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The −514 polymorphism in the hepatic lipase gene (*LIPC*) does not influence androgen-mediated stimulation of hepatic lipase activity

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Abstract The −514T allele of hepatic lipase is associated with increased high density lipoprotein-cholesterol levels in men, but not in women. This observation suggests that the −514C to T polymorphism may diminish the response of hepatic lipase to androgens. To test this hypothesis, five −514T and five −514C homozygous men were treated with the anabolic steroid stanozolol for 6 days. The mean increase in hepatic lipase activity was similar in the two groups (45 ± 10 vs. 51 ± 10 mmol·hr^{-1}·l^{-1}, *P* = 0.5). To evaluate the association between the −514 polymorphism and hepatic lipase activity at different physiological androgen concentrations, hepatic lipase genotypes and activities were measured in 44 men and 40 premenopausal women. The effect of the −514T allele on hepatic lipase activity was significant and quantitatively similar in both sexes. These data indicate that the −514 polymorphism does not influence the response of hepatic lipase activity to androgens, and that the effects of this polymorphism on hepatic lipase activity are independent of androgen action.—Vega, G. L., J. Gao, T. P. Bersot, R. W. Mahley, R. Verstraete, S. M. Grundy, A. White, and J. C. Cohen. **The −514 polymorphism in the hepatic lipase gene (*LIPC*) does not influence androgen-mediated stimulation of hepatic lipase activity. *J. Lipid Res.* 1998.** 39: **1520–1524.**

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A recently identified allele of the hepatic lipase gene (*LIPC*) has been associated with decreased hepatic lipase activity (1) and increased plasma high density lipoprotein-cholesterol (HDL-C) concentrations in men (2). The allele, designated −514T, does not contain mutations in the coding region or intron/exon boundaries, and is defined by four linked polymorphisms in the 5′ flanking region (2). These (or other closely linked) nucleotide substitutions presumably reduce hepatic lipase expression, but the underlying mechanism(s) involved is not known. Interestingly, the −514T allele does not appear to be systematically associated with increased plasma HDL-C concentrations in women (2). This gender dimorphism suggests that the −514 polymorphism may alter the well-characterized stimulation of hepatic lipase activity by androgens (3). If the nucleotide changes in the 5′ flanking region abolish (or significantly attenuate) the response of the hepatic lipase gene to androgens, then men who are homozygous for the −514T allele would retain their prepubertal hepatic lipase activity and HDL-C concentrations into adulthood, and would tend to have lower hepatic lipase activities and higher plasma HDL-C concentrations than do men who are homozygotes for the −514C allele. As androgen levels are low in women, a hepatic lipase allele with decreased androgen responsiveness would be expected to have little effect on their hepatic lipase activity and hence on their plasma HDL-C concentrations.

In the present study we tested this hypothesis by *i*) comparing the responsiveness of hepatic lipase activity to androgen administration in men who are homozygotes for the −514T or the −514C alleles, and *ii*) comparing the relationship between the −514 polymorphism and hepatic lipase activity in men and premenopausal women. Our data indicate that the effect of the −514T allele on hepatic lipase activity is independent of androgen action.

METHODS

The study was approved by the Internal Review Board at the University of Texas Southwestern Medical Center.

Abbreviations: HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; PCR, polymerase chain reaction.

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Subjects

All subjects in this study were apparently healthy men and women who did not have diabetes or use steroid hormones. The effects of the −514 polymorphism on androgen responsiveness of hepatic lipase activity were examined in ten white men (five pairs). One member of each pair of men was homozygous for the −514T allele of hepatic lipase, while the other was homozygous for the −514C allele. The members of each pair were matched for plasma HDL-C concentrations. Each man took 0.2 mg/kg per day of the oral synthetic anabolic steroid stanozolol (Winstrol, Winthrop, Pittsburg, PA) up to a maximum of 16 mg/day for 6 consecutive days. Compliance was monitored by pill counting. Hepatic lipase activity was measured after an overnight fast on the morning before the first dose of stanozolol was ingested, and on the morning after the last dose of stanozolol.

To compare the effects of the −514T allele on hepatic lipase activity in men and women, hepatic lipase activity and genotypes were determined in 44 Turkish men, and in 40 premenopausal Turkish women aged 21 to 45 years. These subjects were participants in the Turkish Heart Study (4) who met the criteria given above, and from whom both postheparin plasma and genomic DNA were available.

Assay of plasma lipoproteins

Plasma concentrations of cholesterol and triglyceride were measured enzymatically using commercial reagents. Plasma HDL-C concentrations were measured by sodium phosphotungstate (0.55 mm) precipitation.

Assay of post-heparin plasma hepatic lipase activity

Hepatic lipase activity was measured in post-heparin plasma as described previously (5).

