either thermoneutral (TN; 70°F or 21°C) or hot (HC; 88°F or 31°C) ambient temperatures. At this time, rats were placed in the assigned environments for 7 - 10 days of adaptation and monitoring of food intake and body weight prior to the injection period. The 10 day treatment period began at 50 or 100 days of age with daily injections of either: 1) sterile water (control); 2) bGH (Monsanto Company; St. Louis) at 20 mg/day/kg bw ; 3) bPL (Monsanto Company; St. Louis) at 20 mg/day/kg bw ; or 4) bPRL (Monsanto Company; St. Louis) at 2 mg/day/kg bw . During this period, there are daily measurements of body weight and food

consumption. Each rat is tested on the last day of chronic injection to determine resting metabolic rate and body temperatures at the 2 ambient temperatures used in this study. Food is removed from all animals 12 h prior to testing. On the test day, each rat is weighed to 0.1 g. Thermocouple wires (40-gauge copper-constantan), with melting ice bath reference junctions, are inserted approximately 2 cm beyond the anal sphincter to measure rectal temperature, and attached to the mid-dorsal surface of the tail base to record tail skin temperature. Tests are conducted using a water-jacketed plexiglas metabolic chamber, as described by Spiers and Adair (1986). Oxygen content of influent and effluent air are measured with a paramagnetic oxygen analyzer. Each metabolic value is adjusted to STPD conditions, and metabolic heat production estimated assuming a respiratory quotient of 0.83. Rate of heat production is expressed as watts (W) relative to whole body weight (kg). Animals are allowed a minimum of 1 h equilibration to both 21 and 31°C. *All tests described below were not a part of the original proposal, and have been added to increase our understanding of animal response to these growth promotants.* Rats are anesthetized after the last injection day and total body electrical conductivity measured using an electromagnetic scanner (Em-Scan, Springfield) to determine fat free (FFM) and total body water (TBW) masses (Morbach and Brans, 1992). Blood is then collected by cardiac puncture, and serum analyzed for IGF-I using the RIA described by Gargosky et al. (1994). Heart, spleen, kidneys, liver, gastrointestinal tract, and reproductive tract are removed following euthanasia, blotted dry, and immediately weighed to the nearest milligram. Lengths of femur and tibia bones from the left side of each animal are measured to the nearest millimeter using calipers. Repeated-measure ANOVA is used to determine time-related differences between treatments, and 2-way ANOVA used for comparisons of animals maintained at 21 and 31°C and injected with sterile water and bGH solutions.

d.2. Chronic experimental results

bGH Results:

Both prepubertal (Figure 7) and adult rats (Figure 8) responded to treatment with bGH with an increase in growth rate. However, adult rats were more responsive to both bGH treatment and ambient temperature than prepubertal animals. For example, the ADG of 100 day-old rats at 31°C was 57% of the rate at 21°C (Table 1). In addition, the bGH-induced increase in ADG at 100 days of age was 2.5-4.9 compared to only 1.5-1.6 in 50 day-old rats. This would suggest that bGH was more effective in stimulating an increase in body mass during slower growth phases (i.e., 100 days of age). Relative feed intake was depressed by heat stress in both age groups, with no bGH effect (Table 1). Adult rats also exhibited changes in body composition that were not observed in younger animals. Treatment with bGH increased relative fat free mass, change in relative fat free mass, relative total body water, and change in relative total body water. None of these changes occurred in prepubertal rats. Likewise, serum insulin-like growth factor (IGF-I) in adult rats only was decreased by heat challenge, followed by increase due to bGH treatment. Similar changes in IGF-1 were not seen in the 50 day-old rats.

Table 1. Growth and Body Composition Values After BST Treatment at 21 and 31°C

Ambient Temp. Injection	21°C		31°C		P Values		
	Control	BST	Control	BST	Ta	Trt	Ta X Trt
50 Day-Olds							
ADG (g/day)	3.24	5.10	3.35	5.03	.95	<.0001	.78
Serum IGF-1(ng/ml)	388.53	356.13	357.41	429.37	.61	.63	.21
Rel. Fat Free Mass at Study End*	74.13	74.93	75.37	75.87	.29	.53	.88
Change Rel. Fat Free Mass*†	-4.98	-5.24	0.89	-2.25	.23	.64	.69
Rel. Total Body Water at Study End*	55.81	56.37	56.75	57.11	.28	.56	.90
Change Rel. Total Body Water*†	-3.54	-4.83	0.51	-1.84	.20	.50	.84
Relative Feed Intake (8-9 dys Trt)*	9.54	9.46	6.63	6.57	<.0001	.86	.99
100 Day-Olds							
ADG (g/day)	1.64	4.04	0.93	4.58	.84	<.0001	.11
Serum IGF-1(ng/ml)	434.64	668.73	298.96	396.61	.0008	.005	.23
Rel. Fat Free Mass at Study End*	68.11	70.26	63.83	67.10	.0004	.0082	.57
Change Rel. Fat Free Mass*†	2.66	3.69	-2.27	0.91	.0026	.07	.30
Rel. Total Body Water at Study End*	51.18	52.75	47.61	50.46	<.0001	.0020	.35
Change Rel. Total Body Water*†	1.96	2.69	-2.52	0.54	.0007	.04	.20
Relative Feed Intake (8-9 dys Trt)*	7.18	7.07	4.52	4.80	<.0001	.79	.52

* Relative values = grams per 100 grams body mass

† Values are change from days 2 to 9 days of treatment

Table 2. Relative Organ Weight (gm/gm) and Bone Length (mm/gm) Values*

Ambient Temp.	21°C		31°C		P Values		
Injection	Control	BST	Control	BST	Ta	Trt	Ta X Trt
50 Day-Olds							
Liver	4.68	4.58	4.56	4.33	.34	.40	.72
GI Tract	9.78	10.65	8.31	8.18	<.0001	.24	.12
Kidney	0.84	0.86	0.86	0.82	.77	.85	.41
Heart	0.34	0.35	0.35	0.33	.93	.69	.31
Spleen	0.26	0.32	0.26	0.26	.20	.22	.23
Reproductive Tract	0.28	0.29	0.23	0.29	.22	.11	.30
Femur	15.99	14.70	16.10	15.44	.28	.01	.41
Tibia	18.50	17.00	19.05	18.34	.06	.03	.43
100 Day-Olds							
Liver	4.00	3.97	3.72	3.61	.01	.59	.73
GI Tract	8.13	8.44	5.85	6.50	<.0001	.14	.59
Kidney	0.82	0.78	0.72	0.67	.0002	.11	.91
Heart	0.32	0.29	0.30	0.28	.17	.004	.61
Spleen	0.23	0.24	0.23	0.26	.51	.13	.59
Reproductive Tract	0.21	0.22	0.24	0.25	.04	.62	.93
Femur	13.24	12.67	13.17	11.81	.14	.003	.21
Tibia	15.05	14.43	14.92	13.34	.10	.004	.18

* Relative values = value per 100 grams body mass

Mass of measured body organs increased as expected in most cases with an increase in growth and overall body mass alone. For this reason, relative body mass was calculated (Table 2) to determine if there were differences that were separate from the change in body mass. In both age groups, there was a decrease in gastrointestinal tract weight for animals maintained at 31°C. This was likely a result of the reduced feed intake at this ambient temperature and less material in the tract. Other relative organ mass differences only occurred in 100 day-old animals. They included heat-induced decreases in relative liver and kidney masses, and an increase in relative reproductive tract mass at 31°C. The only bGH effect was a decrease in heart mass as a result of this treatment. This would suggest that cardiac muscle is not as responsive as other tissues to growth stimulation by bGH, and does not increase with a total increase in body size. Relative bone length also decreased with bGH treatment in both age groups to indicate that bone growth was not accelerated to the same level as overall body growth.

