

Growth hormone improves lipoprotein concentration and arylesterase activity in mice with an atherogenic lipid profile induced by lactalbumin

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The effect of growth hormone (GH) on arylesterase (AE), one of the activities of paraoxonase, has never been studied. The aims of the present study in mice were: (a) to compare the effect of age and sex on serum lipid and lipoprotein levels after consumption of lactalbumin- v. chow-based diets and (b) to study the effect of GH administration, age and sex on serum AE activity, lipid and lipoprotein and body fat levels in mice fed a lactalbumin diet. Seventy-two mice were divided into three age- and sex-matched experimental groups: (1) control chow (CC), (2) non-GH lactalbumin (NGL) and (3) GH-treated lactalbumin (GL) mice. Lactalbumin increased total cholesterol, (LDL + VLDL)-cholesterol and TAG and diminished HDL-cholesterol in all animals ($P < 0.05$). In comparison with their NGL counterparts, old GL males presented lower total cholesterol (15%) and (LDL + VLDL)-cholesterol (17%) levels ($P < 0.05$), whereas values of the same parameters were higher in adult GL males ($P < 0.05$) (22 and 23%, respectively). Adult GL females displayed higher serum HDL-cholesterol concentrations (26%) ($P < 0.05$) than adult NGL females. AE activity was lower in old GL females (78%) and old GL males (20%) ($P < 0.05$), but higher in adult GL males (100%) ($P < 0.01$). GH, that was inversely related to food intake, decreased abdominal and gonadal fat in all mice ($P < 0.05$). To conclude, lactalbumin induced an atherogenic lipoprotein profile in NGL mice that was reverted by GH, preferentially in old males, suggesting that GH therapy will be more effective in aged men. The present results suggest that AE activity was age-, sex- and body fat level-dependent and that it diminished as a consequence of improved antioxidant status.

Ageing: Arylesterase: Body fat deposits: Growth hormone: Lactalbumin: Lipoproteins

The effect of dietary protein on lipid metabolism and atherosclerosis has been extensively tested^(1,2). Most of the studies have compared the effect of casein and soya proteins in young animal models (mainly rabbits and rats)^(3,4), while only a few have been performed in adult mice and no information is available for old mice⁽⁵⁾. It has been suggested that lactalbumin is the most atherogenic animal protein in rabbits⁽³⁾, though no data are available for adult and old mice.

The BALB/c mouse strain has been widely used to study the effect of atherogenic diets⁽⁶⁾ and has also been used in some growth hormone (GH) studies⁽⁷⁾. GH is known to exert important regulatory effects on cholesterol metabolism in mice⁽⁸⁾. Both decreased production and increased production of GH have been associated with increased frequency of CVD⁽⁸⁾. GH treatment in hypophysectomised LDL-receptor-deficient mice decreased total cholesterol (TC) and LDL-cholesterol levels⁽⁷⁾. Transgenic mice overexpressing GH show high TC and LDL-cholesterol concentrations and low TAG levels⁽⁹⁾. The effects of GH treatment on lipoproteins

in mice with normal GH secretion have not been studied previously.

The age-related decline in pituitary GH secretion has been linked to many alterations associated with ageing⁽¹⁰⁾ such as fat deposition and detrimental alterations in lipoprotein metabolism and lipid peroxidation impairments. Total-body and abdominal obesity are known to be risk factors of increased CVD morbidity and mortality⁽¹¹⁾. It has been established that GH can increase lean body mass and reduce adipose tissue mass in elderly subjects and obese animal models^(12–14). Results of GH administration on lipoprotein metabolism in healthy and elderly individuals have been contradictory^(15,16) and its use has also produced many side effects, including insulin resistance and probably diabetes⁽¹²⁾. Furthermore, sex hormones affect the results of GH administration differently in males and females⁽¹⁷⁾.

Although the mechanisms underlying the ageing process are poorly understood, forms of DNA, proteins and lipids altered by oxidation found in aged organisms support the hypothesis

Abbreviations: AE, arylesterase; CC, control chow; GH, growth hormone; GL, GH-treated lactalbumin; NGL, non-GH lactalbumin; TC, total cholesterol.

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that an increased oxidative status may be associated with ageing⁽¹⁸⁾. Epidemiological studies in GH-deficient subjects⁽¹⁹⁾ and GH-transgenic mice⁽²⁰⁾ indicate that these animals display reduced expression and/or activity of enzymes involved in antioxidant defences and increased lipid peroxidation. Lipid peroxidation and high LDL-cholesterol serum levels have been known to increase with age, thus putting the elderly at greater risk of atherosclerosis and CVD⁽²¹⁾. It has been suggested that HDL-bound paraoxonase 1 (*EC* 3.1.1.2), an enzyme that exhibits arylesterase (AE) activity, is involved in lipoprotein-phospholipid metabolism, and could inhibit lipid peroxide generation in LDL^(22,23). Thus, GH treatment of aged animals could improve their antioxidant status by increasing AE activity and helping to maintain a less atherogenic lipoprotein profile. Nonetheless, GH has been reported to inhibit expression of the paraoxonase 1 hepatic gene⁽²⁴⁾. To the best of our knowledge, the effect of the administration of GH on AE activity in adult and old mice has never been tested.