Western blotting of hepatic lipase protein

Aliquots of postheparin plasma were resolved on 4–10% SDS-polyacrylamide gels and immunoblotted as described (6), using a monoclonal anti-human hepatic lipase antibody (8C9) kindly provided by Dr. Linda Curtiss. Bands were detected by chemiluminescence using a secondary antibody labeled with horseradish peroxidase.

Assay of hepatic lipase polymorphisms

The −514 polymorphism was assayed by polymerase chain reaction (PCR) amplification and restriction digestion as described previously (2).

Assay of hepatic lipase promoter activity

A 3kb fragment of the hepatic lipase 5′ flanking sequence (−2928 to +31) was PCR amplified using genomic DNA from a −514CC homozygote and from a −514TT homozygote. PCR fragments were cloned into the pGL3-Basic vector (Promega Corp., Madison, WI). The fidelity of the inserts was verified by DNA sequencing, and the constructs, together with an internal control (pRL-CMV, Promega) and the rat androgen receptor (kindly provided by Dr. David Russell) were transfected (Superfect, Qiagen Inc., Santa Clarita, CA) into HepG2 cells (ATCC, Rockville, MD). Cells were incubated in DMEM with 10% charcoal/dextran-treated FBS (HyClone, Logan, UT) and 50 nm methyltrienolone (R1881, DuPont NEN, Wilmington, DE), or 100 nm 5α dihydrotestosterone, or vehicle (ethanol) alone. Luciferase activity (Dual-Luciferase Reporter Assay System, Promega Corp) was assayed after 16 h. Parallel experiments were performed using a mouse mammary tumour virus (MMTV)–luciferase reporter construct (kindly provided by Dr. David Mangelsdorf) as a positive control for androgen-mediated stimulation of transcriptional activity.

Statistical methods

In men taking stanozolol, differences in the mean values of all measured parameters were compared between −514C and −514T homozygotes using paired *t*-tests. In Turkish subjects, the mean values of hepatic lipase activity in −514CT heterozygotes and −514C homozygotes were compared using unpaired *t*-tests.

RESULTS

Effects of anabolic steroid administration

Ages, body mass indices, and plasma lipid and lipoprotein concentrations of the 10 men who took stanozolol are given in **Table 1**. All of the men completed the drug treatment regimen and no adverse reactions were reported.

TABLE 1. Age, body mass index, and plasma lipid concentrations in men treated with stanozolol

			TC		TG		LDL-C	
Subject	Age	BMI	Baseline	Stanozol	Baseline	Stanozol	Baseline	Stanozol
					mg/dl			
1) −514C								
1C	23	25	167	149	128	85	115	113
2C	29	26	150	113	149	104	83	72
3C	20	22	122	118	65	49	78	73
4C	27	30	177	177	25	148	126	127
5C	20	23	143	130	69	76	66	83
Mean ± SD	24 ± 4	25 ± 3	152 ± 19	137 ± 23	87 ± 45	92 ± 33	94 ± 23	94 ± 22
2) −514T								
1T	50	32	161	159	102	169	108	114
2T	25	24	182	172	276	131	90	119
3T	25	24	141	133	60	68	85	94
4T	23	22	183	164	58	38	121	122
5T	28	29	147	114	39	18	86	78
Mean ± SD	30 ± 10	26 ± 4	163 ± 17	148 ± 22	107 ± 87	85 ± 57	98 ± 14	105 ± 17

BMI, body mass index; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein-cholesterol.

TABLE 2. Effects of stanozolol on plasma HDL-C concentration and hepatic lipase activity

Subject	HDL		Hepatic Lipase	
	Baseline	Stanozolol	Baseline	Stanozolol
	mg/dl		*mmol·h⁻¹·l⁻¹*	
1) −514CC				
1C	35	20	54	112
2C	37	23	25	87
3C	41	37	35	69
4C	46	23	48	92
5C	55	34	34	89
Mean ± SD	43 ± 7	27 ± 7	39 ± 10[a]	90 ± 14
2) −514TT				
1T	32	21	37	92
2T	33	24	19	58
3T	41	23	12	41
4T	47	31	16	73
5T	54	31	11	55
Mean ± SD	41 ± 8	26 ± 4	19 ± 9	64 ± 17

[a] $P < 0.05$ for −514CC versus −514TT (paired *t*-test).

Stanozolol treatment did not significantly affect plasma triglyceride or low density lipoprotein-cholesterol (LDL-C) concentrations (Table 1). The men were matched for plasma HDL-C concentrations, therefore mean plasma HDL-C levels before stanozolol treatment were similar in −514T and −514C homozygotes (**Table 2**). Stanozolol treatment decreased plasma HDL-C concentrations in all of the men, and the magnitude of the decrease was similar in −514T and −514C homozygotes (Table 2).

