



Le Tour 2006: Daily Analysis Report

Le Tour 2000. Daily Allaryole Report	
्रक्तुः : १० Bayers Medserrandee ta Montelimus, 238 km	Seneday, July 13th
Floyd Landis	Temp: 95 °F / 35 °C Humidity: 60 % Heat Index: 113 °F / 45.05 °C
Pioju Lanus	100 1 1 1 3 1 1 3 1 1 1 1 1 1 1 1 1 1 1
Place: 29 Time: 5 hrs 54 min 33 sec Gap: 29 min 57 sec	Place: 2 Time: 59 hrs 52 min 3 sec Gap: 0 hrs 1 min 2 sec
Place: 29 Time: 5 hrs 54 min 33 sec Gap: 29 min 57 sec	race. 2 fills. 33 fts 32 ftm 3 sec oup. 5 fts 1 ftm 2 sec

Race Notes: It was so freaking hot today. Way too hot to do anything except nothing. Why was it so hot. Peirero and Voight and Chavenal, and

some other guy got away and built a 30 minute gap. Floyd gives up yellow. Super easy. That's a good thing. Time to get some rest the next two days.

Power, RPE,	Heart Rat	e, Wast, Ri	Evs Power	· TIR vs. Powe							
	Power wh	en Moving	Power w	hen Pedaling	Stra	ain	Work o	r Stress in Kjoules F	rom:	RPE	<u>HR</u>
	Watts	Watts/kg	Watts	Watts/kg	RPE	HR	Power	RPE	HR.	Pwr	Pwr
Today	178	2.56	217	3.12	5	NA	3,787	5,683	NA	1.50	. NA
Tour Avs	218	3.14	266	3.84	4.5	NA	3,926	4,528	NA.	1.13	NA
Tour Min	178	2.56	217	3.12	3.5	NA	2,624	2,682	NA	1.01	NA
Tour Max	267	3.84	314	4.52	7	NΑ	5,870	7,619	NA	1.50	NA
Today Tour Avs						N/A			NA		NA
Tour Avs	223	3.28	268	3.94	6.3	NA	3,911	5,348	NA	1.34	NA
Tour Min	164 (S21)	2.41	214	3.15	4	NA	2,174 (S21)	2,651 (S21)	NA	0.88	NA .
Tour Max	285 (S11)	4.19	314	4.62	10 (S15)	NA	5,620 (S15)	11,286 (S15)	NA	2.01	NA

Power Distri	Ze			********	******	3000000		<u> </u>	*******	**********	P	er Kil	одтап	of I	ody.	Weig	ht		*******	-00000000		20022000	******				Relat	ive to RP	E (1-10)	
	Wa			-				Tir	ne (%))									Time (min)					Т	ime (%	9		Time (m	nin)
	%	min		0-1	1-2	2-3	3-4	4-5	5-5	松 丁	7-8	8-9	>9	,	0-1	1-2	2-3	3-4	4-5	3. 6	6.7	7-8	8-9	>9	<h< td=""><td>Н</td><td>>H</td><td><h< td=""><td>H</td><td>>H</td></h<></td></h<>	Н	>H	<h< td=""><td>H</td><td>>H</td></h<>	H	>H
Today	17.8	63.0		24.4	16	19.3	18	12	5.4	2.4	1.1	0.4	0.6		86	57	68	64	42	19	8	4	1	2	78	17	4	276.5	61.6	15.9
Tour Avs	18	55.0	100	23.1	13	14.5	14	14	10.8	6.09	2.81	1.3	1.2		69	36	42	42	43	34	18	8.2	3.7	3.5	64	24.5	11	189.9	76.05	33.51
Today Tour Avs Tour Min	13.4	29.0	188	17.2	5.5	7.9	10	9.8	5.4	2.4	1.1	0.4	0.6	%	44	20	29	31	21	15	8.5	3.9	1.4	2.1	42	16.7	4.5	154.5	35.91	15.93
Tour Max	23.9	80.0	1888	30	19	19.3	18	24	21.3	9.4	3.8	1.7	1.7	<u> </u>	101	57	68	64	89	78	29	11	5	5	78	45.5	16		166.5	47.95
Today Tour Avs Tour Min				******					*****	*******				Ĺ					*******											
Tour Avs	15	41.2		20.4	11	12.4	14	16	14	7.22	2.91	1.2	1.1	<u> </u>	55	29	34	38	45	40	20	8	3	3			—	<u> </u>		
Tour Min		26.0		12.6	5.7	6.9	11	9.1	6.8	3.7	1.7	0.9	0.7	<u></u>	37	16	19	26	20	15	8	4	2	2						
Tour Max		84.0	₩	33.8	16	16.8	17	23	27.9	10.3	4.7	1.7	1.7		104	42	46	48	73	85	31	14	5	5				×		

	k Power	Outpi	n.	5 mm	33	2 W	6			m		3.54		wa.					Surges:			Hydra	tion (. Kaei	8y 51a	18 90	
				Αv	erage P	ower (v	vatts)					Dis	stan	e fre	om Sta	rt (km)(# of	surges	>		Weig	ht (kg)		Bottle	Sweat
		Se	ec		M	in		I	Iour		Sec			1	Min		Н	our	thar	w/kg c	f.	Pre	Post	Δ	%∆	Count	Loss (I)
	·	5	30	1	5	10	30	1	2	5	3	30	1	5	10	30	l	2	> 6	> 8	> 10	69	67	2	2.8986	20	11.6
	Today	833	569	471	340	296	269	223	n	18	1 6	5.4	6.1	3.7	175	158	14.3	n	201	49	14	Est	L	Sweat R	ates	%	
ű	Tour Avs	894	622	529	414	360	305	275	n									n	373	101	29	Loss (1)	. 1/1	ır l	/mjoule	GME	Kcals
Ruder	Tour Min	825	528	471	340	296	252	214	n		T	П						n	201	49	14	5.12 -	1.9	6	3.06	24	3769.509476
1888	Tour Max	972	912	674	465	426	377	346	n		T.							ħ	487	141		Race Foo	d Eaten	;			
	Today Tour Avs Tour Min Tour Max	******	******	******	*******	*******			**********	7	T	T	****						T	T				_			
	Tour Avs	841	555	479	400	360	314	283	255																		
	Tour Min	731	465	403	308	283	249	231	193																		
	Tour Max	965	645	555	478	435	386	359	315	(<u> </u>						L			<u></u>	L	L						