The aims of the present study in mice were: (a) to compare the effect of age and sex on serum lipid and lipoprotein-cholesterol levels after consumption of lactalbumin- v. chow-based diets; (b) to study the effect of GH administration, age and sex on serum AE activity, lipid and lipoprotein-cholesterol and body fat levels in mice fed a lactalbumin diet. Furthermore, the relationships between serum AE activity, serum lipid and lipoprotein-cholesterol concentrations and body fat levels were also tested.

Materials and methods

Materials

Recombinant human GH (Norditropin) was donated by Novo Nordisk Pharma S.A. (Madrid, Spain). Control mouse BALB/c serum employed for the AE calibration curves was purchased from Harlan Laboratories (London, UK). Phenylacetate was obtained from Aldrich (Barcelona, Spain). Phenol was purchased from Panreac (Barcelona, Spain) and purified with a glass oven kugelrohr (Büchi, Flawil, Switzerland). All other chemicals and solvents used were of the highest purity commercially available.

Animals and diets

All procedures were carried out in accordance with the National Institutes of Health guidelines for animal care and experimentation published in the Spanish Royal Order 223/88⁽²⁵⁾. The study was approved by the Institutional Committee for the Care and Use of Laboratory Animals of the Universidad Complutense de Madrid, Spain.

BALB/c mice were obtained from the breeding centre of Departamentos de Nutrición y Fisiología de la Facultad de Farmacia de la Universidad Complutense (Spanish Government licence: ES280790000085) and maintained at the animal facilities until experimental age. Mice were individually housed in metabolism cages (room temperature 23°C ± 1°C and 12 h light–dark cycles) and given free access to water and diets.

Seventy-two mice were fed the A04 chow diet (Panlab S. L., Barcelona, Spain) after weaning. The animals were divided into three age- and sex-matched experimental groups at 6 months

(adults) and 18 months (old mice) of age. Group 1 contained twenty-four control chow (CC) mice who received a commercial chow diet for mouse maintenance (A04; Panlab, Madrid, Spain) and two subcutaneous injections of saline solution daily (at 08.00 and 17.00 hours) for 1 month. Group 2 was composed of twenty-four non-GH lactalbumin (NGL) mice who were fed a 14% lactalbumin diet and administered two subcutaneous injections of saline solution daily (at 08.00 and 17.00 hours) for 1 month. Group 3 consisted of twenty-four GH-treated lactalbumin (GL) mice given a 14% lactalbumin diet and two subcutaneous injections of 2 µg GH/g body weight daily (at 08.00 and 17.00 hours) for 1 month. Each group included twelve adult (six males and six females) and twelve old (six males and six females) mice. On the last day of the trial, when the mice were 7 and 19 months old, respectively, they were anaesthetised by intraperitoneal injection of ketamine (100 mg/kg body weight) plus xylazine (5 mg/kg body weight), and blood was drawn via retro-orbital plexus puncture. The mice were euthanised by decapitation. Serum was separated from whole blood by centrifugation (1500g; 10 min; 4°C) and stored at –80°C until use.

The main characteristics of the control chow diet were: moisture, 11.9%; crude protein, 16.1%; crude oil, 3.1%; crude fibre, 3.9%; N-free extract, 60.0% (of which starch, 45.8% and total sugars, 2.0%); total minerals, 5.1%; vitamin A, 2 mg (6600 IU)/kg; vitamin D₃, 22.5 µg (900 IU)/kg; vitamin E, 30 mg/kg. The experimental diet containing lactalbumin was formulated according to AIN-93M standards⁽²⁶⁾, substituting lactalbumin for casein while maintaining all other components (Table 1).

In NGL and GL mice, peri-intestinal, peritoneal and perirenal fat was excised and was referred to as 'abdominal fat'. Periovarian and periepididymal fat pads were cut free and were referred to as 'gonadal fat'. After dissection, fat pads were weighed and frozen at –80°C.

Serum measurements

Serum of all three mice groups (CC, NGL, GL) were analysed for TC, HDL-cholesterol and TAG with enzymic colorimetric commercial kits (Biolabo S.A., Materlab, Madrid, Spain). The estimation of the non-HDL-cholesterol ((LDL + VLDL)-cholesterol) was calculated by the equation: (LDL + VLDL)-cholesterol = TC – HDL-cholesterol. The TC:HDL-cholesterol and the (LDL + VLDL)-cholesterol:HDL-cholesterol ratios were employed as cardiovascular risk indexes.