Before stanozolol administration, mean hepatic lipase activity was significantly lower in the −514T homozygotes than in the −514C homozygotes (Table 2). Stanozolol administration increased hepatic lipase activity in all of the men (**Fig. 1**). Western blotting with a monoclonal anti-hepatic lipase antibody confirmed that stanozolol administration increased the amount of hepatic lipase protein in postheparin plasma (**Fig. 2**). The increase in hepatic lipase activity (calculated as post-stanozolol activity minus pre-stanozolol activity) was similar in −514T and −514C homozygotes (45 ± 10 vs. 51 ± 10 mmol·hr^{-1}·l^{-1}, $P = 0.5$).

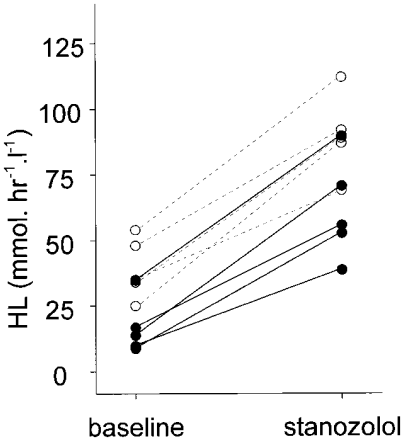

Fig. 1. Effects of stanozolol on post-heparin plasma hepatic lipase activity. Men who were homozygotes for the −514T (●) or −514C (○) allele of *LIPC* were treated for 6 days with stanozolol (0.2 mg·kg^{-1}·day^{-1}).

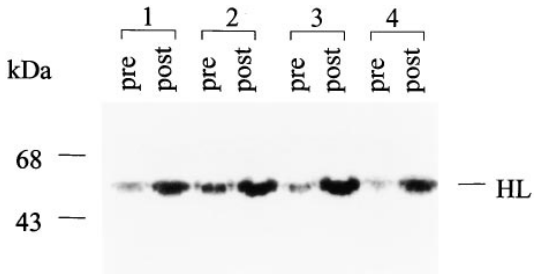

Fig. 2. Hepatic lipase mass in post-heparin plasma increases after stanozolol treatment. Aliquots (2 μl) of post-heparin plasma from four individuals before (pre) and after (post) treatment with stanozolol (0.2 mg·kg^{-1}·day^{-1}), were resolved by 4–10% SDS-polyacrylamide gel electrophoresis and immunoblotted with an anti-human hepatic lipase monoclonal antibody. The positions of molecular weight standards (albumin, 68kDa; ovalbumin, 43 kDa) and hepatic lipase (HL) are indicated.

Association between the −514 polymorphism and hepatic lipase activity in Turkish men and women

Mean hepatic lipase activities were lower in women than in men (**Table 3**). In both sexes, mean hepatic lipase was significantly lower in −514CT heterozygotes than in −514C homozygotes (Table 3). The average difference between −514CT heterozygotes and −514C homozygotes was 7 mmol·hr^{-1}·l^{-1} in women and 9 mmol·hr^{-1}·l^{-1} in men.

Assay of hepatic lipase promoter activity

Methyltrienolone caused a 50-fold increase in transcription from the MMTV promoter, but did not increase transcription from either the −514C or the −514T hepatic lipase promoters (**Fig. 3**). Essentially identical results were obtained in Huh-7 cells, and with 100 nm 5α-dihydrotestosterone (not shown).

DISCUSSION

Androgens increase hepatic lipase activity in humans (3). An androgen-mediated increase in hepatic lipase ac-

TABLE 3. *LIPC* genotypes and hepatic lipase activity in Turkish men and women

LIPC Genotype	Men		Women	
	HL Activity	n	HL Activity	n
	mmol·h⁻¹·l⁻¹		*mmol·h⁻¹·l⁻¹*	
All	51 ± 14[a]	44	35 ± 11	40
−514 CC	55 ± 12[b]	24	37 ± 8[b]	27
−514 CT	46 ± 14	20	30 ± 11	13

Values in the table are means ± SD for hepatic lipase activity; n, number of subjects.

[a] $P < 0.0001$ for men versus women (*t*-test).

[b] $P < 0.05$ for CC versus CT (*t*-test).