Thermoregulatory tests conducted at the end of the 10-day injection period showed few significant effects of bGH treatment on thermal status or metabolism (Figures 9, 10, 11, 12, 13, 14). Long-term exposure to different thermal environments did, however, have an effect on thermoregulatory ability. Rats at 50 days of age and maintained at 31°C had a lower rectal temperature ($P < .03$) than those maintained at 21°C (Figure 9). Likewise, MR was reduced in 50 day-old rats maintained at 31°C (Figure 10) compared to rats maintained at 21°C ($P < .0014$). Rats treated with bGH at 100 days of age and housed at 31°C had a lower metabolic rate than control animals maintained at 31°C ($P < .05$) (Figure 13). These responses are similar to those seen in large domestic animals during chronic exposure to heat stress. The reduction in metabolic heat production is a controlled effort to reduce body heat content that results in a lower body temperature if successful. As a result of the decreased heat content, there is less energy expenditure for heat dissipation and reduced overall energy intake. All of these activities work together for the maintenance of homeostasis during heat stress conditions.

bGH Dose-Response Results

A separate study was conducted to determine the growth-related dose-response to bGH. Rats at both 50 and 100 days of age were maintained at 31°C and injected daily with 0, 10, or 20 mg bGH/kg bw. It was decided to test rat responsiveness to bGH under heat stress conditions, because it was earlier thought to yield a greater response than at thermoneutrality. All treatment and test procedures were identical to those previously described for the chronic bGH experiments. As noted earlier, the 100 day-old rat is more responsive than the 50 day-old rat to bGH treatment (Table 3). Average daily gain increased linearly (4.5 fold increase from 0 to 20 mg/kg bw) in the 100 day-old rat with bGH treatment (Table 3). The increase in ADG of the 50 day-old rats was only 1.4-fold and was nonlinear. Relative fat free mass and total body water were unaffected by bGH treatment at either age. Likewise, there was no effect of bGH on relative feed intake (Table 3). Relative organ mass was almost completely unaffected by bGH dose treatments (Table 4). In no case was there a significant linear response or effect. Only relative bone length was significantly decreased with bGH treatment. This was only in the 100 day-old rat, and was attributed to an increase in body mass without a comparable increase in bone length. In the younger 50 day-old rat, which was experiencing rapid bone

Table 3. Growth and Body Composition Values for Different BST Doses at 31°C

	BST Dose (mg)			P Value
	0	10	20	Dose
50 Day-Olds				
ADG (g/day)	3.48	3.00	5.03	<.0001
Rel. Fat Free Mass at Study End*	75.53	75.63	75.87	.95
Change Rel. Fat Free Mass*†	1.45	2.59	-2.25	.04
Rel. Total Body Water at Study End*	56.86	56.94	57.11	.96
Change Rel. Total Body Water*†	0.77	1.88	-1.84	.04
Relative Feed Intake (8-9 dys Trt)*	6.52	6.44	6.57	.96
100 Day-Olds				
ADG (g/day)	1.02	2.55	4.58	<.0001
Rel. Fat Free Mass at Study End*	66.30	68.68	67.10	.18
Change Rel. Fat Free Mass*†	-1.26	0.52	0.92	.33
Rel. Total Body Water at Study End*	49.62	51.92	50.46	.06
Change Rel. Total Body Water*†	-1.17	0.67	0.54	.16
Relative Feed Intake (8-9 dys Trt)*	4.58	5.24	4.80	.16

* Relative value = value per 100 grams body mass

† Values are change from days 2 to 9 days of treatment

Table 4. Relative Organ Weight (gm/gm) and Bone Length (mm/gm) Values*

	BST Dose (mg)			P Value
	0	10	20	Dose
50 Day-Olds				
Liver	4.32	3.71	4.33	.02
GI Tract	8.53	8.04	8.18	.58
Kidney	0.81	0.77	0.82	.53
Heart	0.34	0.33	0.33	.76
Spleen	0.27	0.28	0.26	.62
Reproductive Tract	0.25	0.28	0.29	.15
Femur	16.00	15.92	15.44	.46
Tibia	18.81	18.48	18.34	.70
100 Day-Olds				
Liver	3.45	3.18	3.61	.06
GI Tract	6.21	7.02	6.50	.17
Kidney	0.68	0.63	0.67	.14
Heart	0.30	0.30	0.28	.10
Spleen	0.30	0.22	0.26	.68
Reproductive Tract	0.26	0.27	0.25	.91
Femur	13.49	13.39	11.81	.001
Tibia	15.41	15.02	13.34	.0003

* Relative value = value per 100 grams body mass

growth, there was no effect of bGH. Therefore, an increase in body mass at 50 days of age was accompanied by a comparable increase in bone length.

Implications

Treatment of prepubertal and adult rats with bGH results in similar increases in growth rate in both thermoneutral and hot environments. However, there are age-related differences in response. Prepubertal rats respond with a simple increase in body size, with no detectable changes in body composition. In contrast, adult rats exhibit an increase in body size and a change in body composition (i.e., increased relative fat free mass and total body water). There are no detectable changes in thermoregulatory ability (i.e., rectal temperature, tail-skin temperature, metabolic rate) at either age following repeat treatment with bGH at either 21 or 31°C. A determination of chronic dose-response to bGH showed an increase in ADG for both age groups from 0 to 20 mg/kg bw.

bPL Results:

Growth rate was increased from preinjection level in both age groups receiving bPL (Figures 15, 16; Table 5), with the greater increase occurring in the adult rat. Relative feed intake was decreased by heat challenge in both age groups (Table 5). Relative fat free mass and total body water were unaffected by heat stress in prepubertal animals, but were decreased in adult rats. These relative values were not altered by bPL administration in any treatment group. In the prepubertal rat, serum IGF-1 was decreased by heat challenge, and increased with injection of bPL, while there was no effect of temperature or bPL in the adult rat. This suggests an age-dependent difference in the growth stimulation pathways for the two age groups.

Relative liver, GI tract and kidney weights were decreased by heat challenge in the prepubertal animal, but were not affected by bPL treatment (Table 6). Relative organ weights affected by heat challenge in the adult rat were GI tract and heart, which were decreased, and the reproductive tract, which was increased. The heart also showed a decrease with bPL treatment, suggesting that cardiac muscle is not as responsive as other tissues to growth stimulation by bPL.

Treatment with bPL had no effects on metabolism or thermal status in either age group (Figures 17, 18, 19, 20, 21, 22). Resting metabolic rate in prepubertal rats was decreased during acute heat challenge ($P < .003$), and in animals maintained at 31°C ($P < .002$). Body temperatures were increased during acute heat challenge ($P < .05$), and were decreased in animals with long-term exposure to 31°C ($P < .04$), indicating adaptation to the higher temperature environment. In adult rats, metabolic rate decreased during acute heat challenge ($P < .0001$), while rectal temperature was unchanged. A comparison of responses from animals maintained in 21 and 31°C environments showed no thermoregulatory differences in response to suggest that adaptation had occurred at either temperature.