	Start	3.00	Total		Calc	Start	Tep	Total	Tu	**	Speed	VAM	E	stimate	l Pow	er		tual	%
Climb/Feature:	(km)	9094	ost	Grade	Grade	Elen	Дlev	Gain	486		km/hr	m/tur	Roll	Acro	Grav	Total	Power	wse	Diff
I. Cote de Puechabon	54.9	57.5	2.6	5.2	5.201252	154.95	290	135.05	7	13	21.61663	1123	23	40	236	297	280	4.03	6.4282440
2. Col de la Cardonille	75.3	77.5	2.2	5.5	5,5014885	209.15	330	120.85	•	4	26.05263	1431	27	70	301	100	312	4.49	21.7823362
3. Cote de l'Arbousset	118	119.5	1.5	4.5	4.5012197	162.55	230	67.45	3	29	25.83732	1162	27	68	245	340	306	4.40	10.061158
4. Cote de Saint-Maurice	194.4	195.5	1.1	4.6	4.6003146	255.45	306	50.55	2	42.54	24.36323	1120	26	57	236	319	305	4.39	4.31048082
5. Cote de Villeneuve de Berg	203.1	205	1.9	5.3	5.3021742	291.4	392	100.6	4	48	23.75	1258	25	53	265	343	319	4.59	6.97218274
* All climbs were cat 4's.																			
															i				



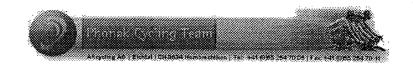


Le To						-		epo	rt																			S4	mday .	uly 1	in
Floyd	Land	is													Ten	ıp:	90	_ 'F	/_32	2 °C	Н	umidity	/ ;	60	_%	Hea	t Index:	99	<u>.7</u> F	/_37.	6 .C
Stage	tesuit			90	421		koi	le.		26.4		מעוני	8		GC	R.	utts			3,636.0		n neu	814		4	99	low h		25	e e	v.
Place:							ec	Gap:	0	min		7 sec	 C		Plac		2	_ T	ime:	64	hrs	5 n	nin _	4	sec	Gap	o: <u>0</u>	hrs	1_ min	29	sec
Race N										_	Rre	akaway	r that	t was	beld	to 5 π	ninute	s for r	most	of the r	ace. St	mne on	ıt and	 1							
going m	_							iom v	Vao .	пурпц.	ы	anamay	Little	· Wac	, iiciu		in tale	1011	11000	01 410 1	100. 00	ung co									
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Power	aroene.	Riear	t Nat	(333)	HR. K	1							***	****		*****		******	****		****	******			****	****	******	W .		******	
	į		ver wh			╀.				Pedaling		╂	RPE		rain .	HR	-		Do.	Worl	k or St	ress in) RF		les F	rom:	H	2	133 -	PE wr		<u>HR</u> Pwr
Too	lay	Wa 26			tts/kg .81	+	Watts 313	T		Watts/kį 4.50	5	\dagger	6	-		NA.	-			047		4,6				N.		***	1.16		NA
Tot	ır Avs	22	1	-	.19		269			3.88			4.63			NA				934		4,5				N.		I	1.13		NA
1000000	r Min	17 26	_		.56	┼	217 314	-+		3.12 4.52		-	3.5 7		+	NA NA				624 870		2,6 7,6				N/ N/		₩-	1.01	 	NA NA
Too	ır Max				.04	+	314	-	2222	4.J2 	2022	╬	rein		1	NA	-	********	will				<u></u>		*******	N/	and the same	-			NA
100000	ir Avs	22	3	3	.28	1	268	十		3.94			6.3			NA			3,5	911		5,3	48			N/			1.34		NA
Tot	ır Min	164 (S21)	2	.41		214	\Box		3.15			4			NA				(S21)	_	2,651				N/		II	0.88		NA
Tot	и Мах	285 (S11)	4	.19	<u> </u>	314			4.62		10	(8)	15)	L	NA		نـــــن	5,620	(S15)	i_	11,286	(S1:	9		N/	A	И	2.01	l	NA
Power	Distri	butier	l.																												
		Z	ero									P	er K	ilogr	am of	Body	Weig	ght								_		Relati	ve to RP	E (1-10)
			atts	_						ne (%)										Time (1						×	Time (9	7 3	<u> </u>	Time (T
8888 m.		%	min	-833331				$\overline{}$			8.5	7-8	2.4			0-1 47	1·2	2-3	3-4	4-5	34		7-8 12	8-9 6	>9 6	<i 51</i 		>H 18	<h 128.5</h 	79.5	>H 46
Too	n Avs	15.3 17.8	39.0 54.0	-199994				_	14		5.28		1.4	-	T (4	68	35	41	42	43	34		8.4	3.9	3.7	63		12	185.8	76.28	-
Z Tot	ır Min	13.4	29.0		17.2	5.5	7.9	10 9	8.0	5.4	2.4	1.1	0.4	1 0.	6	44	17	25	31	21	15		3.9	1.4	2.1	42		1 10	129	35.91	
Tot	ır Max	23.9	80.0	<u>.</u>	30	19 1	9.3	18 2	24	21.3	9.4	5	2.4	3	4	101	57	68	64	89	78	29	12		.6.	78	45.5	16	276.5	166.5	47.95
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DERKY.	3933.5553	ez uepa	18-0000						89888	********	Ť	5990555555			ce fron				22222	Ø	of sur			*****			ht (kg)	3923333	Bottle	Swea	<u></u>
		Se	c	AV	erage P M		(watt	<u>"</u>	Н	lour	il	Sec	\neg	istali		lin	II (KII)	Ho	ш	19	han w/	-		Pr		Post	Δ	% /		Loss	
		5	30	1	5	10	30	0 :	ı	2	1	5 3	30	1	5	10	30	1	2	> 6		8 >	10	69	9	68	1	1.449	3 15		8.2
Too	- 1	892	567	536	443	418			05	n	H	63 1	1.1	11	11	9.2	156	2.8	n	498			50	i	st	10	Sweat R		- %		771-
10000	ır Avs ır Min	894 825	528	530 471	417 340	364 296	$\overline{}$			n D	H	\dashv	\dashv	\neg	+				n п	381 201			31 14	Los 5.	47	1.5		/mjoule 2.03	GMI 24		Kcals 28.308648
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2000		202	2000000	2222		2000000	0000000	0000000	202000	0000000000	3000 3000	000000000	<u>-</u>	-	*****	*****	25120000		999999		0000000	********			88888	2002000	*****	99999999		88888888	0040000000000000
Climb	and !	specia	l Fea	T	1000000	****				Gain	::: 		0	000001	meter	1000	0		feet I		,,,,,,,,,	0.005						, ,, 			<u></u>
Climb/F		•		Start (km)	100000		Total Dist		de:	Calc Grade		Start Elev	8		Tota Gair		Tie Ma	800	<u></u>	km/hr	VAM m/hr	Rol	11	tima Aer	o G	rav	Total				% Diff
1. Cote			les	8.9		_	5.1	4.2		4.19977		230		44	214	12.55	9	8	*	3.50365	1406	1		149	\neg	96	480	430			0.49385033 2.70533595
2. Col de				88.2	72.		5.5 8.8	5.1		4.80006 5.09980	\neg	556.3 854.8		20 303	263. 448.		14 23	37.5	: ·	3.45972 2.34921	1125	25		- 51 44		37 40	313 307	300			431261394
4. Cote 6				165.	1	\neg	5.3	5.5		5.00058	\neg	716.3		81	264.		i	31.7	201	7.58262	1378	-		83		90	402	407			164510382
								Ľ																							
5 Attack	s > 10 v	v/kg or									\Box												\Box								
last clim				ļ	-			<u> </u>	_		4		-	_		-						ļ	4	-	+					-	
16 attacl	cs > 8 w	//kg on	last	-		-			-		\dashv			\dashv		-			4-			-	-		+	- 8		-	+	+	
climb				-	+	+		+-	\dashv		\dashv		-	-		-						 	-		+	-8		-	+	+	