Table 1. Composition of semi-synthetic experimental lactalbumin diet

Ingredient	Lactalbumin diet (g/kg diet)
Lactalbumin	140
Maize starch	465.692
Dextrinised maize starch	155
Sucrose	100
Cellulose	50
Mineral mix (AIN-93M)	35
Vitamin mix (AIN-93VX)	10
L-Cystine	1.50
L-Arginine	0.70
Choline bitartrate	2.5
t-Butylhydroquinone	0.0008
Soyabean oil	40

Serum arylesterase activity

Serum AE activity of NGL and GL mice was measured using the method of Nus *et al.* (27), with simulated body fluid as the buffer and phenylacetate (0.25 mM) as the substrate after adaptation to the mouse model (28). The molar absorption coefficient of phenol, determined spectrophotometrically at 270 nm, 25°C and pH 8, was 1297/M per cm. A quantity of 1 unit AE activity is equal to 1 mol phenylacetate hydrolysed/litre per min. The AE:HDL-cholesterol ratio was employed as a potential marker of HDL antioxidant capacity.

Statistical analysis

Data are presented as mean values and standard deviations. Spearman correlation coefficients were used to evaluate the relationships between continuous variables. CC and NGL mice data were initially evaluated using two-way ANOVA. When significant differences were detected the minimum significant difference (MSD) procedure was used to assess the differences between the groups with a prespecified *P* value of 0.05. Three-way ANOVA was employed to study the effect of treatment, sex and age. An unpaired Student's *t* test was employed to analyse the effect of GH treatment, segmenting data by sex and age groups. The Kruskal–Wallis H test was employed to analyse data of TAG and AE activity because they were not distributed normally. Non-parametric multiple comparisons were made using a modification of the Hollander and Wolfe formula by Sánchez *et al.* (29) using SAS 7.0 statistical software (SAS Institute, Inc., Cary, NC, USA). All statistical analyses were performed using the statistical package SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). Progress curves of the AE reactions were fitted to first-order kinetics using the SIMFIT 5.4 statistical package (Manchester University, Manchester, UK) (30).

Results

Effect of age, sex, diet and treatment on feed intake

Fig. 1 shows the feed intake of CC, NGL and GL mice. No significant differences were found for feed intake between CC and NGL mice (Fig. 1). Furthermore, GH administration increased feed intake in adults, and this effect was more important in males (*P*<0.05) (Fig. 1).

Effects of age and sex on serum lipoproteins and TAG in control chow mice

Old CC mice (males and females) had higher TC, (LDL + VLDL)-cholesterol and TAG, and lower HDL-cholesterol levels than adult CC mice (males and females) (*P*<0.05) (Fig. 2). A significant age × sex interaction was found for TC (*P*<0.001) (Fig. 2). Old CC females and adult CC males had significantly higher TC levels than adult CC females (*P*<0.05) (Fig. 2).

Effect of dietary protein on serum lipoprotein levels

In comparison with their CC counterparts, all NGL mice (males, females, adults and old mice) displayed higher TC and (LDL + VLDL)-cholesterol (*P*<0.05) and lower

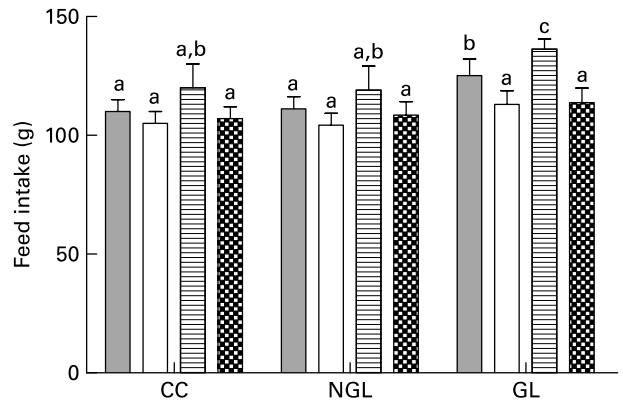


Fig. 1. Feed intake of control chow (CC), non-growth hormone lactalbumin (NGL) and growth hormone lactalbumin (GL) mice. (■), Adult females; (□), old females; (▤) adult males; (▥) old males. Values are means, with standard deviations represented by vertical bars. ^{a,b,c} Mean values with unlike letters were significantly different (*P*<0.05; one-way ANOVA followed by the DMS test).