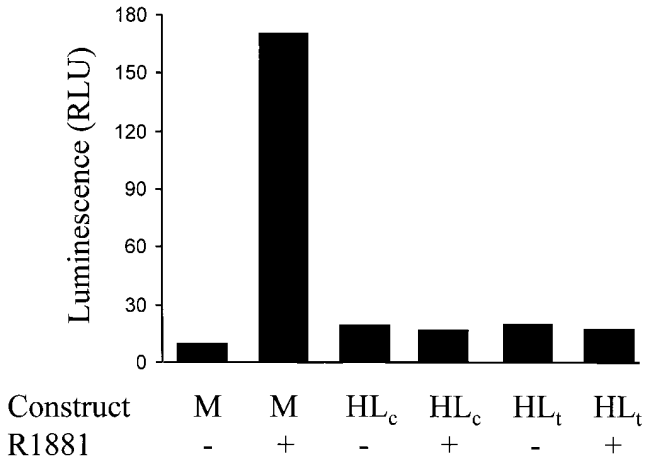

Fig. 3. Effects of methyltrienolone on luciferase activity. Constructs are MMTV-luciferase (M), 3 kb hepatic lipase-luciferase −514C (HL_c) and −514T (HL_t). Cells were grown in DMEM supplemented with 10% charcoal/dextran stripped FBS, and exposed to methyltrienolone (R1881) in ethanol, or ethanol alone for 16 h prior to assay. Luciferase activity from the test constructs was standardized by cotransfection with a control plasmid. Values are means from duplicate dishes. The experiment was repeated 3 times.

tivity appears to be responsible, at least in part (7), for the peri-pubertal decrease in HDL-C concentrations that occurs in males but not in females (8), and hence for the sex-difference in HDL-C concentrations between adult men and women. In the present study we assessed the relationship between androgens, the −514 polymorphism, and hepatic lipase activity in two ways. First, the responsiveness of hepatic lipase to androgen administration was compared in men who were homozygotes for the −514T or −514C allele. The androgen-mediated increase in hepatic lipase activity was very similar in these two groups. Second, the association between hepatic lipase activity and the −514 polymorphism was compared in men and women. The −514T allele was associated with significantly lower hepatic lipase activity both in women and men, and the magnitude of the effect was similar in the two sexes. Taken together, these results indicate that the nucleotide substitutions associated with the −514T allele do not attenuate the response of hepatic lipase to androgens.

To directly assess the effects of the −514 polymorphism on androgen responsiveness we administered stanozolol, a non-aromatizable analog of testosterone, to men who were homozygous for the −514T or the −514C alleles of *LIPC*. The resulting increase in hepatic lipase activity was consistent with the increase observed in a previous study using this drug (9), and was essentially identical in −514T and −514C homozygotes. Western blotting with a monoclonal antibody directed against hepatic lipase confirmed that stanozolol administration increased the amount of hepatic lipase protein in postheparin plasma. This result suggests that the nucleotide substitutions in the −514T allele do not attenuate the effects of androgens on hepatic lipase activity. However, we cannot exclude the possibility that allele-specific differences in androgen response may be observed at lower doses of stanozolol than those used in the present study. Alternatively, stanozolol may not mimic the action of endogenous androgens on hepatic lipase expression. Accordingly we sought to corroborate the results of the stanozolol study by comparing the effect of the −514 polymorphism on hepatic lipase activity in men and women.

The −514T allele was associated with decreased hepatic lipase activity in women, indicating that the −514 polymorphism influences hepatic lipase activity under conditions where endogenous androgen concentrations are very low. The decrease in hepatic lipase activity associated with the −514T allele was quantitatively similar in men and women, and was comparable with the effect observed previously in white men (10). As plasma androgen concentrations are about 20-fold lower in women than in men (11), this finding indicates that the effect of the −514 polymorphism on hepatic lipase activity is similar over a very wide range of endogenous androgen concentrations.

To determine whether the −514T allele showed a decreased transcriptional response to androgens, HepG2 cells were co-transfected with the androgen receptor and with reporter constructs containing 3 kb fragments of the hepatic lipase promoter, and exposed to methyltrienolone, a synthetic androgen. Androgens markedly increased the rate of transcription from the MMTV promoter (which is known to respond to androgens [12]) in this system, but the −514C and the −514T hepatic lipase promoter constructs showed no transcriptional response to androgens under these conditions. Essentially identical results (not shown) were obtained in a different hepatoma-derived cell line (Huh-7), and with a natural androgen (5α-dihydrotestosterone). The failure of these hepatic lipase constructs to respond to androgens may indicate that the androgen response element in *LIPC* does not reside in the proximal 3 kb of 5′ flanking sequence. Alternatively it is possible that one or more transcriptional co-activators required for androgen stimulation of hepatic lipase are not expressed in the cell lines we have tested, or that the effect of androgens on hepatic lipase activity is post-transcriptional.

In summary, we find no evidence for *LIPC* allele-specific responses to androgens at concentrations ranging from supraphysiological (stanozolol treatment) to low (normal women). These data indicate that the effect of the −514 polymorphism on hepatic lipase activity is independent of androgen action. Therefore other factors must be responsible for the effects of the −514T allele on hepatic lipase activity.

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