Properation	Le 7	Four 2	2006	: Da	ily /	Anal	ysi	s R	еро	rt									•													
Place A Time A Pa S S S S S S S S S	1000	. +5 · C	ap te	L'A	lpe d	Hue	. 18	7 &																					ues	đạy J	uly IN	n
Place 4 Time 4 Time 5 Time 20 Time	Floy	d Land	is												Tem	p: _	90	_ 'F	<u>, 32</u>	c	I	łumi	dity:	6	0_%	Hea	t Index	:: _	99.7	·F	/_37.6	_·c
Race Notes: Floyd last the broten sole today and the mechanises forget to avoid hour the computer with the wheel change. So we load date.	Stage	Result	41 /8	70	4	38		160	i i		3.9	osph	8		GC	Resul	16			16200		en e	SAGA				low)			233	e e e	
Part	Place:	4 Ti	me: 4	hrs	53 n	nin	32 s	ec	Gap:	1 m	in	10 s	ec		Place	: .		Ti	me:	69					*******		**********		0			
Part	Race	Notes:	Floyd h	ad the	broke:	n axle i	lođav	r and	the me	chanic	s fore	ot to sw	itch on	t the	comn	uter wi	ith tl	he wh	eel cl	hange.	So we	lost	- data	-	_			•		-		•
Power but Moring		_																														
Power but Moring	Poses	. Rep	Hear	e Rat	e 01.0	a R	er.			iii	***					***				****	***		***			***	****	***				
Today	mmm.						T	******	*****			-	9999999	Stra	20020022 sin		M			Wor	kors	ooooo bress	in Kid	orles I	grom.	*****	******	T	RDE	<u>*************************************</u>	20000000	HR
Total May 178 2.5 2.17 3.12 3.5 3.12 3.5 3.1 3.12 3.5 3.1 3.12 3.5 3.1 3.12 3.5 3.1 3.12 3.1								_					RPE			IR			Pov		X OI B			dica i	TOIN.	H	2	701				
Today	E-000003		27	(8)	3.	94		321		4,	59	- -	8	-					4,7	55	4		6,804		ļ			I	1.4	43		
Today Toda	Ž T		17	18	· 2.	.56	+-	217	\dashv	3.	12	-	3.5	\dashv			░		2,6	24	\dashv		2,682		\vdash			╫	1.0	01		
Total Aria 223 328 296 3.94 6.3 5.4 5.4 5.4 5.5 6.7 7.8 8.9 5.4 1.2 1.	Т	our Max	27	()				321							N	A		******								_		II.				
Tour Min 164 (S21) 2.41 2.14 3.15 4 NA 2.174 (S21) 1,651 (S21) NA 0.88 NA 1.00 (S55) NA 2.00 (S55) NA 2.	VAKKA .	-					<u> </u>					- -		_			▓-				\perp											
Tour Max 25 (811) 4.19 314 4.62 10 (815) NA 5.600 (815) 11,266 (815) NA 2.01 NA	100000						\vdash		\dashv					\dashv			░	2			+			1)	\vdash			₩-				
Per Kilogram of Body Weight Time (vin)	WWW.													0														I				
Per Kilogram of Body Weight Time (vin)	Powe	r Distri	butier	10000																												
Walts Walt	2444							*******]	er Kil	ograi	m of B	ody W	eigh	nt			******		******				*********	Rela	tive	to RPF	(1-10)	
Today 15.3 39.0 18.5 6.7 10 15 18 13.2 8.5 4.7 24 2.5 4.7 17 25 39 46 34 22 12 6 6 6 51 31 18 128.5 79.5 46										ime (%	6)									Time (min)						Time (in)
Tour Avs	(333) ₄			_	-10001		~~	$\overline{}$			-	7	$\overline{}$	_	₩ -			-					_		7	-	 	+	₩-			
Today Tour Average Power (wats) See Min Hour See Min Hour Today Tour Average Power (wats) Tour A	3323	· 1	15.5	39.0	₩		_					\rightarrow		_	101		-	-	_				1		1	3 - -			100			
Today	Ž To	our Min		29.0		17.2	5.5	7.9	10 9.		-		_	0.6	4	14 1	_		31	21	15	8.5			1 - 1:	-	+	-	1001			
Tour Aws 15 41.2 20.4 11 12.4 14 16 14 7.22 2.91 1.2 1.1 5.5 2.9 3.4 38 45 40 20 8 3 3 0 0 0 0 0 0 0 0		***************************************	23.9	80.0	₩.	30	19 1	9.3	18 2	1 21.3	9.4	1 5	2.4	3	<u></u>	01 5	7	68	64	89	78	29	12	6	6	78	45.5	16		276.5	166.5	47.95
Tour Min	00000		15	41.2	-₩	20.4	11 1	24	14 14	5 14	72	2 2 91	12	1.1	-	55 2	0	34	38	45	40	20	R	3	1	-		-	₩-	-+		
Peak Power Output 3,10	100000	ì		 	-19999-		-	_					-	_	103 1										 							
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Sec Min Hour Sec Min Sec Sec Min Min Sec Min	Peak	Power i	Jutou	ık 💮	soute:	100000	446 (AA4			369	mm, #	mun	# 1	va.					Surge	est			Hy	arati	n é	Ene	ey i	tati	J9t		
Today					Ave	rage P	ower	(watt	s)				Dist	ance	from	Start (km)			#	ofsu	rges :	>	_	٠ .	Weig	ht (kg)]1	Bottle	Sweat	
Today					,			1 2	1							-	+							и—		-		$\overline{}$			_	
Tour Avs 894 618 530 417 364 307 277 n Tour Min 825 528 471 340 296 252 214 n Tour Max 972 912 674 465 426 377 346 n Tour Min 731 465 403 308 283 249 231 193 Tour Max 965 645 555 478 435 386 359 315 Climbs and Special Features Start 69 700 700 700 700 700 700 700 700 700 70	To	oday		30		-	-10	13	1			-	30	+	, , ,	0 3	+	+	— и		-T-	,,	> 10	и				-	493		 	8.2
Total Max 972 912 674 465 426 377 346 n n 498 188 50 Race Food Estent Total Max 972 912 674 465 426 377 346 n n 498 188 50 Race Food Estent Total Max 841 555 479 400 360 314 283 255	₹ To	our Avs		$\overline{}$		417		-	7 27	7 .	<u>. </u>						1		- и	381		07	31	DJ DJ					lc	GME	F	Kcals
Total V 841 555 479 400 360 314 283 255	200000	- 1							_	1		-	-	+		+	+	\dashv	— В		_			и—			8	1.72		24	473	33.0387
Tour Avs	7777	**********	ستنش	المثثث	****	7	720	-	4	'		_		#	+	+	+	-		478	₩	200	20	N Vacc	. r u ca 1	actri:						
Climbs and Special Features: Total Revalue Cam 9 meter 0 feet Ci 0.003 k 0.18 Bac W	WWW.	ŕł	841	555	479	400	360	31	4 283	3 2:	55			1		士	\top				土											
Climbis and Special Features: Total Elevation Gam:	WWW.		_					_	-				+	+	+		+	+	_		+			<u> </u>								
Start Store Stor	200110	ou waxi	200	043	333]	4/6	433		0 33		2_8			. I										и					******			
Climb/Feature: (sm) 699 699 699 Grade 890 880 Gain 86 8 km/hr m/hr Roll Acro Grav Total 899 840 Diff 1. Col de l'Izoard 71.8 86 14.2 7 6.9959308 1369 2360 991 80 23 21.09781 1472 22 37 310 388 342 4.92 7.437836622 2. Col du Lautaret* 121.9 134 12.1 4.4 4.40095 1526 2058 532 34 30 29.63265 1303 31 103 274 808 390 5.61 4.577309629 3. Adjus #Hissz 173.2 187 13.8 7.9 7.9013614 763 1850 1087 38 34 21.46932 1691 23 39 356 348 410 5.90 1.898076839 Actual time up L'Alpe d'Huez. Time given by official race course timers	Climb	as and S	pecia	l Fear	tures	1200000		*****	*****				400000	9934	neter				eet	·····	0000000	0.005	*******			****		******	******		953	
1. Col de l'Izoard 71.8 86 14.2 7 6.9959308 1369 2360 991 369 23 21.09781 1472 22 37 310 369 342 4.92 7.437836622 2. Col du Lautaret* 121.9 134 121 4.4 4.40095 1526 2058 532 24 36 29.63265 1303 31 103 274 469 390 5.61 4.577309629 3. 2. 2. 2. 2. 2. 2. 2. 2. 2. 2. 2. 2. 2.	Climb/	Feature:			ŀ							lessesses.		*** 1					3	- 1		<u></u>										
2. Col du Lautaret* 121.9 134 121 4.4 4.40095 1526 2058 532 34 36 29.63265 1303 31 103 274 469 390 5.61 4.577309629 3. Lautaret* 173.2 187 13.8 7.9 7.9013614 763 1850 1087 38 34 21.46932 1691 23 39 356 488 410 5.90 1.80005039 Actual time up L'Alpe d'Huez. Time given by official race course timers			d			1	т			1			1									1			_	- 83					-4	
Actual time up L'Alpe d'Huez. Time given by official race course timers	2. Col c	du Lautar	et*						î	1			1	\neg			000					1				100		:				
d'Huez. Time given by Official race course timers	3 1 1	e d'Hare			173.2	187	1	13.8	7.9	7.90	13614	763	185	0	1087	- 38	4	34	21.	46932	1691	1	23	39	3:	6	418	43	0	5,90	1.89	6626839
d'Huez. Time given by Official race course timers	Actual	time vo !	'Alna				+		-	+-			-	+			+		-			+		_	+-	-#		-	\dashv		+	
official race course timers						<u> </u>	+			+		<u> </u>	\vdash	+			#		-			+			+-	- 18			\dashv		-	
who timed the climb.				ers			\perp										1															
					<u> </u>	_	-		_	-	<u>. </u>	ļ		1			II.		_	[L			\perp	_			\perp		4	
* Time Estimated	I* Time	Estimate	d			<u> </u>	+		<u> </u>			ļ	-	+			4		L			-				-8		<u> </u>	+			