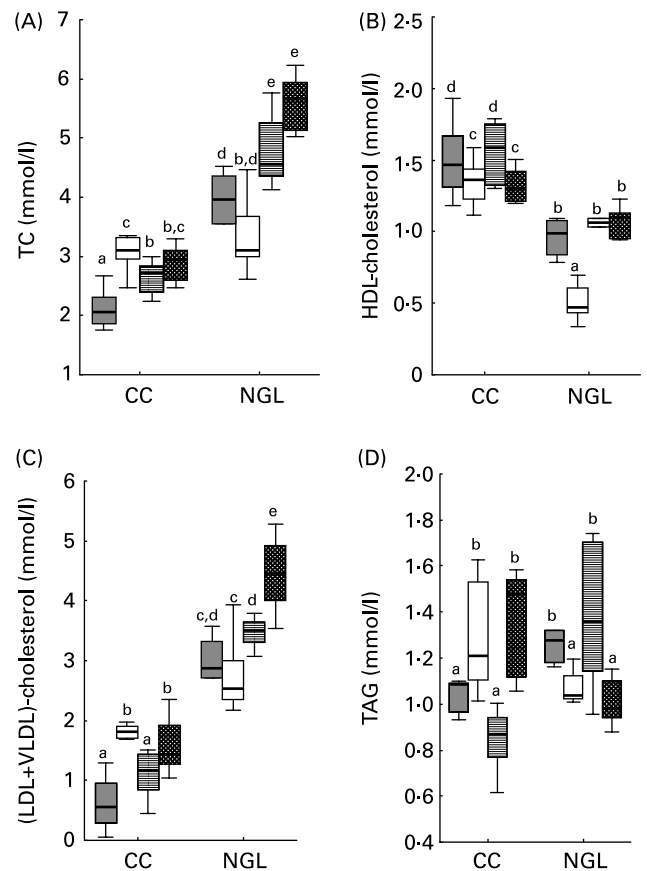


Fig. 2. Serum total cholesterol (TC) (A), HDL-cholesterol (B), (LDL + VLDL)-cholesterol (C) and TAG (D) levels of control chow (CC) and non-growth hormone lactalbumin (NGL) mice. (■) Adult females; (□), old females; (▤), adult males; (▥), old males. Each box represents the median, and 25th and 75th percentiles; lines outside the box correspond to the maximum and minimum values of the analysed variables. ^{a,b,c,d,e} Median values with unlike letters were significantly different (*P*<0.05; one-way ANOVA followed by the DMS test and Kruskal–Wallis H test followed by non-parametric multiple comparisons (29)). For details of procedures, see Materials and methods.

HDL-cholesterol ($P < 0.05$) levels, while adult NGL mice presented higher TAG levels ($P < 0.001$) and old NGL mice had lower TAG levels ($P < 0.05$) (Fig. 2).

Effects of age and sex on serum arylesterase activity, serum lipoprotein and body fat levels in non-growth-hormone lactalbumin mice

A significant sex \times age interaction was found in NGL mice for serum TC, HDL-cholesterol, (LDL + VLDL)-cholesterol concentrations, the TC:HDL-cholesterol ratio, and quantities of gonadal and abdominal fat (Fig. 2 and Table 2). Old NGL females had significantly lower serum HDL-cholesterol ($P < 0.001$) and TAG ($P < 0.05$) concentrations and higher TC:HDL-cholesterol and (LDL + VLDL)-cholesterol:HDL-cholesterol ratios ($P < 0.01$) than adult NGL females (Fig. 2 and Table 2). Old NGL males had significantly higher serum (LDL + VLDL)-cholesterol concentrations ($P < 0.05$), serum AE activity levels ($P < 0.01$), AE:HDL-cholesterol ratio ($P < 0.01$), gonadal fat ($P < 0.01$) and significantly lower serum TAG ($P < 0.001$) levels than adult NGL males (Fig. 2 and Table 2). Serum AE activity was higher in adult females than in adults males ($P < 0.01$), while the opposite was observed in old male mice, whose AE was higher than that of the old females ($P < 0.001$).

NGL males (adult + old) showed significantly higher serum TC, HDL-cholesterol and (LDL + VLDL)-cholesterol concentrations ($P < 0.05$), and a lower AE:HDL-cholesterol ratio (data not shown) than NGL females (adult and old), in addition to a smaller amount of abdominal and gonadal fat ($P < 0.05$). Old NGL mice (males and females) had higher AE activity levels ($P < 0.01$) and TC:HDL-cholesterol, (LDL + VLDL)-cholesterol:HDL-cholesterol, AE:HDL-cholesterol ratios ($P < 0.05$) (data not shown), and lower HDL-cholesterol ($P < 0.05$) and TAG ($P < 0.001$) concentrations than adult NGL mice.

Effect of growth hormone administration on arylesterase activity, lipoproteins-cholesterol and body fat levels

Three-way ANOVA revealed significant main effects of age, sex and treatment, as well as significant double (age \times treatment, sex \times treatment) and triple (age \times sex \times treatment) interactions for the variables studied. To clarify all these main effects and interactions, the percentage of change attributable to the effect of treatment for every group was calculated using the next expression ((NGL mean – GL mean) \times 100)/NGL mean). All results are summarised in Table 3.