Table 5. Body Composition Values

Ambient Temp. Injection	21°C		31°C		Ta	P Values	
	Control	PL	Control	PL		Trt	Ta x Trt
50 Day-Olds							
Average BW Change from Pre-injection (g)	30.82	34.55	17.27	25.56	<.0001	.0002	.16
Serum IGF-1 (ng/ml)	478.97	806.74	387.80	478.86	.04	.04	.24
Relative Fat Free Mass *	73.92	74.10	75.62	74.29	.41	.61	.51
Relative Total Body Water *	55.62	55.70	56.96	55.92	.36	.57	.51
Relative Feed Intake Average * (Days 2-9)	10.92	10.74	6.71	7.21	<.0001	.52	.18
100 Day-Olds							
Average BW Change from Pre-injecion (g)	0.25	14.44	-0.24	15.60	.80	<.0001	.54
Serum IGF-1 (ng/ml)*	387.59	466.65	428.86	379.42	.65	.77	.21
Relative Fat Free Mass *	70.30	68.60	65.80	66.76	.005	.73	.22
Relative Total Body Water *	52.83	51.49	49.43	50.12	.005	.68	.21
Relative Feed Intake Average * (Days 2-9)	6.70	6.84	4.85	4.93	<.0001	.38	.84

* Relative value = value per 100 grams body mass

Table 6. Relative Organ Weight Values*

Ambient Temp.	21°C		31°C		P Values		
Injection	Control	PL	Control	PL	Ta	Trt	Ta x Trt
50 Day-Olds							
Liver	4.54	4.52	4.13	4.50	.05	.10	.06
GI Tract	10.45	10.78	8.83	9.92	.01	.13	.41
Kidney	0.88	0.87	0.80	0.81	<.001	.98	.51
Heart	0.35	0.33	0.33	0.32	.06	.09	.71
Spleen	0.25	0.27	0.27	0.27	.41	.61	.33
Reproductive Tract	0.24	0.23	0.25	0.24	.39	.59	.93
100 Day-Olds							
Liver	3.64	3.69	3.55	3.58	.44	.76	.94
GI Tract	8.41	8.97	6.62	7.17	<.0001	.13	.99
Kidney	0.72	0.67	0.70	0.65	.34	.08	1.00
Heart	0.31	0.28	0.29	0.27	.02	.001	.49
Spleen	0.21	0.23	0.24	0.22	.56	.90	.11
Reproductive Tract	0.22	0.23	0.27	0.25	.02	.89	.56

* Relative value = value per 100 grams body mass

Implications

There were age-related differences in response to treatment with bPL. The greater growth rate seen in adult rats receiving bPL suggests that bPL treatment affects growth at this age. Less of an increase in growth rate was noted in the prepubertal rat. These results are different from the bGH studies which showed similar increases in growth rate in 50 and 100 day-old rats. In addition, treatment with bPL produced no significant change in body composition (e.g., relative fat free mass and total body water), but treatment with bGH produced an increase in relative fat free mass and total body water in 100 day-old rats.

bPRL Results

The previously reported studies showed that injection of adult rats with bGH or bPL improved growth in both thermoneutral and heat-challenge conditions. Results of the bPRL have only recently been analyzed and reported. The general finding was that bPRL had no effect on growth rate (Figure 23) or feed intake (Figure 24) in prepubertal rats. Fat free mass, total body water (Figure 25) and liver mass at this age were not affected by bPRL, but were decreased in rats maintained at 31°C. Reproductive tract mass was not altered by T_a , but was increased in rats receiving bPRL compared to control rats ($P \leq .01$). Metabolic rate decreased ($P \leq .002$) (Figure 26) and tail-skin temperature increased ($P \leq .0001$) from 21 to 31°C environments, but were not affected by bPRL treatment. Serum IGF-1 was not affected by bPRL treatment, but was reduced in both age groups maintained at 31°C (Figure 27). The heat-induced reduction in IGF-1 was consistently found in rats maintained at 31°C. Adult rats treated with bPRL increased body weight above control level only under the thermoneutral condition ($P \leq .04$) (Figure 28). Feed intake was lower in rats maintained at 31°C (Figure 29), but was not affected by bPRL treatment. Fat free mass and total body water were lower in adult rats maintained at 31°C compared to rats at 21°C ($P \leq .0001$ -.0002) (Figure 25), but were not affected by bPRL treatment. In general, organ mass in adult rats was not altered by bPRL treatment, but spleen mass was greater in bPRL rats ($P \leq .0002$). Metabolic rate decreased ($P \leq .0001$) (Figure 26) and tail-skin temperature increased ($P \leq .0001$) in adult rats from 21 to 31°C, but was unaffected by bPRL treatment.

Implication

Injection of bPRL at 2 mg/kg bw was not reliably effective in altering growth or thermoregulatory ability at different ages or ambient temperatures. Growth responses to repeat treatment with bGH and bPL were greater than for treatment with bPRL.

e. Chronic lactation experiments

e.1. Design

First-parity female Sprague-Dawley rats were randomly assigned to either vehicle or lactogenic hormone treatments in either thermoneutral ($n = 81$) or heat-challenge ($n =$

48) environments (approx. eight rats /group). Rats were moved into the thermoneutral (21°C) or hot (31°C) environment one day after pups were born (day 2), and maintained in these conditions until the end of the measurement period (day 15). Litters were culled to nine pups immediately prior to moving into the different environments. Rats were provided ad libitum access to chow (Formulab Diet #5008, PMI Feeds, St. Louis, MO) and water, and lights were on from 0700 to 1900 daily.

Dams and litters were each weighed to .1 g at approximately 1300 - 1500 h daily, and dam's feed consumption was measured to .1 g daily. On day 5, dams began receiving daily i.p. injections of either bGH, bPRL, T188F analog or vehicle (sterile water) at both 21 and 31°C. *Studies using bPL were conducted at 31°C. However, sufficient bPL material was not available to conduct tests at 21°C. Likewise, bPL analogs, 73A and 73D, were available for testing at 21°C, but not at 31°C. We plan to acquire additional analogs and bPL in the future to complete our tests at the other environmental conditions.* Bovine growth hormone, bPL and bPL analogs were administered at a rate of 20 mg/kg/d and bPRL was given at a rate of 2 mg/kg/d for 10 days. On day 15, litters and dams were euthanized. Dams' body composition (fat free mass and total body water) was determined by EM-Scan (EM-Scan; Springfield, IL). Dams' organs, including left inguinal-abdominal mammary gland, gastrointestinal tract, liver, heart, kidneys, spleen and reproductive tract, were removed and weighed to .0001 g. Left femur and tibia were dissected out and length measured to .01 mm.

e.2. Results of lactation studies

Studies at thermoneutrality (21°C)

Dams injected with 20 mg bGH/kg bw/day showed no significant increase in growth rate above control level ($P < .16$) (Figure 30; Table 7), but litter growth rate was augmented by this treatment ($P < .04$) (Figure 31; Table 7). A comparison of the difference between dam body weight changes for treatment and control groups administered bGH, bPRL, and bPL analog T188F (Figure 32) showed that while bGH-treated dams maintained a constant or slightly elevated body weight above control level, dams treated with bPRL or T188F exhibited a reduction in weight. In contrast, litter weight gain above control level was greatly augmented by T188F, compared to either bGH or bPRL (Figure 33). This would suggest that T188F is highly effective in stimulating milk production. Both T188F and bGH resulted in significant increases in average litter daily gain ($P < .04$) (Table 7). Neither bPRL nor the other bPL analogs (73A, 73D) were effective in producing an increase in litter weight gain. A decrease in relative feed intake was noted with bGH, bPRL, 73A and T188F at 21°C ($P < .06$) (Table 7). The increased growth of litters from bGH and T188F dams was supported by a significant increase in relative mammary gland size ($P = .009-.09$). Likewise, there was an increase in relative mammary gland size for dams treated with 73A and 73D, but not for bPRL (Table 8). This would suggest that the bGH and bPL analog compounds were not acting through the prolactin system.