Le Tour 2006: Daily Analysis Report

Seege to Bourg D'Osmis to La Toussuits, 182 km	Wednesday, Luty 19th	
Floyd Landis	Temp: 90 'F / 32 'C Humidity: 60 % Heat Index: 99.7 'F / 37.6 'C	3
Stage Resulta: 257.12 des	GC Results 17810 Imposes 60.0 Inches 14.5 Appli	
Place: 23 Time: 5 hrs 46 min 8 sec Gap: 10 min 4 sec	Place:11	c
Race Notes: Bad Day. Floyd bonked on last climb. Didn't eat or drink enough. Missing l	st 6 km of Toussuire	

Power, RPF.	Heart Rat	e, Wast, RI	E vs Powe	r IIR 98 Pawe							
	Power wh	en Moving	Power w	hen Pedaling	Str	ain	Work c	r Stress in Kjoules F	rom:	RPE	HR
	Watts	Watts/kg	Watts	Watts/kg	RPE	HR	Power	RPE	HR	Pwr	Pwr
Today	259	3.73	312	4.48	10	NA	5,379	8,848	NA	1.64	NA
Tour Avs						NA			NA		NA
	178	2.56	217	3.12	3.5		2,624	2,682	NA	1.01	NA
Tour Max	267	3.84	314	4.52	7	NA	5,870	7,619	NA	1.50	NA
Today						NA			NA		NA
Tour Avs	223	3.28	268	3.94	6.3	NA NA	3,911	5,348	NA	1.34	NA
Tour Min	164 (S21)	2.41	214	3.15	4	NA.	2,174 (521)	2,651 (S21)	NA	0.88	NA
Tour Max	285 (\$11)	4.19	314	4.62	10 (S15)	NA	5,620 (S15)	11,286 (S15)	NA	2.01	NA

Power Distr	bution																														
	Ze	го									P	er Kil	ograr	n o	f Body	weiş	<u>ş</u> ht										Rela	tive to	RPI	E (1-10)	
	Wa	atts		L				Tir	me (%)									Time (min)					1	ime (%	6)			Time (n	nin)
(name of the	%	min		0-1	1-2	2-3	3-4	4-5	5.5	67	7-8	8-9	>9		0-1	1-2	2-3	3-4	4-5	.5.6	6-7	7-8	8-9	>9	<h< td=""><td>Н</td><td>>H</td><td>≋ -</td><td>H</td><td>Н</td><td>>H</td></h<>	Н	>H	≋ -	H	Н	>H
Today	16.9	58.0	M	20.1	4.8	5.5	12	26	20.8	6.8	1.9	1.9	0		70	17	19	42	90	72	24	7	7	0	43	46.8	11	14	7.4	161.9	36.7
Tour Avs	L		M								L														Г						
Today Tour Avs Z Tour Min	13.4	29.0		17.2	5.5	7.9	10	9.8	5.4	2.4	1.1	0.4	0.6		44	20	29	31	21	15	8.5	3.9	1.4	2.1	42	16.7	4.5	15	4.5	35.91	15.93
Tour Max	23.9	80.0		30	19	19.3	18	24	21.3	9.4	3.8	1.7	1.7		101	57	68	64	89	78	29	11	5	5	78	45.5	16	27	6.5	166.5	47.95
Today			1								Γ	1		T	******				************		******	*****	*****		-	******		~~~	7	**********	
Tour Avs	15	41.2		20.4	11	12.4	14	16	14	7.22	2.91	1.2	1.1	Ī	55	29	34	38	45	40	20	8	3	3					\neg		
Tour Avs Tour Min		26.0		12.6	5.7	6.9	11	9.1	6.8	3.7	1.7	0.9	0.7	ı	37	16	19	26	20	15	8	4	2	2			П	#	ヿ		
Tour Max		84.0		33.8	16	16.8	17	23	27.9	10.3	4.7	1.7	1.7		104	42	46	48	73	85	31	14	5	5							