A triple significant age \times sex \times treatment interaction was found for serum TC ($P < 0.05$), (LDL + VLDL)-cholesterol ($P < 0.05$) and gonadal fat ($P < 0.01$). TC and (LDL + VLDL)-cholesterol levels were significantly higher in adult GL males (22 and 23 %, respectively) and significantly lower in old GL males (15 and 17 %, respectively) than in their NGL counterparts (Table 3). The amount of gonadal fat in all GL mice except adult males was significantly lower (26–75 %) than that of the NGL mice (Table 3).

A significant age \times treatment interaction was found for serum HDL-cholesterol ($P < 0.01$) concentrations and abdominal fat ($P < 0.05$). GH administration resulted in significantly higher serum HDL-cholesterol concentrations (23 %) in adults and, particularly, in adult females (26 %) than in

Table 2. Effects of age and sex on serum total cholesterol (TC);HDL-cholesterol, (LDL + VLDL)-cholesterol and arylesterase activity (AE);HDL-cholesterol ratios and body fat levels in non-growth-hormone lactalbumin mice (Means and standard deviations for six animals per group)

	Adult mice				Old mice				ANOVA		
	Female		Male		Female		Male		Sex	Age	Sex \times age
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
TC:HDL-cholesterol ratio (LDL + VLDL)-cholesterol:HDL-cholesterol ratio	4.22 ^a	0.81	4.67 ^a	0.79	6.91 ^b	1.90	5.28 ^a	0.47	NS	**	*
AE:HDL-cholesterol ratio (units/mmol)	3.20 ^a	0.68	3.40 ^{a,c}	0.25	5.77 ^b	1.73	4.27 ^{b,c}	0.48	NS	**	NS
Abdominal fat (g)	58.60 ^a	37.41	15.42 ^b	4.44	93.37 ^a	42.63	77.95 ^a	9.05	NS	**	NS
Gonadal fat (g)	0.21 ^a	0.02	0.12 ^b	0.03	0.16 ^b	0.04	0.15 ^b	0.04	***	NS	**
	0.57 ^a	0.14	0.29 ^b	0.07	0.44 ^c	0.08	0.43 ^c	0.04	***	NS	***

^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P < 0.050$; one-way ANOVA followed by the DMS test).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (two-way ANOVA).

Table 3. Effects of recombinant human growth hormone treatment on serum TAG, total cholesterol (TC) and lipoprotein-cholesterol concentrations, arylesterase activity (AE), and gonadal fat (GF) and abdominal fat (AF) fat levels in growth-hormone-treated lactalbumin (GL) mice (Mean values and standard deviations)

	n	TC (mmol/l)		LDL + VLDL-cholesterol (mmol/l)		HDL-cholesterol (mmol/l)		TAG (mmol/l)		AE (units/l)		GF (g)		AF (g)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Adult mice															
NGL	12	4.44	0.67	3.32	0.53	1.01†	0.12	1.33†	0.24	36.22†	27.84	0.37	0.18	0.16	0.05
GL	12	4.96	1.03	3.60	1.02	1.24	0.28	1.59	0.25	30.52	10.74	0.25	0.06	0.05	0.03
%‡		-12		-8		-23*		-20***		16		40**		61***	
Old mice															
NGL	12	4.39	1.28	3.61	1.06	0.76	0.31	1.04	0.09	63.62	23.93	0.44	0.07	0.16	0.04
GL	12	4.00	0.87	3.23	0.70	0.76	0.32	1.24	0.17	38.52	30.41	0.32	0.07	0.08	0.03
%		9		10		0		-18***		39*		30**		53***	
Females															
NGL	12	3.66§	0.58	2.93§	0.52	0.71§	0.26	1.16	0.11	50.47	24.51	0.51§	0.12	0.18§	0.04
GL	12	3.84	0.87	2.94	0.74	0.83	0.40	1.36	0.17	16.67	17.96	0.28	0.09	0.07	0.03
%		-5		0		-18		-17***		67***		48**		59***	
Males															
NGL	12	5.16	0.72	4.06	0.73	1.07	0.10	1.22	0.31	50.47	34.20	0.31	0.09	0.14	0.04
GL	12	5.28	0.66	4.05	0.59	1.16	0.28	1.46	0.37	49.25	18.68	0.30	0.04	0.06	0.04
%		-2		0		-8		-21*		2		3		52***	
Adult females															
NGL	6	3.98 ^a	0.40	3.01 ^a	0.35	0.96 ^a	0.13	1.26 ^a	0.07	54.41 ^a	29.89	0.57 ^a	0.14	0.21 ^a	0.02
GL	6	4.39 ^{a,b}	0.86	3.13 ^a	0.85	1.21 ^b	0.26	1.45 ^b	0.08	22.99 ^a	1.60	0.23 ^b	0.08	0.07 ^b	0.03
%		-10		-4		-26*		-15***		58		60**		67***	
Adult males															
NGL	6	4.78 ^b	0.64	3.59 ^{a,c}	0.52	1.07 ^{a,b}	0.10	1.39 ^{a,b}	0.32	18.04 ^b	4.30	0.29 ^b	0.29	0.12 ^c	0.03
GL	6	5.82 ^c	0.63	4.41 ^b	0.74	1.27 ^b	0.32	1.81 ^c	0.24	36.17 ^a	11.29	0.28 ^b	0.01	0.03 ^b	0.01
%		-22**		-23*		-19		-30**		-100**		-3		75***	
Old females															
NGL	6	3.42 ^{a,d}	0.61	2.86 ^a	0.63	0.52 ^c	0.13	1.08 ^d	0.07	47.09 ^a	20.71	0.44 ^c	0.08	0.16 ^{ac}	0.04
GL	6	3.30 ^d	0.47	2.75 ^a	0.56	0.55 ^c	0.19	1.27 ^a	0.18	10.36 ^b	6.42	0.32 ^b	0.09	0.07 ^b	0.03
%		4		4		-6		-18***		78**		27**		56***	
Old males															
NGL	6	5.68 ^c	0.49	4.60 ^b	0.54	1.08 ^{a,b}	0.11	0.99 ^d	0.10	82.90 ^c	5.56	0.43 ^c	0.04	0.15 ^c	0.04
GL	6	4.85 ^c	0.20	3.80 ^c	0.28	1.05 ^{a,b}	0.0-0.19	1.20 ^a	0.16	66.68 ^a	9.59	0.32 ^b	0.04	0.09 ^b	0.02
%		15*		17**		3		-21*		20*		26**		40***	