Table 7. Growth and Body Composition Values at 21°C

Injection	Control	BST	P	Control	PRL	P	Control	73A	P	Control	73D	P	Control	T188F	P
Litter Average															
Daily Gain (g/d)	18.24	20.47	.04	19.46	19.69	.85	17.72	18.60	.47	17.68	19.62	.11	16.70	20.30	.007
Dam Average															
Daily Gain (g/d)	4.67	5.78	.16	4.61	3.42	.18	3.92	5.15	.16	3.95	3.31	.47	5.10	3.83	.18
Relative Fat															
Free Mass *	72.11	74.60	.24	72.87	72.83	.98	78.15	76.04	.37	74.12	75.18	.66	79.78	75.56	.98
Relative Total															
Body Water *	54.04	55.88	.34	54.61	54.59	.99	58.57	56.97	.46	55.55	56.33	.72	57.50	56.61	.70
Relative Feed															
Intake Average *															
(Days 6-14)	15.98	14.92	.02	15.47	14.51	.06	15.93	14.91	.04	15.58	15.04	.29	16.09	15.09	.06

* Relative values = grams per 100 grams body mass

Table 8. Relative Dam Organ Weight and Bone Length Values*

Injection	Control	BST	P	Control	PRL	P	Control	73A	P	Control	73D	P	Control	T188F	P
Mammary Gland	1.58	1.76	.09	1.98	1.90	.50	1.82	2.06	.06	1.68	1.91	.07	1.69	2.05	.009
Liver	6.11	5.88	.26	5.61	5.69	.73	5.88	6.11	.32	5.98	6.05	.76	6.63	6.01	.02
GI Tract	18.20	17.25	.41	16.79	14.98	.16	17.36	17.79	.74	17.62	17.08	.68	21.45	16.17	.0002
Kidney	0.96	0.91	.14	0.91	0.87	.24	0.90	0.86	.26	0.92	0.91	.83	0.98	0.90	.02
Heart	0.32	0.30	.05	0.33	0.33	.89	0.34	0.31	.003	0.30	0.33	.01	0.35	0.31	.003
Spleen	0.17	0.20	.04	0.18	0.18	.89	0.17	0.19	.07	0.17	0.22	.0001	0.19	0.19	.95
Reproductive Tract	0.25	0.16	.001	0.14	0.15	.85	0.15	0.16	.77	0.19	0.15	.18	0.21	0.16	.08
Femur	10.19	9.87	.42	10.81	10.84	.94	11.14	10.46	.12	10.54	10.67	.77	10.97	10.58	.42
Tibia	12.01	11.15	.13	11.93	12.55	.32	12.45	11.90	.39	12.44	12.09	.58	12.30	11.89	.54

* Relative values = grams per 100 grams body mass

Studies during heat stress (31°C)

Dam growth rate was increased above control level by injection of bGH (Figures 34,35). However, analysis of dam and litter ADG (Table 9) showed significant differences between bGH and control values at 31°C. Weight differences between treatment and control values for dams (Figure 36) and litters (Figure 37) were also increased over time with bGH treatment. A comparison with T188F groups showed an increase in the dam minus control weight interval (Figure 36), but no change in the litter response. Treatment with bPRL had no effect on either endpoint. There were few effects of bGH, bPL, bPRL or T188F on growth of dams and litters at 31°C (Tables 9, 10). Treatment with T188F reduced relative fat free mass, relative total body water, and relative feed intake at 31°C. However, there was no effect of any test compound on relative mammary gland size at this temperature (Table 10).

Although bGH and bPL were able to increase growth of rats at 21 and 31°C, there was less effect of any of the tested compounds on growth or body composition at 31°C. It is possible that a higher dose of growth promotant is needed to stimulate lactation, than is required for augmentation of growth at 31°C.

Lactation dose response to bGH

Growth rate was increased in dams receiving 40 or 80 mg bGH/kg/d (Figure 38) and in litters of dams receiving 40 mg bGH/kg/d (Figure 39). Relative fat free mass and total body water were increased by bGH at all doses (Table 11). Dams receiving 20 mg bGH/kg/d had a decreased relative feed intake.

Relative mammary gland weight was increased in animals receiving the 20 or 40 mg bGH treatment (Table 12). Other relative organ weights affected by the 20 mg dose were heart and reproductive tract, which were both decreased. Relative heart and kidney weights were decreased with the 40 mg dose. Injection with 80 mg bGH resulted in a decrease in relative liver weight. In contrast, relative femur and tibia lengths were not affected by any treatment.

The results of this study show that administration of 40 mg bGH/kg bw/day results in the largest increase in growth rate and body composition. In fact, treatment with the higher dose (i.e., 80 mg/kg/day) diminished changes in body growth rate and composition.

Implication

Both bGH and T188F at 20 mg/kg bw/day are effective in stimulating mammary gland growth and increased lactation at 21°C, as reflected by an increase in pup growth. These compounds had less effect on these endpoints in the heat stress environment. This temperature-dependent response is different from the effect of bGH on growth of 50 and 100 day-old rats, where bGH was equally effective in increasing growth. One suggestion derived from the different responses is that a higher dose of growth promotant might be needed to elicit a response when there are extra internal (e.g.,

lactation) or external (e.g., heat stress) demands placed on an animal's system. Future studies are needed to determine what the delicate balance is between stressor level in an animal and effective dose of growth promotant.

Table 9. Growth and Body Composition Values at 31°C

Injection	Control	BST	P	Control	PL	P	Control	PRL	P	Control	T188F	P
Litter Average												
Daily Gain (g/d)	14.32	15.79	.10	15.76	14.76	.27	14.83	15.39	.52	15.77	15.73	.98
Dam Average												
Daily Gain (g/d)	-0.46	0.72	.33	-2.50	-2.37	.92	-0.47	-1.95	.21	-1.37	-1.03	.85
Relative Fat Free Mass *	70.38	71.39	.40	70.31	71.87	.21	69.58	69.08	.68	73.15	66.98	.001
Relative Total Body Water *	52.83	53.56	.42	52.77	53.92	.22	52.22	51.83	.66	54.89	50.23	.001
Relative Feed Intake Average * (Days 6-14)	11.93	11.99	.87	11.00	9.02	<.0001	11.50	9.95	.0001	11.47	9.99	.02

* Relative values = grams per 100 grams body mass

Table 10. Relative Dam Organ Weight and Bone Length Values*

Injection	Control	BST	P	Control	PL	P	Control	PRL	P	Control	T188F	P
Mammary Gland	1.54	1.56	.80	1.47	1.47	1.00	1.53	1.60	.51	1.69	1.53	.28
Liver	4.98	4.82	.54	4.78	4.40	.17	5.10	4.53	.03	4.75	4.53	.58
GI Tract	12.71	11.27	.12	11.12	9.59	.12	13.45	10.58	.003	11.82	8.78	.03
Kidney	0.87	0.85	.56	0.90	0.80	.02	0.92	0.86	.09	0.83	0.79	.49
Heart	0.33	0.32	.83	0.37	0.32	.02	0.33	0.32	.62	0.33	0.30	.39
Spleen	0.18	0.18	.53	0.17	0.17	.41	0.18	0.18	.54	0.18	0.18	.80
Reproductive Tract	0.17	0.16	.57	0.17	0.16	.63	0.17	0.18	.49	0.16	0.15	.66
Femur	12.65	12.19	.69	12.75	12.08	.57	12.76	11.02	.12	12.38	11.36	.56
Tibia	14.29	13.70	.15	13.65	13.62	.94	13.85	13.62	.56	14.01	13.01	.10

* Relative values = grams per 100 grams body mass

Table 11. Growth and Body Composition Values

Dose (mg/kg/d) Injection	20 mg		P	40 mg		P	80 mg		P
	Control	BST		Control	BST		Control	BST	
Litter Average Daily Gain (g/d)	18.08	20.10	.06	17.12	20.19	.003	20.41	20.40	.99
Dam Average Daily Gain (g/d)	5.16	5.39	.82	2.86	6.77	<.0001	2.69	4.68	.04
Relative Fat Free Mass *	71.91	74.74	.05	74.19	77.23	.03	70.27	73.72	.02
Relative Total Body Water *	53.89	55.99	.05	55.60	57.84	.03	52.68	55.24	.08
Relative Feed Intake Average * (Days 6-14)	16.35	14.95	.004	15.65	16.06	.37	15.45	15.58	.82