ř.	k Power	Outpi	int	\$ ma	3.	t# 14	Ng.		,	9.trum	•	23	WA.					Surges:			Hydra	tion .	& Enc	ny Sta	119	
				Av	erage I	ower (watts)				I	Distar	ice fi	om Sta	rt (km	1)	Ţ	# of	surges		4		ht (kg)		ı	Sweat
		Se	ec		M	lin			Hour	<u></u>	Sec		,	Min	,	H	our	than	w/kg c	f:	Pre	Post	Δ	% ∆	Count	Loss (i)
processor.		5	30	1	5	10	30	1	2	5	30	1	5	10	30	1	2	> 6	> 8	> 10	69	68	1	1.4493	20.	10.6
	Today	828	722	478	406	378	358	344	n	136	27	21	20	159	20	20	n				Est	T	Sweat R	ates	%	
3ggt	Tour Avs								n								n				Loss (I)	и	ır l	/mjoule	GME	Kcals
2	Tour Min	825	528	471	340	296	252	214	n								n	201	49	14	7.27	1.5	34	1.97	24	5354,156713
	Tour Max	972	912	674	465	426	377	346	n								n	487	141	43	Race Foo	d Eaten	:			
	Today					T	1			7	·····	Ī				******	*****	<u> </u>	********	***************************************						
	Today Tour Avs Tour Min	841	555	479	400	360	314	283	255	1		\vdash	П													
	Tour Min	731	465	403	308	283	249	231	193			1														
	Tour Max	965	645	555	478	435	386	359	315																	

Climbs and Special Fea	tures:		Total E	levation	Gain:		θ	meter		0 1	feet	Ct (1.005 k	0.18	138ke	wi	Wi	68.5	75.5
•	Start	300	Total	- %	Calc	Jan 1	Tep	Total		89 0	Speed	VAM	E	stimate	d Pow	er	A.	tual	%
Climb/Feature:	(km)	Om.	Ost	Grade	Grade	Ebr	Blev	Gain	7484	340	km/hr	m/hr	Roll	Acro	Grav	Total.	Pewa	w/kg	Diff
Col du Galibier	2.7	45.5	42.8	4.5	4.5045632	720	2646	1926	96	32	26.60221	1197	28	75	252	355	333	4.79	6.12343085
2.Col de La Croix de Fer	103.8	126.5	22.7	6.9	6.8974059	505	2067	1562	67	33	20.16284	1387	21	33	292	346	334	4.81	3.432036413
3. Col du Mollard	140.7	146.5	5.8	6.8	6.8036233	1244.3	1638	393.7	16	29	21.11223	1433	22	37	302	361	348	5.01	3.680263055
4. La Toussuire	163	182	18.7	5.9	5.9033076	603	1705	1102	51	202	20.63765	1216	22	35	256	313	310	4.46	0.852670958
Toussuire first 3.12 km	163.3	166.416	3.116		7.7898956	603	845	242	8	59	20.81187	1616	22	36	340	398	382	5.50	4.018823171
Toussuire to 6.24 km	166.4	169.532	3.116		7.7251305	845	1085	240	Q	51	18.98071	1462	20	27	308	355	342	4.92	3.642213029
Toussuire to 9.36 km	169,5	172.648	3.116		7.7251305	1085	1325	240	12	26	15.037	1158	16	13	244	273	269	3.87	1.531307335
Tousuire to 12.48 km	172.6	175.764	3.116		7.0779971	1325	1545	220	10	36	17.63774	1245	19	22	262	303	302	4.35	0.17772058
Toussuire to 15.60 km	175.8	178.88	3.116		2.7288717	1545	1630	85	6	30	28.76308	784.6	30	94	165	290		0.00	100
Tousuire to 18.72 km	178.9	181.996	3.116		2.4076295	1630	1705	75.	6	0	31.16	750	33	120	158	311		0.00	100





Le Tour 2006: Daily Analysis Report

Stage 1. Saint-Jean-De-Maurienne to Morsine, 200.5 km	Thursday, July 20th
Floyd Landis	Temp: 95 'F 1 35 'C Humidity: 60 % Heat Index: 113 'F / 45.05 'C
Stage Resulta: 200 km 3/3 km/hr 23/3 mph	GC Results 2.624-0 berretiss 22 berr 20.3 eigh
Place: 1 Time: 5 hrs 23 min 36 sec Gap: 0 min 0 sec	Place: <u>3</u> Time: <u>80 hrs 9 min 19 sec Gap: 0 hrs 0 min 30 sec</u>
Race Notes: The comeback kid.	

	Power wi	en Moving	Power w	hen Pedaling	Stra	ain	Work	or Stress in Kjoules F	rom:	RPE	HR
·	Watts	Watts/kg	Watts	Watts/kg	RPE	HR	Power	RPE	HR	Pwr	Pwr
Today	281	4.16	324	4.80	10	NA	5,456	9,043	NA	1.66	NA
Tour Avs Tour Min	226	3.27	274	3.95	5	NA	4,054	4,834	NA	1.16	NA
Tour Min	178	2.56	217	3.12	3.5	NA	2,624	2,682	NA	1.01	NA
Tour Max	281	4.16	324 4.52		10	NA	5,870	9.043	NA	1.66	NA.
Today Tour Avs Tour Min						NA .			NA		NA
Tour Avs	223	3.28	268	3.94	6.3	NA	3,911	5,348	NA NA	1.34	NA.
Tour Min	164 (S21)	2.41	214	3.15	4	NA	2,174 (S21)	2,651 (S21)	NA	0.88	NA.
Tour Max	285 (S11)	4.19	314	4.62	10 (S15)	NA	5,620 (S15)	11,286 (S15)	NA	2.01	NA.