NGL, non-growth-hormone lactalbumin.

^{a,b,c,d} Mean values within a column with unlike superscript letters were significantly different ($P < 0.05$; one-way ANOVA followed by the DMS test and Kruskal–Wallis H test followed by non-parametric multiple comparisons⁽²⁹⁾).

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (unpaired Student's *t* test for parametric variables and Kruskal–Wallis H test for TAG and AE).

† Significantly different from their old NGL counterparts.

‡ Percentage change is calculated as ((NGL mean – GL mean)/NGL mean) × 100.

§ Significantly different from their male NGL counterparts.

NGL mice (Table 3). The amount of abdominal fat in adult and old GL mice (61 % and 53 %, respectively) was significantly lower than that of NGL animals (Table 3).

A significant sex × treatment interaction was found for gonadal and abdominal fat ($P < 0.05$). GL mice of both sexes displayed less abdominal fat than their NGL counterparts, although this was more noticeable in the females (59 %) than in the males (52 %) (Table 3). Female GL mice had lower gonadal fat (48 %) than their NGL counterparts.

AE was significantly lower in old (39 %) and female (67 %) GL mice than in NGL mice (Table 3). Compared with NGL animals, AE concentrations were significantly lower in old GL females (78 %) and males (20 %) but were higher in adult GL males (100 %) (Table 3).

Serum TAG concentrations were higher (15–30 %) ($P < 0.05$) in all GL mice (Table 3). TC:HDL-cholesterol, (LDL + VLDL)-cholesterol:HDL-cholesterol and AE:HDL-cholesterol ratios did not differ significantly between GL and NGL groups except in old GL male mice, whose

AE:HDL-cholesterol ratio was significantly lower ($P < 0.050$) than that of their NGL counterparts (data not shown).

Relationships between arylesterase activity and lipid and lipoprotein-cholesterol concentrations and amount of body fat in non-growth-hormone lactalbumin and growth-hormone-treated lactalbumin mice

Table 4 shows the correlations of serum AE activity levels, serum lipid and lipoprotein-cholesterol concentrations and amounts of abdominal and gonadal fat in NGL and GL mice. Serum AE activity was positively correlated with serum TC and (LDL + VLDL)-cholesterol concentrations and negatively correlated with serum levels in all NGL animals. In adult NGL mice, serum AE activity was negatively correlated with serum TC and (LDL + VLDL)-cholesterol levels in males and positively correlated with these same levels in NGL females while in the adult GL males, AE activity only displayed a significant positive correlation with

Table 4. Plasma arylesterase activity correlations with lipoprotein-cholesterol concentrations and body fat levels in non-growth hormone lactalbumin (NGL) and growth hormone-treated lactalbumin (GL) mice