* Relative values = grams per 100 grams body mass

Table 12. Relative Dam Organ Weight and Bone Length Values*

Dose (mg/kg/d) Injection	20 mg		P	40 mg		P	80 mg		P
	Control	BST		Control	BST		Control	BST	
Mammary Gland	1.49	1.69	.06	1.52	1.91	.0004	1.69	1.75	.59
Liver	6.22	5.89	.12	6.14	6.26	.52	5.74	6.63	.0004
GI Tract	18.91	17.35	.15	18.22	18.65	.67	16.81	17.98	.33
Kidney	0.97	0.94	.25	0.96	0.90	.05	0.85	0.90	.15
Heart	0.33	0.30	.07	0.33	0.30	.02	0.31	0.33	.24
Spleen	0.18	0.20	.87	0.18	0.21	.85	0.21	0.24	.15
Reproductive Tract	0.28	0.16	.002	0.22	0.17	.10	0.18	0.20	.64
Femur	10.00	10.06	.89	10.54	9.99	.13	10.46	10.42	.93
Tibia	11.87	11.41	.41	11.87	11.31	.29	11.65	11.66	.99

* Relative values = value per 100 grams body mass

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g. Legends to Figures

1. Changes in rectal temperature (upper graph), tail-skin temperature (middle graph), and metabolic rate (lower graph) of 50 day-old rats injected with bGH (BST) in 21 (thermoneutral) and 31°C (heat stress) environments.
2. Changes in rectal temperature (upper graph), tail-skin temperature (middle graph), and metabolic rate (lower graph) of 100 day-old rats injected with bGH (BST) in 21 (thermoneutral) and 31°C (heat stress) environments.

3. Changes in rectal temperature (upper graph), tail-skin temperature (middle graph), and metabolic rate (lower graph) of 50 day-old rats injected with bPL in 21 (thermoneutral) and 31°C (heat stress) environments. An asterisk indicates a significant change ($P < .05$) from the initial -5 minute value.
4. Changes in rectal temperature (upper graph), tail-skin temperature (middle graph), and metabolic rate (lower graph) of 100 day-old rats injected with bPL in 21 (thermoneutral) and 31°C (heat stress) environments. An asterisk indicates a significant change ($P < .05$) from the initial -5 minute value.
5. Changes in rectal temperature (upper graph), tail-skin temperature (middle graph), and metabolic rate (lower graph) of 50 day-old rats injected with bPRL in 21 (thermoneutral) and 31°C (heat stress) environments. An asterisk indicates a significant change ($P < .05$) from the initial -5 minute value.
6. Changes in rectal temperature (upper graph), tail-skin temperature (middle graph), and metabolic rate (lower graph) of 100 day-old rats injected with bPRL in 21 (thermoneutral) and 31°C (heat stress) environments. An asterisk indicates a significant change ($P < .05$) from the initial -5 minute value.
7. Body weight (upper graph) and change in body weight from days -1-2 (lower graph) of 50 day-old rats at 2 to 9 days of treatment with bGH (BST) and vehicle in 21 and 31°C environments.
8. Body weight (upper graph) and change in body weight from days -1-2 (lower graph) of 100 day-old rats at 2 to 9 days of treatment with bGH (BST) and vehicle in 21 and 31°C environments.
9. Rectal temperature of 50 day-old rats treated with bGH (BST) and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
10. Metabolic rate of 50 day-old rats treated with bGH (BST) and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
11. Tail-skin temperature of 50 day-old rats treated with bGH (BST) and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
12. Rectal temperature of 100 day-old rats treated with bGH (BST) and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
13. Metabolic rate of 100 day-old rats treated with bGH (BST) and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
14. Tail-skin temperature of 100 day-old rats treated with bGH (BST) and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.

15. Body weight change from days -1-2 for 50 day-old rats at 2 to 9 days of treatment with bPL and vehicle in 21 and 31°C environments.
16. Body weight change from days -1-2 for 100 day-old rats at 2 to 9 days of treatment with bPL and vehicle in 21 and 31°C environments.
17. Rectal temperature of 50 day-old rats treated with bPL and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
18. Tail-skin temperature of 50 day-old rats treated with bPL and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
19. Metabolic rate of 50 day-old rats treated with bPL and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
20. Rectal temperature of 100 day-old rats treated with bPL and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
21. Tail-skin temperature of 100 day-old rats treated with bPL and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
22. Metabolic rate of 100 day-old rats treated with bPL and vehicle in 21 and 31°C environments, when exposed to 21 and 31°C.
23. Body weight change from days -1-2 for 50 day-old rats at 2 to 11 days of treatment with bPRL and vehicle in 21 and 31°C environments.
24. Feed intake from days -1-2 for 50 day-old rats at 2 to 10 days of treatment with bPRL and vehicle in 21 and 31°C environments.
25. Final relative fat free mass (left graphs) and relative total body water (right graphs) for 50 (upper graphs) and 100 (lower graphs) day-old rats maintained in 21 and 31°C environments, and treated with bPRL and vehicle.
26. Final metabolic rate at 21 and 31°C for rats maintained in 21°C environments (left graphs) and 31°C environments (right graphs) at 50 (upper graphs) and 100 (lower graphs) days of age, and treated with bPRL and vehicle.
27. Final serum IGF-1 levels for 50 (left graph) and 100 (right graph) day-old rats treated with bPRL and vehicle in 21 and 31°C environments.
28. Body weight change from days -1-2 for 100 day-old rats at 2 to 11 days of treatment with bPRL and vehicle in 21 and 31°C environments.
29. Feed intake from days -1-2 for 100 day-old rats at 2 to 10 days of treatment with bPRL and vehicle in 21 and 31°C environments.

30. Change in dam body weight from preinjection level at day 5 for animals maintained at 21°C and injected with bGH (BST) and vehicle solutions.
31. Change in litter weight from preinjection level at day 5 for animals maintained at 21°C and injected with bGH (BST) and vehicle solutions.
32. Difference in dam body weight between treatment and control groups from preinjection level at day 5, for animals maintained at 21°C and injected with bGH (BST), bPRL and T188F solutions.
33. Difference in litter weight between treatment and control groups from preinjection level at day 5, for animals maintained at 21°C and injected with bGH (BST), bPRL and T188F solutions.
34. Change in dam body weight from preinjection level at day 5 for animals maintained at 31°C and injected with bGH (BST) and vehicle solutions.
35. Change in litter weight from preinjection level at day 5 for animals maintained at 31°C and injected with bGH (BST) and vehicle solutions.
36. Difference in dam body weight between treatment and control groups from preinjection level at day 5, for animals maintained at 31°C and injected with bGH (BST), bPRL and T188F solutions.
37. Difference in litter weight between treatment and control groups from preinjection level at day 5, for animals maintained at 31°C and injected with bGH (BST), bPRL and T188F solutions.
38. Difference in dam body weight between treatment and control groups from preinjection level at day 5, for animals maintained at 21°C and injected with different doses of bGH (BST).
39. Difference in litter weight between treatment and control groups from preinjection level at day 5, for animals maintained at 21°C and injected with different doses of bGH (BST).

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FIGURE 38

FIGURE 39

IV. UNIVERSITY OF MISSOURI-COLUMBIA (Bovine Studies)

Results of research (Univ. of Missouri)

Final Report of the Missouri Lab (Assoc. Prof. Donald Spiers)

a. Specific Objective

- 1) Evaluation of the effects of bGH, bPRL, bPL and PL analogs on lactation, food consumption, thermoregulation and endocrine status of lactating dairy cows under thermoneutral, heat and cold conditions.

The original BARD proposal indicated that we would test nonpregnant lactating cows under thermoneutral, heat, and cold conditions using "vehicle, bGH, bPRL, and bPL or its analogs". The sample size for these tests was to be four. We determined that a more suitable sample size would be 10-12 animals per group. Likewise, it was decided that more detailed animal measurements were needed. For example, temperature transmitters were surgically implanted into the peritoneal cavity of each animal to allow for continuous monitoring of core body temperature every 20 minutes for the duration of the study.