		то									P	er Ki	ograr	n of	Body	Weig	ht										Relat	ive to RP	E (1-10)	
	W			·				Tir	ne (%	<u> </u>									Time ((min)					1	îme (9	6)	3	Time (n	nin)
200004	%	min		0-1	1-2	2-3	3-4	4-5	5-9	67	7-8	8-9	>9	<u></u>	0-1	1-2	2-3	3-4	4-5	3.5	6-7	7-8	8-9	>9	<h< td=""><td>Н</td><td>>H</td><td><h< td=""><td>н</td><td>>H</td></h<></td></h<>	Н	>H	<h< td=""><td>н</td><td>>H</td></h<>	н	>H
Today Tour Avs Tour Min	13.2	43.0		16.5	6.9	6.5	6.7	17	33.6	10.1	1.8	0.7	0.8	8	53	22	21	22	53	109	33	6	2	3	37	50	13	118.2	161.8	43
Tour Avs	17.5	53.0		22.3	12	13.4	14	14	12.6	6.55	2.87	1.4	7.9	፠Γ	68	35	41	42	43	34	18	8	4	4	57	25	18	3 1		35.04
Z Tour Min	13.4	29.0		17.2	5.5	7.9	10	9.8	5.4	2.4	1.1	0.4	0.6		44	20	29	31	21	15	8.5	3.9	14	21	42	16.7	4.5	×	35.91	15.93
Tour Max	23.9	80.0		30	19	19.3	18	24	21.3	9.4	3.8	1.7	1.7	*	101	57	68	64	89	466	3	11	5	5	78		16	276.5	166.5	47.95
Today										*******		*****		~	*****	*****	*****	****	*******	1	*****					- Time	الخشم			
Today Tour Avs Tour Min	15	41.2		20.4	11	12.4	14	16	14	7.22	2.91	1.2	1.1	1	55	29	34	38	45	40	20	8	3	3				} —		
Tour Min		26.0		12.6	5.7	6.9	11	9.1	6.8	3.7	1.7	0.9	0.7		37	16	19	26	20	15	8	4	2	7	-	_		 		
Tour Max		84.0	▓	33.8	16	16.8	17	23	27.9	10.3	4.7	1.7	17	1	104	42	46	48	73	85	31	14	5	-	} —		\vdash			

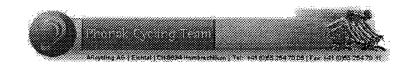
Pe	k Power	Outp	JL.	5 mu	4	8 14	44			0 min	•	9 7	WA.					Surgest			Hydra	ion.	e Reser	ev Sta						
					erage F	ower (T		_	Ι			om Sta				a	surges		g		ht (kg)		Bottle	Sweat				
		Se	-	ļ	M	in		1	Hour		Sec	<u> </u>		Min		He	our	than	w/kg o	f:	Pre	Post	Δ	%∆	Count	Loss (I)				
Person	,	- 5	30	1	5	10	30	1	2	5	30	1	5	10	30	1	2	>6	> 8	> 10	68	66.3	1.7	2.5	17	9.86				
	Today	867	544	484	451	431	401	352	n	9.6	69	71	70	69	69	154	n	564	67	22	Est	П	Sweat Ra	ites	%					
ā	Tour Avs	894	618	530	417	364	307	1 1 1			133	122	81	115	119	74	n	381	107	31	Loss (I)	Lи	1	mioule	GME	Kcals				
2	Tour Min	825	528	471	340	296	252	214	n								'n	201	49	14	7.37	1.3		1.81	24					
	Tour Max	972	912	674	465	431	401	352	п									564	141		Race Food					5430.801083				
	Todov	***************************************	******	_	***************************************	***************************************	*****	***************************************	***************************************			·	 	******			يسد		***************************************		Kace Pood	Baten	. /0	Bottles u	15 10					
w	Louay			-				\vdash		1											20 used to	drink.	All others	poured or	1					
	Tour Avs	841	555	479	400	360	314	283	255	1				i			Ē				himself du	ring rie	ie.							
	Today Tour Avs Tour Min	731	465	403	308	283	249	231	193																					
	Tour Max	965	645	555	478	435	386	359	315																					

	Start	300	(Cole)	100	Calc	Start	Tep	Total		e) e	Speed	VAM	E	stimate	d Powe	er	A	tual	%
Climb/Feature:	(kın)	On:	Oct	Orack	Grade	Plen.	Blec	Gain	146		km/hr	m/hr	Roll	Aero	Grav	Total	Power	w/kg	Diff
1. Col des Saises	67.6	82.5	14.9	6.4	6.2335522	723	1650	927	36	55	24.2167	1507	25	56	317	3190	395	5.68	1.01544622
. Col des Aravis	103.1	109	5.9	7.1	7.1025934	1068	1486	418	16	49	21.05055	1491	22	37	1 3	373	371	5.34	0.58420313
. Col de la Colombiere	122.2	134	11.8	5.8	5.7126887	940	1613	673	27	45	25.51351	1455	27	66		399		5.64	1.78219121
. Cote de Chatillon-sur-Clus	156.9	162	5.1	4.9	4.9078609	485	735	250	11	7	27.52624	1349	29	83		396	;	5.38	5.50764223
. Col de Joux-Plane	176.7	188.5	11.8	8.5	8.3424388	710	1691	981	37	34		1567	20	27		376	372	5.35	1.142676111
											1010100	1307	20	- 27	330	240	3/2	2.33	1.1420/011.
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60000	e Tou					Re	poi	rt																								
*	enge fål	Mor	me	a M	ec.	• 1	97)																							Peida	v July :	<i>it</i> h
1	loyd La	ndis														7	Гетр:		90 .	F /	32 C		Hurr	idity:	(50 %	6 He	at Inde	x :	99.7 1	7 / 37	6 'C
S	age Resi	ilia:	(9/7 D	ASSE		44	98		<i>o</i> , 4				og.			3	CR	e a unit			2,807	ð	len.	28406	9505080		335	los.	 100000000	 8886886666		 #4
PI	ace: 29	Time:	<u>4</u> h	us _ 24	_ m	in .	15	sec	G	ap:_	8 mi	n	0	sec			lace:	3	_	Time	: 84	_hrs	33	min	34	sec	Ga	p: 0		0 п		sec
R	ace Note	s: Floye	just:	sitting	in a	ll day	as b	reak	way	y wit	h Levi a	nd Z	man g	get av	vay.																	_
<u>.</u>							·																									
b	wet, RP	E, He	ert R	ate. Y	Vo	4. R	PE	vs I	099	er,	IR 98	100	e e																			
				when]		_	\perp			whe	n Pedal	ing	┸			Strain					W	ork or	Stress	in Kj	oules	From	:		1	RPE	************	HR
	Today	- V	Vatts	+ \	Vatt	s/kg	╁	Wa	tts	$\overline{}$	Watts	/kg	╬	RF	Έ	+	HR NA	-	! —	P	ower			RPE		\Box	Н		1	Pwr		Pwr
2	Tour Av						1						上			士	NA		-			-+				+-	N.				-	NA NA
2	Tour Mi Tour Ma		178 267	+-	3.8		╀	31		+	3.15 4.55		- -	<u>3.</u>		+	NA		_		,624			2,682			N.	1		1.01		NA
	Today			-			#	zzűő.		+			#	wwi	200		NA NA	-	-		,870		winner	7,619			N.	omme	#	1.50		NA
	Tour Av		223		3.2			268	3	I	3.94	4		6.	3	土	NA			3	911		,	5,348		+	N/		╢	1.34	+	NA NA
	Tour Mi Tour Ma	_	(S21) (S11)		4.1		╀	314		╁	3.13 4.62		-	4 10 (S	_	+	NA				4 (S21)	4		51 (S2			N/			0.88		NA
	0000000000000	000000000	00000000	888888	8888	20000	1		8988	200000	9.02	2000000	 >>>>>>>		00000	000000	NA		a	3,62	0 (S15)		11,	286 (S	15)	<u></u>	N/		₩_	2.01		NA
ro	wer Dist	1			3							<u></u>															ioi -					
•		i i	Zero Vatts		-					Ti	me (%)			Per K	ilog	ram (of Bod	y We	ght		Time	(min)					₩_	Tr' (tive to I	CPE (1-10	
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Confirming testosterone administration by isotope ratio mass spectrometric analysis of urinary androstanediols