	n	NGL		GL	
		r	P	r	P
All	24				
Total cholesterol (mmol/l)		0.31	NS	0.33	*
HDL-cholesterol (mmol/l)		0.15	NS	0.08	NS
(LDL + VLDL)-cholesterol (mmol/l)		0.48	*	0.42	**
TAG (mmol/l)		-0.44	*	-0.35	**
Abdominal fat (g)		0.22	NS	0.28	NS
Gonadal fat (g)		0.36	NS	0.30	NS
Adult males	6				
Total cholesterol (mmol/l)		0.99	***	-0.42	NS
HDL-cholesterol (mmol/l)		-0.4	NS	0.37	NS
(LDL + VLDL)-cholesterol (mmol/l)		-0.99	***	-0.11	NS
TAG (mmol/l)		-0.36	NS	0.75	NS
Abdominal fat (g)		-0.1	NS	-0.10	NS
Gonadal fat (g)		-0.86	*	-0.67	NS
Old males	6				
Total cholesterol (mmol/l)		0.14	NS	-0.75	NS
HDL-cholesterol (mmol/l)		-0.72	NS	0.27	NS
(LDL + VLDL)-cholesterol (mmol/l)		0.11	NS	-0.46	NS
TAG (mmol/l)		-0.36	NS	-0.12	NS
Abdominal fat (g)		-0.11	NS	-0.99	***
Gonadal fat (g)		-0.83	*	0.99	***
Adult females	6				
Total cholesterol (mmol/l)		0.91	**	-0.84	*
HDL-cholesterol (mmol/l)		-0.26	NS	0.27	NS
(LDL + VLDL)-cholesterol (mmol/l)		0.91	**	-0.21	NS
TAG (mmol/l)		-0.91	**	-0.60	NS
Abdominal fat (g)		0.50	NS	0.48	NS
Gonadal fat (g)			NS	0.46	NS
Old females	6				
Total cholesterol (mmol/l)		0.99	***	0.50	NS
HDL-cholesterol (mmol/l)		0.35	NS	0.19	NS
(LDL + VLDL)-cholesterol (mmol/l)		0.97	***	0.80	*
TAG (mmol/l)		0.35	NS	-0.50	NS
Abdominal fat (g)		0.20	NS	0.22	NS
Gonadal fat (g)		0.20	NS	0.15	NS

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Spearman's correlation).

serum TAG levels. In adult and old NGL males, AE was also negatively correlated with gonadal fat. In adult NGL females, serum AE activity was negatively correlated with serum TAG levels. In old GL male mice, AE activity was positively correlated with gonadal fat and negatively correlated with abdominal fat. In old NGL and GL females, serum AE activity was significantly correlated with serum TC and (LDL + VLDL)-cholesterol concentrations.

Discussion

TC and lipoprotein-cholesterol levels of CC adult mice coincide with those of other mice^(6,31) where HDL, which carried at least 60% of TC, was found to be the most abundant lipoprotein. TAG levels of CC adult mice concurred with those obtained by Moen *et al.*⁽³²⁾. Moreover, as expected, lipoprotein-cholesterol and TAG levels increased in CC old mice⁽³³⁾.

The inclusion of 14% lactalbumin in the murine diet induced an atherogenic lipoprotein profile in NGL mice in comparison with their CC counterparts. Non-HDL-cholesterol concentrations in adult NGL mice were four to six times

higher than those of HDL-cholesterol, similar to those reported by Le Boeuf *et al.*⁽³⁴⁾ in the same strain of mice given a high-fat diet. It has been suggested that animal proteins, such as lactalbumin, promote hypercholesterolaemia and atherosclerosis, while plant proteins, such as soya proteins, with a low lysine:arginine ratio, have hypocholesterolaemic and atheroprotective properties^(35,36).

Sex and age affected the lipoprotein-cholesterol concentrations of NGL mice. Oestrogens have been known to act as antioxidants, while testosterone has been shown to inhibit lipoprotein lipase and to increase LDL-cholesterol^(37,38), explaining, at least in part, the present results. Although, in different ways, the serum lipid profile of both NGL and CC mice worsened with age. In fact, old NGL males displayed higher (LDL + VLDL)-cholesterol concentrations than adult NGL males while old NGL females presented lower HDL-cholesterol levels than adult NGL females. These sex- and age-related changes should be associated with the decrease in sex hormone levels in both old NGL males and females^(39,40). As previously indicated, reduced testosterone levels in elderly males would explain the increase in (LDL + VLDL)-cholesterol. The reduction in oestrogen concentrations

after the menopause has been found to lower HDL-cholesterol concentrations in women⁽³⁷⁾.

The fact that old NGL mice presented lower TAG values than adults is not easy to explain, taking into account that ageing is normally associated with high TAG levels in man⁽²¹⁾. It is known that hypercholesterolaemic mice present low TAG levels⁽⁶⁾; thus, the decrease of TAG with age should be related to the presence of cholesterol-enriched VLDL. In fact, the present data on TC, TAG and (LDL + VLDL)-cholesterol suggest that the cholesterol content of the VLDL fraction increases in old NGL mice, producing cholesterol-enriched VLDL particles (for example, β -VLDL).