The increase in animal sample size and additional measurements necessitated a reduction in the number of test conditions. The cold Ta was eliminated from the thermal test conditions, with the understanding that thermoneutral, warm and hot Ta were the conditions of primary interest. In addition, bPRL was eliminated from the compounds to be tested in order to allow for more detailed comparisons of bGH and bPL. We believe that these changes improved our ability to make reliable statistical comparisons and allowed for a more thorough determination of the actions of bGH and bPL.

b. Introduction

Exposure of lactating dairy cows to heat stress results in decreases in feed intake, milk production, and metabolism, to result in a significant loss of productivity during summer months. Treatment of lactating dairy cows with bGH during heat challenge can increase production rates of milk and metabolic heat (Manalu et al., 1991; West, 1994), without necessarily producing a shift in whole-body thermal balance (Johnson et al., 1991). A detailed study of dairy cows was performed to identify time-related changes in thermoregulatory and production parameters during exposure to heat challenge and simultaneous treatment with bGH. An additional study was conducted to compare effects of bGH with bPL under both thermoneutral and warm/hot Ta conditions. The goals of these studies were to first determine the effects of heat stress on performance, and second to identify and compare changes in performance with daily injection of bGH or bPL during heat stress.

c. Experimental designs

Two separate studies were performed to determine the effect(s) of bGH and bPL. The experimental procedures were identical for both studies. Twelve lactating cows were implanted with telemetric temperature transmitters (Mini-Mitter Co., Inc., Sunriver, OR) in each study to continuously measure core body temperature (T_{core}). Following a 7 day recovery period, cows were transferred to the Brody Climatology Chambers for a 5 day acclimation period to 66°F(19°C) (thermoneutral T_a). A blood sample was collected by tail venipuncture, one day prior to the initiation of the injection period (subcutaneous injection of control or bGH solutions) for determination of growth hormone, insulin-like growth factor I (IGF-I), prolactin, insulin, blood urea nitrogen, non-esterified fatty acid, and glucose levels. The 17 day injection period began on day 6 of the experiment, using a daily dose of 31 mg bGH or bPL/animal. This dose was split to occur at 0930 and 2000 h. Test compounds were injected for 4 days at thermoneutrality, prior to the increase in T_a (Figure 1). During this time, there were determinations of metabolic heat production and sweat rates, with blood sampled for analysis as previously described. Elevation of T_a occurred over a 2 day period from 66(19) to 83°F(28.5°C) (warm T_a challenge). Air temperature was set at this level for 7 days, during which time there were the previously described measurements of thermoregulatory and production indicators. After 3 days of exposure to a constant T_a of 83°F(28.5°C), T_a cycling was initiated for a 5.4°F(3.0°C) reduction in T_a from day to night levels. This allowed for a determination of the benefit derived from a slight night time reduction in T_a . After 7 days of exposure to 83°F(28.5°C), T_a was lowered back to 66°F(19°C) for 3 days of measurement of the recovery response. In addition to the previously described measurements, there were daily determinations of milk production, feed and water intakes, respiratory rates, and skin temperatures. Following the first trial, cows were provided 15 days of recovery before switched across treatment (i.e., control to growth promotant, growth promotant to control) and run through a second trial.

Results of bGH Study

Treatment with bGH did not result in a significant change in T_{core} at any sampled time during the test period. Increase in T_a from 19.0 to 28.5°C (i.e., heat exposure)(see Figure 2 for sample times of T_{core}) resulted in an increase in daily average T_{core} from 101.6(38.66) to 103.9°F(39.92°C) ($P<.0001$) after 4 days of exposure to heat condition (Figure 3). With cycling T_a (i.e., 83.0 to 78.0°F), daily average T_{core} decreased only to 103.4°F(39.69°C). Return of T_a to 66°F(19°C) resulted in a decrease in daily average T_{core} to 101.2°F(38.47°C), which was below the initial value ($P<.0001$). This undershoot represented a potential overcompensation for the relief from heat stress.

A daily shift in T_{core} ($P<.0001$) occurred under all test conditions. High and low values for T_{core} , as shown in Figure 4, occurred at the following times:

Table 1. Occurrence times for high and low values of core body temperature during the entire 20 day exposure period.

Study Day	T _a (°F)	High Value (Hours)	Low Value (Hours)
5	66.0	2300 - 2400	0700 - 1100
12	83.0	0300 - 0400	0800 - 1000
13	78.0 - 83.0	2000 - 2200	0700
15	78.0 - 83.0	1700 - 1800	0700
20	66.0	2300	0600 - 0800

Exposure to heat stress did not shift daily time for low T_{core}, but did push peak T_{core} to early morning hours. Night cooling resulted in a shift in peak T_{core} to an earlier time than at 66°F (19°C). This was likely due to the initiation of a reduction in T_{core} with a nighttime reduction in T_a. Return to thermoneutrality shifted peak T_{core} time back to the initial hour.

Treatment with bGH resulted in a 5.22 lbs/day higher level of milk production than for control animals (Figure 5). Increase in milk production with bGH began within 24 hours following initiation of injection, and remained in effect throughout most of the injection period. The only time when milk production of bGH- and vehicle-injected animals were similar was during the transition period for heat stress, when milk production was rapidly decreasing in both groups. Return to the 5 lb/day group difference occurred after 3 days of exposure to peak heat stress when milk production was at its lowest level in the study. During the heat stress recovery period, milk production of the bGH-treated animals remained at the same level above that of vehicle-treated animals.

Time-relative changes in feed and water intakes are shown in Figure 6. Changes in feed (P<.9201) and water (P<.3681) intakes were not different across treatments with bGH and vehicle, but were shifted as functions of time (P<.0001 - .0032). Water intake increased to the middle of the heat cycle period, followed by a rapid decline to below the initial pretreatment level at the end of the T_a cycling period. Reduction in feed intake with heat stress was similar to the reduction in milk production. Recovery of heat-induced depression in feed intake began near the end of the heat cycle period, and preceded the recovery of milk production. The difference in the recovery times for milk production and feed intake was expected, since an increase in total energy intake level would have to occur prior to an increase in production level.

Avenues of heat exchange were not altered by bGH treatment (i.e., metabolic rate - $P < 0.591$; cutaneous evaporative heat loss - $P < 0.372$; respiratory evaporative heat loss - $P < 0.475$) (Figures 7,8). However, there were significant time effects, which reflected the impact of heat stress. Metabolic rate decreased ($P < 0.086$) over time (Figure 7). This change in heat production was not rapid, only reaching low level on the last day of cycling T_a . Likewise, the increase in sweat rate did not peak until 1 day prior to the reduction in T_a to neutral level. These results indicate that reductions in heat production and heat loss by sweating occur slowly during heat stress (i.e., at least 3 days). In contrast, increases in respiratory evaporation and heat loss (Figure 8) peaked more rapidly within 3 days of exposure to heat stress, and remained elevated until T_a was lowered to neutral level. Respiration rate (Figure 8) also increased during heat stress to facilitate evaporative cooling, and averaged higher in bGH-treated animals (67 breaths/min) compared to controls (64 breaths/min) ($P < 0.0001$), although the difference was of no biological significance. In the present study, respiration rate increased with the initial rise in T_a ($P < 0.0001$), and was twice that at T_a 66°F (19°C) within one day of T_a plateau at the upper level (Figure 8). Respiration rate remained at this upper level even during T_a cycling, but decreased rapidly with a progressive reduction in T_a .