Cedric H.L. Shackleton,* Andy Phillips,† Tony Chang,* and Ye Li‡

*Children's Hospital Oakland Research Institute, Oakland, California, USA; †Micromass UK Limited, Manchester, England, UK; and, ‡National Research Institute of Sports Medicine, Beijing, China

A gas chromatographic combustion isotope ratio mass spectrometric (GC/C/IRMS) method was used for studying the incorporation of exogenous testosterone enanthate into excreted urinary 5α - and 5β -androstane- 3α , 17β diols. A multistep but straightforward work-up procedure produced a simple GC chromatogram of urinary steroid acetates composed principally of two androstanediols and pregnanediol. It is anticipated that such a method may form the basis of a doping control test for testosterone that could be used as a primary method during major sporting events or alternatively as a verification technique. Urine samples from five individuals were collected before and after administration of testosterone enanthate (250 mg). The $\delta^{13}C^{0}/00$ value of androstanediols was around -26 to -28 during the baseline period and decreased to about -29 to -30 in the days following synthetic testosterone administration. One of the other major steroids in the chromatogram, pregnanediol, was utilized as the "internal standard," because its $\delta^{13}C^0/00$ values did not markedly change following testosterone administration, remaining at -25 to -27. In all subjects studied, the $\delta^{13}C^{0}/00$ values for androstanediols were reduced sufficiently over 8 days to confirm administration of synthetic testosterone. Although steroids isolated from urine of normal individuals from 12 different countries gave values between -24 and -28, this seemed not to be related to nationality or region. The most likely variable is the proportion of plants with low and high carbon 13 content in the diet. This variable is likely to be more affected by individual food preferences than broad ethnic food divisions. In this paper, we propose a ratio of $\delta^{13}C^0/00$ for and rostanediols to pregnanediol as a useful discriminant of testosterone misuse, a value above 1.1:1.0 being indicative of such misuse. The work-up procedure was designed for batch analysis and to use only simple techniques, rather than employ further instrumentation, such as high-performance liquid chromatography (HPLC), in purifying steroids for GC/C/IRMS. (Steroids 62:379-387, 1997) © 1997 by Elsevier Science Inc.

Keywords: isotope ratio mass spectrometry (IRMS); doping control; testosterone

Introduction

The increasing need in sport for proving testosterone misuse requires new methodologies. For many years, a testosterone/epitestosterone (T/E) excretion ratio determined by gas chromatography-mass spectroscopy (GC-MS) of greater than 6:1 has been used as the hallmark for confirmation of drug administration, but this method is fallible. For one thing, occasional drug-free individuals give a ratio >6, and

a high ratio can also be adjusted downward by simultaneous administration of epitestosterone. We have found that in eight Chinese subjects given 250 mg testosterone enanthate, only three gave T/E values >6 on more than 1 day, demonstrating a high rate of false negatives, at least in this racial group.

In 1990, Southan and co-workers² used isotope ratio mass spectrometry (IRMS) to show that synthetic testosterone had a different ¹³C content than endogenous hormone. This is a reflection of the origin of the materials, because all testosterone, both endogenous and synthetic, is ultimately of plant origin. Gonadal testosterone is made from precursor molecules derived from a wide variety of vegetable mate-

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Steroids 62:379-387, 1997 © 1997 by Elsevier Science Inc. 655 Avenue of the Americas, New York, NY 10010 rials eaten by humans or by the animals humans eat. Individual plants discriminate to different degrees against ¹³CO₂, and particular species are known to have high or low levels of ¹³C in their biomolecules.³ In human bodies, the ¹³C content, therefore, reflects an average of the ¹³C content of all the plant material eaten by humans and our animal protein providers. Synthetic testosterone, in contrast, is generally made from a single plant species, mostly soy, so the ¹³C content has a defined value reflecting the relatively low ¹³C content of this plant. Thus, a significant difference in ¹³C content between gonadal and soy testosterone could provide the basis for developing a definitive test for hormone misuse. Differences in carbon isotope ratios referred to by the symbol δ , defined as the difference in isotope ratio between the sample and an international carbonate standard "PDB." Although this is the accepted standard, for common usage a calibrated international standard of CO₂ is used. The values reported for δ carbon isotope ratios are

$$\delta^{13}$$
C⁰/₀₀ = $\left(\frac{\text{Ratio sample - Ratio PDB}}{\text{Ratio PDP}}\right) \times 1000$

Becchi and coinvestigators have published pioneering studies on development of a method employing IRMS for determination of carbon isotope ratio of testosterone extracted from urine.^{4,5} They demonstrated that, providing sufficient urine was available, the endogenous or exogenous origin of testosterone could be readily determined. A major remaining problem demonstrated by these studies relates to sensitivity of the analysis, because the quantity of urine collected from athletes is relatively small (about 75 mL), and this is divided into two, a primary (A) and a secondary sample (B). On each sample, nonsteroidal drug metabolites must be analyzed, as well as anabolic steroid screening and measurement of the T/E ratio.

We have attempted to improve the methodology to allow more sensitive analysis. It was decided to forgo any attempt to analyze testosterone itself and to concentrate on analysis of its metabolites 5α -androstane- 3α , 17β -diol ($5\alpha AD$) and 5β -androstane- 3α , 17β -diol (5β AD). Our overall objective was to easily produce a single sample for analysis containing a few defined steroids to include the androstanediols and steroids we call "endogenous reference compounds" (ERCs). An ERC, in this instance, is a steroid whose carbon isotope ratio could not be altered through administration of exogenous testosterone. Aguilera, Becchi, and co-workers in their most recent publication use cholesterol and 5-androstene-3β,17β-diol as ERC.5 To achieve our overall objective, we developed simple methodology adaptable to batch analysis, which required no liquid chromatographic (HPLC) instrumentation. Using this methodology, we determined the $\delta^{13}C^0/00$ of the androstanediols present in urine following administration of testosterone enanthate to five volunteers. This communication presents the results of this study.

Experimental

Materials

Testosterone enanthate, Testoviron depot® was obtained from Schering, Japan. Reference steroids were obtained from Sigma (St. Louis, Missouri, USA), which was also the supplier of Girard reagent T (carboxymethyl, trimethyl ammonium chloride hydrazide) and sodium bismuthate. Sephadex LH 20 was a product of Pharmacia AB and Sep-pak® cartridges, a product of Waters Corp. (Milford, Massachusetts, USA). B-glucuronidase/aryl sulfatase was obtained from Sigma (Type H1) and Boehringer Mannheim (Mannheim, Germany). Solvents were of analytical grade.