In concordance with other studies in mice⁽⁴¹⁾, adult NGL females showed higher AE activity than adult NGL males. These differences should be ascribed to their different lipoprotein profiles and body fat contents, as suggested by the data and the different significant correlations observed. However, while AE activity increased in old NGL males, it remained stable in old NGL females. Thus, the sex-related differences in AE activity observed in the adult NGL mice were inverted with ageing. These findings coincide with recent results in rats⁽⁴²⁾. The explanation for this age–sex interaction on AE activity may be that AE activity is constant in old NGL females despite reduced HDL-cholesterol levels. Thus, VLDL-cholesterol may stabilise paraoxonase 1 activity, as proposed by Deakin *et al.*⁽⁴³⁾. This hypothesis is supported by the strong positive correlation found in the present study between AE and (VLDL + LDL)-cholesterol in old NGL females. Therefore, as Thomás-Moyá *et al.*⁽⁴²⁾ suggest, and the negative correlation between AE activity and (VLDL + LDL)-cholesterol in these animals supports, the increase in AE activity in old NGL males may be due to a mechanism designed to counterbalance the age-related increase in (VLDL + LDL)-cholesterol concentrations.

The AE:HDL-cholesterol ratio should be considered an indicator of the antioxidant potential of HDL. In fact, adult NGL mice had lower ratios than old NGL mice, and NGL females had higher ratios than NGL males, suggesting a powerful age- and sex-related response against peroxidation.

The present results show for the first time that GH administration in mice interacts with age and sex hormones, resulting in different effects on lipids, lipoproteins and AE in young and old and male and female mice with an atherogenic lipid profile induced by lactalbumin. In contradiction to the findings of other trials⁽⁸⁾, the effects of GH on TC, (LDL + VLDL)-cholesterol and gonadal fat were age and sex dependent. These effects were in general more important in males than in females, probably due to the negative modulating effect of oestrogens on GH action⁽⁴⁴⁾. Furthermore, in accordance with other studies^(8,45,46), the present results demonstrate that exogenous GH administration improved the atherogenic profile of old GL males, while it worsened the lipoprotein profile of adult GL males.

Contradictory results have been published regarding the effect of GH on HDL metabolism, including both a decrease⁽⁴⁵⁾ and an increase in HDL-cholesterol levels⁽⁴⁶⁾. Nevertheless, the present results on HDL-cholesterol coincide with those of Bänsch *et al.*⁽³⁷⁾, who found a positive correlation between GH and HDL-cholesterol concentrations in healthy women but not in men. Bänsch *et al.*⁽³⁷⁾ found that synergy exists between GH and oestrogens with regard to the regulation of HDL metabolism, explaining why levels of

HDL-cholesterol in the present study increased only in adult and not in old GL female mice.

To the best of our knowledge, this is the first time that AE activity has been related to body fat content. The strong negative correlation found between AE activity and gonadal fat in NGL adult and old male mice is very interesting. At present, we have no clear hypothesis to explain these results, which are probably related to the high (LDL + VLDL)-C levels and the binding of AE to the VLDL fraction⁽⁴³⁾. A large gonadal fat deposit has previously been related to high VLDL concentrations in mice⁽⁴⁴⁾. Furthermore, since administration of GH is known to affect abdominal fat first⁽¹²⁾, the strong negative correlation observed between AE activity and abdominal fat in old GL males suggests that AE could represent a new marker of adiposity in old GL male mice.

The reduction in abdominal and gonadal fats in old GL mice concurred with results previously described in rats and human subjects⁽⁴⁷⁾ and may be related to the parallel decrease of AE activity in these animals, and could also be due to reduced lipid peroxidation, as previously demonstrated in old Wistar rats⁽⁴⁸⁾. GL females displayed low AE activity and these females also displayed the lowest amount of abdominal fat. Thus, we conclude that differences regarding AE activity between the sexes in both NGL and GL mice were related to differences in body fat levels.

Noteworthy is the observation of the diminution of body fat levels due to GH administration despite the increase in feed intake. Previous studies have demonstrated that the effect of GH on food intake is species, age and sex dependent^(49,50). Our own study shows that GH administration diminished body fat levels in all animals independently of age and sex, and paradoxically there was an increased feed intake in adult mice and to a greater magnitude in males than in females, suggesting important effects of sex and ageing hormone regulation. One interpretation of this finding is that the lowering of adipocyte critical mass due to GH administration would be expected to affect negatively the production of leptin⁽⁵⁰⁾ and in turn induce an increase in food intake.

In short, the results of the present study suggest that the effects of GH on some CVD markers are highly dependent on age and sex. Old male mice seem to be the target for GH treatment, which improves the lipoprotein profile and decreases abdominal and gonadal fat. AE activity in mice diminished as a consequence of improved antioxidant status, as we reported previously in human subjects⁽²³⁾. These results also suggest that GH therapy would be more effective in aged men.

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E. L.-O. and M. N. should be considered as the first author. E. L.-O., A. A.-T. and W. V. took care of the animals and determined lipoproteins, TC and TAG, and helped in the data analysis. M. N. determined AE activity and analysed and discussed all results. J. M. S.-M., E. M.-M. and F. J. S.-M. supervised

and led all experiments and the analysis and discussion of all data.

The authors declare that there is no conflict of interest.

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