Blood level of bGH in bGH-injected animals was elevated above that of control animals ($p < 0.0001$) throughout the treatment period (Figure 9). Likewise, blood level of IGF I, which is considered to be a primary mediator of bGH action, was elevated in bGH-treated animals throughout this period ($P < 0.0001$) (Figure 9). Both compounds increased progressively and were not affected by heat exposure. Plasma urea nitrogen was reduced in bGH animals below control level ($P < 0.0046$) even during heat stress conditions (Figure 10). Blood level of non-esterified fatty acids was increased with bGH treatment above control level ($P < 0.0014$) (Figure 10). This would suggest that fatty acids are being mobilized to support an increase in milk production without an increase in feed intake. Blood levels of glucose for both treatment groups decreased with heat stress ($P < 0.0001$) to reflect the decrease in feed intake during this period (Figure 11). Prolactin level was not affected by bGH treatment ($P < 0.12$), but rapidly doubled in value during heat stress (Figure 12).

Implications

Short-term exposure to heat stress decreased both feed intake and milk production in lactating dairy cows. Daily treatment with recombinant bovine growth hormone increases milk production above control level by approximately 5.22 lbs/cow/day. This occurs during all phases of heat stress, including onset and recovery periods. Neither feed intake nor thermoregulatory control were altered during the heat stress period as a result of treatment with recombinant bovine growth hormone. These results indicate that recombinant bovine growth hormone is effective at 31 mg/animal/day in significantly elevating milk production above control level during heat stress, without compromising thermal status.

Results of bPL Study

Treatment with bPL did not result in a significant change in T_{core} at 19°C (Figure 13), with periods of daily high and low values occurring at the same time as for bGH (Table 1). Likewise, exposure to 28.5°C resulted in a 1°C increase in T_{core} (Figure 14), and a slight shift in both high and low T_{core} values as noted in the bGH study (Table 1). The difference from the bGH study is that treatment with bPL resulted in an increase in T_{core} above control level during the second day of exposure to 28.5°C (time x treatment interaction effect; $P < .0001$). The increase in T_{core} was 0.3 to 0.4°C, and occurred during the rise and plateau in daily T_{core} movement. This elevation in T_{core} of bPL cows was still evident the next day (time x treatment interaction effect; $P < .0001$), but only during the early morning hours when T_{core} was at its highest level (Figure 15). There were no significant treatment differences in T_{core} after the first 2-3 days of heat exposure (Figures 16,17). Return of T_a to 19°C resulted in a decrease in T_{core} below that seen at the start of the experiment (Figure 18). As noted in the bGH description, this is likely a result of overcompensation as a part of the recovery process.

Milk production was not affected by treatment with bPL ($P < .58$) (Figure 19). This is opposite from the bGH response and suggest that bPL is not as effective as bGH in stimulating milk production. A slight, but insignificant increase in milk production, did occur as a result of bPL treatment prior to heat stress. Exposure to 28.5°C resulted in a slow decline in milk production which reached nadir after 6 days of peak heat exposure. Cycling of T_a to reduce heat stress level was not effective in increasing milk production until after 3 days. Feed intake declined ($P < .0001$) with heat stress (Figure 20) to reach a low point 1 day prior to the nadir for milk production. It is likely that the preceding decline in feed intake had a direct influence on milk production. Treatment with bPL resulted in a significantly higher level of feed intake ($P < .04$) at a difference of 1.7 lbs/day. In contrast, water intake was less in bPL-treated cows (Figure 20; $P < .097$) with a difference of only 0.13 gallons/day. It is unlikely that such small differences in feed and water intakes would constitute physiologically significant differences in overall performance.

Heat exchange was unaltered by bPL treatment (i.e., metabolic rate- $P < .61$; cutaneous evaporative heat loss- $P < .92$; respiratory evaporative heat loss- $P < .36$). This response was similar to the responses to bGH treatment. However, all measured avenues of heat exchange changed over time ($P < .0001$) with exposure to heat stress (Figure 21). Heat production slowly decreased by approximately 70 Kcal/hr after 7-8 days at 28.5°C, and remained depressed even after several days return to 19°C. Both cutaneous and respiratory avenues for evaporative heat loss increased rapidly ($P < .02$) to a plateau at 2-3 days at 28.5°C, and rapidly returned to thermoneutral level with the return to 19°C. Respiration rate of bPL cows was below control level ($P < .03$) by 1.5 breaths/min (Figure 22). This was only a 3% difference in performance, and unlikely a significant contributor to overall performance. The pattern of change in respiration rate during heat stress was very similar to that seen during the bGH study. In both cases, it was obvious that respiration rate is a sensitive indicator of heat strain in the dairy cow.

No blood parameter in the bPL study was altered by treatment (Figures 23, 24, 25). Prolactin, insulin, and PUN levels (Figures 23, 24) were increased ($P < .0001$) during heat stress, as was noted during the bGH study. Likewise, blood glucose level was decreased at 28.5°C ($P < .0001$), reflecting the overall reduction in feed intake.

Implications

Bovine placental lactogen at 31 mg/animal/day is not as effective as bovine growth hormone in increasing milk production, and maintaining this level during heat stress. In addition, there is some indication that bovine placental lactogen increases body core temperature during the initial stages of heat exposure at that time of the day when core temperature is increasing or reaching peak level.

Results of Antioxidant Activity Study

A sample of blood collected from cows in the initial heat stress study (i.e., bGH) was sent to Dr. Robert J. Harmon in the Animal Sciences Department at the University of Kentucky for analysis and determination of antioxidant activity. Oxidative stress occurs when there is a disturbance in the equilibrium status of pro-oxidant/antioxidant systems. Oxidative damage to lipids, proteins, carbohydrates, and nucleic acids can occur with a decrease in antioxidant activity. Known stressors of the pro-oxidant/antioxidant systems include environmental pollutants and challenges to the immune system. We wanted to take the opportunity to determine if the level of heat stress used in this study (i.e., 28.5°C) was capable of altering antioxidant activity in the dairy cow.

Implications

The results of bGH heat stress (Trial I in the attached document) showed a reduction in bovine plasma antioxidant activity. Such a shift in activity due to heat stress could affect immune function, and result in increased susceptibility to infection. The information derived from this study represents a potentially important link between heat stress and disease susceptibility.

Attached are excerpts (Methods and Results) from the thesis of Mei Lu for review. In addition, three copies of the entire thesis are included.

d. Literature

1. Gargosky, S.E., P. Tapanainen, and R.G. Rosenfeld. 1994. Administration of growth hormone (GH), but not insulin-like growth factor-I (IGF-I), by continuous infusion can induce the formation of the 150-kilodalton IGF-binding protein-3 complex in GH-deficient rats. Endocrinol. 134: 2267.

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5. Spiers, D.E. and E.R. Adair. 1986. Ontogeny of homeothermy in the immature rat: Metabolic and thermal responses. J. Appl. Physiol. 60: 1190.
6. West, J.W. 1994. Interactions of energy and bovine somatotropin with heat stress. J. Dairy Sci. 77: 2091.

e. Legends to Figures

1. Average chamber air temperature during the bGH (BST) study.
2. Average chamber air temperature with sample times for core body temperature analysis.
3. Average core body temperature before, during, and after heat stress in the bGH (BST) study.
4. Average upper and lower core body temperature values in the bGH (BST) study for thermoneutral and heat stress periods.
5. Average changes in milk production for bGH (BST) and control cows during all phases of the bGH study, including thermoneutral and heat stress sessions.
6. Average changes in water (upper graph) and feed (lower graph) intakes for bGH (BST) and control cows during all phases of the bGH study, including thermoneutral and heat stress sessions.
7. Average metabolic (upper graph) and cutaneous evaporation rates for bGH (BST) and control cows during all phases of the bGH study, including thermoneutral and heat stress sessions.
8. Average respiratory vaporization (upper graph) and respiration (lower graph) rates for bGH (BST) and control cows during all phases of the bGH study, including thermoneutral and heat stress sessions.