Individuals studied

Eight Chinese male subjects aged 19-22 were studied, although GC/C/IRMS analysis was only conducted on five. Permission for undertaking these experiments was obtained from the Chinese National Research Institute of Sports Medicine, and consent was obtained from the participants. Spot morning urine samples were collected for 2 days prior to an intramuscular injection of 250 mg testosterone enanthate. Two urine samples (0-8 h and 8-24 h) were collected for the first 4 days after administration, although only aliquots of the early morning sample were subject to analysis. Morning spot urine samples were collected on the 5-9th days after administration and on days 11, 13, and 15.

Determination of testosterone/epitestosterone ratio (T/E)

Urinary testosterone and epitestosterone were quantified in all the samples using an adaptation of the method of Donike et al.⁶ These measurements were carried out by one of us (YL) in China at the National Institute of Sports Medicine. T/E ratios were then determined.

Preparation of steroid extract for GC/C/IRMS

A flowsheet summarizing the methodology is shown in Figure 1. Urine (typically 25 mL) was extracted by Sep-pak® cartridge according to the method of Shackleton and Whitney. Once dried, the extract was dissolved in 3 mL 0.1 M acetate buffer pH 5 and Helix pomatia-derived β-glucuronidase/sulfatase (12 mg Sigma

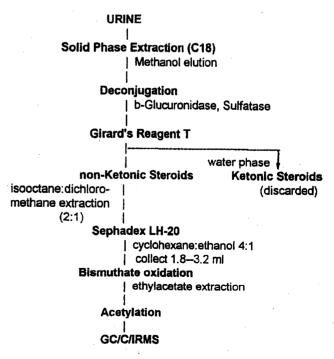


Figure 1 Flow sheet of the urinary extraction method.

type H1 powder, 100 µL Boehringer liquid enzyme) was added. Hydrolysis was allowed to proceed for 3 h at 55°C. A Girard separation was carried out to separate carbonyl-containing steroids (ketonic) from noncarbonyl-containing (nonketonic) steroids.8,9 To the hydrolyzed urine mixture, 2 mL glacial acetic acid and 100 mg Girard reagent T was added. The solutions were placed in an oven at 100°C for 30 min. The nonketonic steroids were extracted by 2 \times 5 ml isooctane: dichloromethane 2:1 (v/v), and the solvent was dried. Small columns of 0.5 Sephadex LH-20 were prepared in Pasteur pipettes, the Sephadex being allowed to swell in the cyclohexane: ethanol (4:1) solvent system before preparation. 10 The steroid extract dissolved in 100 µL of the same solvent mixture was added to these columns. Solvent eluting between 1.8 and 3.2 mL was collected and dried. Acetic acid (0.1 mL), water (0.1 mL). and 5 mg sodium bismuthate were added. 9 Oxidation was allowed to proceed for 2 h, and after neutralization (0.5 mL of 0.5 M acetate buffer), the mixture was extracted with 4 mL ethyl acetate. After drying, steroid acetates were prepared overnight with 50 µL acetic anhydride and 50 µL pyridine. The acetates were analyzed by GC/C/IRMS.

Gas chromatography combustion isotope mass spectrometry (GC/C/IRMS)

A schematic representation of the GC/C/IRMS instrumentation is illustrated in Figure 2. The acetylated steroid samples were kept refrigerated until analysis. Cyclohexane (20 μ L; 99.9% pure from Sigma Chemicals 27-0625-8) was added to each vial, and one-tenth (2 μ L) of each sample was injected splitless onto a J&W 30 m DB17 capillary column housed in a Fisons 8000 series GC. The injector was kept at 220°C. The temperature program was as follows: starting temperature 50°C (1 min), followed by rapid temperature increase (25° min) to 300°C, where it was held for 15 min.

The separated components were heart-split into the combustion furnace filled with copper oxide wires (Elemental Microanalysis Limited, UK) held at 850°C. The combustion gases were passed through a nafion membrane water removal trap, and the remaining $\rm CO_2$ was analyzed on a Micromass isochrom isotope mass spectrometer. The mass spectrometer consisted of an electron impact source running at 400 μ A trap current, the ionized $\rm CO_2$ gas, was focused by a magnet onto three Faraday collectors. The ions collected were those at masses 44, 45, and 46. The Micromass data system calculated the areas of the beams and subtracted any background; whereupon, calculation of the $^{13}\rm C$ δ values for the successive peaks were carried out.

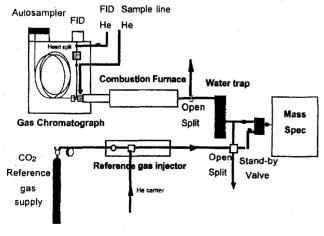


Figure 2 Schematic of the Micromass GC/C/IRMS instrument.

Results and discussion

Evaluation of gas chromatographic columns

The objective of the study was to measure $\delta^{13}C^0/00$ for urinary androstanediols formed as metabolites of testosterone. In our initial studies (Subject 1), we undertook chromatography on DB1 capillary columns but did not get resolution of the two diacetylated epimers (Figure 3A). Later employment of a DB17 column (Figure 3B) allowed separation of the epimers as well as the ERC, pregnanediol diacetate, and pregnanetriol diacetate (Figure 3B).

Confirmation of identity of steroids in extracts

Prior to sending the first samples for GC/C/IRMS analyses in England, the identities of the principal components of the chromatogram were confirmed by GC/MS. This was carried out on a Hewlett-Packard 5970 instrument housing a 15 meter DB1 capillary column. The peaks chosen for GC/C/IRMS analyses had retention times and electron impact mass spectra identical to those of 5α - and 5β -androstanediol diacetate and pregnanediol diacetate. Reference steroids for these compounds were also analyzed on the GC/C/IRMS instrument using both DB1 and DB17 columns, and these gave identical retention times to the urinary steroids. Pregnanetriol could also be analyzed by GC/C/IRMS.

Achievement of work-up procedure objective

The method developed and utilized had the following qualities. First, the Girard separation almost completely removed carbonyl containing steroids from the hydrolyzed extracts, which probably represent 75% of urinary steroids. Exceptions may be the 11-carbonyl containing steroids that probably do not react because of the hindered nature of that functional group. Second, a crude micro Sephadex LH-20 column separation effectively produced a fraction containing steroids with two and three functional groups. Third, sodium bismuthate oxidation was designed into the procedure as a means of removing remaining long-retention time pregnane metabolites, thus allowing shorter periods between injection. Many of the quantitatively more important metabolites are converted into 17-oxygenated C₁₉ steroids by the procedure. Fourth, acetylation provided steroids with good gas chromatographic properties that were readily separated. Fifth, despite the complexity of the steroid fraction of urine, the final chromatogram was simple and composed of only a few peaks for which $\delta^{13}C^{0}/00$ could be determined with accuracy.

$\delta^{13}C^{0}/00$ value of the synthetic testosterone

Testosterone acetate prepared from the Japanese testosterone enanthate used for injection in these studies gave a 13 C 0 /00 value of -30.41, a value close to the lowest value obtained for androstanediol diacetate measurements obtained in the subjects studied following testosterone administration

We also analyzed five other current products and one synthetic sample made more than 40 years ago. The following results were obtained: testosterone of Chinese manufacture -30.40; U.S. manufacture -30.38; two Czech products,