9. Average blood BST (upper graph) and IGF-1 (lower graph) levels for bGH (BST) and control cows during all phases of the bGH study, including thermoneutral and heat stress sessions.
10. Average blood PUN (upper graph) and NEFA (lower graph) levels for bGH (BST) and control cows during all phases of the bGH study, including thermoneutral and heat stress sessions.
11. Average blood insulin (upper graph) and glucose (lower graph) levels for bGH (BST) and control cows during all phases of the bGH study, including thermoneutral and heat stress sessions.
12. Average blood prolactin levels for bGH (BST) and control cows during all phases of the bGH study, including thermoneutral and heat stress sessions.
13. Average core body temperature for animals in the bPL study as a function of time of day, before and after initiation of bPL treatment at thermoneutrality.
14. Average core body temperature for animals in the bPL study as a function of time of day, before and during initial stages of heat stress at 28.5°C.
15. Average core body temperature for animals in the bPL study as a function of time of day, during the second and third days of exposure to heat stress.
16. Average core body temperature for animals in the bPL study as a function of time of day, before and after night cycling down of air temperature.
17. Average core body temperature for animals in the bPL study as a function of time of day, with night cycling down of air temperature during heat stress.
18. Average core body temperature for animals in the bPL study as a function of time of day, at the final stages of heat stress and during the early stages of recovery at thermoneutrality.
19. Changes in milk production of bPL and control cows during the entire period of study.
20. Feed (upper graph) and water (lower graph) intakes of bPL and control cows during the entire period of study.
21. Metabolic heat production (upper graph), cutaneous evaporative heat loss (middle graph), and respiratory evaporative heat loss (lower graph) of bPL and control cows during the entire period of study.
22. Respiration rate of bPL and control cows during the entire period of study.
23. Blood prolactin (upper graph), BST (middle graph), and IGF-1 (lower graph)

levels of bPL and control cows during the entire period of study.

24. Blood glucose (upper graph), insulin (middle graph), and PUN (lower graph) levels of bPL and control cows during the entire period of study.
25. Blood NEFA levels of bPL and control cows during the entire period of study.

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V. Description of Cooperation

All placental lactogen analogs used by the University of Missouri in this project were derived from the Israeli laboratory. Monsanto Co. (St. Louis, MO) was instrumental in the development of this project. Drs. Robert Collier, John Byatt, and Nick Staten were our contacts for most of this exchange. Assistance provided by the Monsanto Co. included 1) information regarding the generation of placental lactogen analogs; and 2) generation of all recombinantly-derived growth promotants including bovine growth hormone, bovine placental lactogen, and bovine prolactin. Dr. Robert Harmon and his graduate student M. Lu were responsible for the determination of antioxidant activity in blood samples of dairy cows exposed to heat stress. A meeting for the exchange of information between Drs. Gertler and Spiers was held at Monsanto during the second year of this project.

VI. Evaluation of Research Achievements and Benefits to Agriculture

Studies in our laboratories will have both immediate and future benefit to animal producers. Our work with dairy cows has allowed us to determine the strain of heat stress on thermal balance and, in turn, the impact on production. In addition, we have identified the natural sequence of events utilized by the dairy cow to maintain thermal balance during heat stress. Treatment with bovine growth hormone increases milk production equally in both thermoneutral and heat stress environments, without increasing feed intake or compromising avenues of heat production and heat loss. Injection of an equal dose of bovine placental lactogen over the same time period had no effect on milk production in either thermoneutral or heat stress environments. In fact, there is suggestion that bovine placental lactogen results in a significant, albeit slight, increase in core body temperature over control level during the early stages of heat exposure. Rat studies confirmed that chronic treatment with bovine growth hormone or placental lactogen has no effect on thermoregulatory ability. Injection of young and adult rats with bovine growth hormone increased growth rate in both thermoneutral and heat stress environments. However, increase in fat free mass only occurred in the adult animals. Treatment with bovine placental lactogen was less effective than growth hormone in increasing growth at different ages. In addition, bovine placental lactogen had no effects on fat free mass. There was no effect of bovine prolactin on any treatment group. Lactation studies showed that the T188F analog from Israel is as effective as bovine growth hormone in increasing mammary gland growth and stimulating milk production in the rat. Within this test model, there is no effect of bovine prolactin. Additional analogs, 73A and 73D, show some lactogenic activity in our rat model. However, additional tests are needed to confirm this activity. Following further tests of these analogs, primary candidates may be selected for further testing in dairy cows. Placental lactogen analogs will eventually be developed that selectively stimulate lactogenic and/or somatogenic receptors in dairy cows for optimum productivity.

Three copies of the documents in Sections VI, VII, VIII, and IX are attached at the end of this final project report for review.

VII. Publications (all publications are enclosed in the body of the final report)

1. D. Vashdi, N.R. Staten, E. Sakal, G.G. Krivi and A. Gertler. N-terminus-truncated recombinant analogues of bovine placental lactogen: interaction with receptors of human growth hormone and somatogen receptor mediated IGF-I secretion in rat hepatocytes. *Endocrinology* 136, 1258-1266. (1995).
2. D. Vashdi-Elberg, N.R. Staten, E. Sakal, R.E. McKinnie, J. Djiane, G.G. Krivi and A. Gertler. Selective modification of recombinant bovine placental lactogen by site-directed mutagenesis at its C-terminus. *J. Biol. Chem.* 271:5558-5564 (1996).
3. D. Helman, N.R. Staten, J. Byatt, J. Grosclaude, R.E. McKinnie, A. Shamay, J. Djiane and A. Gertler Site-directed mutagenesis of recombinant bovine placental lactogen at lysine-73 leads to a selective reduction of its somatogenic activity. *Endocrinology* (October 1997, in press).
4. D. Helman, N.R. Staten, J. Grosclaude, C. Nespoulous, J. Djiane and A. Gertler. Novel recombinant analogues of bovine placental lactogen: G133K/R provide a tool to understand the difference between the action of prolactin and growth hormone receptors. *J. Biol. Chem* (submitted).

VII. Abstracts

1. Spiers, D.E., P.A. Eichen, R.L. Matteri, and J.C. Byatt. 1995. Recombinant bovine somatotropin stimulation of growth in prepubertal rats at different ambient temperatures. *J. Animal Science* 73 (Supp. 1): 150.
2. Spiers, D.E., P.A. Eichen, E.L. Robinson, R.L. Matteri, J.C. Byatt. Treatment of adult rat with recombinant bovine somatotropin increases growth during heat challenge. *FASEB J.* 10: A113, 1996.
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somatotropin. J. Dairy Sci. 80 (Suppl. 1): 177.

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7. Eichen, P.A., D.E. Spiers, K.E. Westhoff, and J.C. Byatt. 1997. Recombinant bovine prolactin effects in prepubertal and adult rats at different ambient temperatures. J. Anim. Sci. 75 (Suppl. 1): 158.

VIII. Departmental Reports

1. Eichen, P.A., D.E. Spiers, E.L. Robinson, and R.L. Matteri. 1997. Recombinant bovine placental lactogen effects in prepubertal and adult rats at different ambient temperatures. Animal Sciences Departmental Report, University of Missouri-Columbia, pp. 15-18.
2. Spiers, D.E., P.A. Eichen, and R.L. Matteri. 1997. Age-related differences in the effect of bovine somatotropin administered to rats in different thermal environments. Animal Sciences Departmental Report, University of Missouri-Columbia, pp. 35-39.
3. Spiers, D.E. J.N. Spain, and B.L. Snyder. 1997. Stimulation of milk production in dairy cows during heat stress using recombinant bovine somatotropin. Animal Sciences Departmental Report, University of Missouri-Columbia, pp. 175-180.

IX. Thesis (Some material gathered from this project was used to generate a portion of the following thesis)

1. Lu, M. 1996. Influence of heat stress and calving on antioxidant activity in bovine blood. Department of Animal Science, Univ. Of Kentucky.