

Effects of dihydrotestosterone on adipose tissue measured by serial analysis of gene expression

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Abstract

Intra-abdominal fat accumulation is related to several diseases, especially diabetes and heart disease. Molecular mechanisms associated with this independent risk factor are not well established. Through the serial analysis of gene expression (SAGE) strategy, we have studied the transcriptomic effects of castration and dihydrotestosterone (DHT) in retroperitoneal adipose tissue of C57BL6 male mice. Approximately 50 000 SAGE tags were isolated in intact and gonadectomized mice, as well as 3 and 24 h after DHT administration. Transcripts involved in energy metabolism, such as glyceraldehyde-3-phosphate dehydrogenase, malic enzyme supernatant, fatty acid synthase, lipoprotein lipase, hormone-sensitive lipase and monoglyceride lipase, were upregulated by DHT. Transcripts involved in adipogenesis, and cell cycle and cell shape organization, such as DDX5, C/EBP α , cyclin I, procollagen types I, III, IV, V and VI, SPARC and matrix metalloproteinase 2, were upregulated by DHT. Cell defense, division and signaling, protein expression and many novel transcripts were regulated by castration and DHT. The present results provide global genomic evidence for a stimulation of glycolysis, fatty acids and triacylglycerol production, lipolysis and cell shape reorganization, as well as cell proliferation and differentiation, by DHT. The novel transcripts regulated by DHT may contribute to identify new mechanisms involved in the action of sex hormones and their potential role in obesity.

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Introduction

Obesity is a growing epidemic in many countries, especially in sedentary populations (Seidell 2000). Important health disorders, such as cardiovascular diseases, atherosclerosis, diabetes mellitus and cancer (Bray 1985, Kissebah *et al.* 1989, Calle *et al.* 2003), are associated with obesity. In fact, there is a correlation between the distribution of fat accumulation and health disorders (Despres *et al.* 1990, Vague 1956, Sheehan & Jensen 2000). Sex hormones partly define the localization of fat accumulation. Men tend to gain fat in the abdominal region while women accumulate fat mainly in the gluteal-femoral area (Sjostrom *et al.* 1972). The male pattern of fat accumulation is more closely associated with health disorders (Vague 1956, Sheehan & Jensen 2000). A link may thus exist between androgen levels, fat accumulation and fat distribution. However, until

now, the global molecular effects of androgens on adipose tissue have not been elucidated.

With the advent of DNA microarrays (Schna *et al.* 1995) and serial analysis of gene expression (SAGE) (Velculescu *et al.* 1995), new possibilities arose for large-scale transcriptome analysis and comparison. Both techniques enable characterization of the transcriptome under multiple experimental conditions. DNA microarrays are restricted to known sequences and have some limitations in quantification (Novak *et al.* 2002). However, the SAGE method is highly quantitative and does not require previous knowledge of the sequences under study. This powerful strategy allows us to characterize the entire transcriptome and perhaps discover novel genes (Velculescu *et al.* 1997, St-Amand *et al.* 2001).

We have already presented the transcriptome of normal adipose tissue in mice (Bolduc *et al.* 2004). In

the present study, using the SAGE strategy, we show the effects of castration and dihydrotestosterone (DHT), the most potent androgen, on adipose tissue of male mice. The transcripts involved in fat metabolism are discussed, as well as many transcripts involved in various functions modulated by androgens in this tissue. These findings constitute the first step towards a precise understanding of the molecular mechanisms involved in the physiological effects of androgens on adipose tissue.

Materials and methods

Sample preparation

Retroperitoneal adipose tissue was obtained from 10 male C57BL/6 12–14-week-old mice per group, purchased from Charles River Canada Inc (St Constant, Canada). The animals had access to Lab Rodent Diet No. 5002 and water *ad libitum*. A sham gonadectomy was performed 7 days prior to organ collection for the intact group, while gonadectomy was performed at the same time for the three gonadectomized (GDX) groups. DHT (0.1 mg) was injected 3 h (DHT3h) and 24 h (DHT24h) prior to killing in groups 3 and 4. The dose of DHT selected was the smallest dose that could restore the prostate weight of GDX mice to the level of intact mice. The control groups (intact and GDX) received vehicle solution (0.4% (w/v) Methocel A15LV Premium/ 5% ethanol) instead of DHT. All animal experimentation was conducted in accord with accepted standards of humane animal care. The retroperitoneal adipose tissue was dissected between 0900 and 1215 h. The samples from all mice of the same group were pooled to eliminate interindividual variations and to extract sufficient amount of mRNA. The tissues were stored at -80°C until RNA extraction.

Transcriptome analysis

The SAGE method was performed as previously described (Velculescu *et al.* 1995, 1997, Kenzelmann & Muhlemann 1999, St-Amand *et al.* 2001). Polyadenylated RNA was extracted with the mRNA direct kit (DynaL, Oslo, Norway), annealed with the biotin-5'-T₁₈-3' primer and converted to cDNA with the cDNA synthesis kit (Invitrogen). The resulting cDNA library was digested with NlaIII (anchoring enzyme), and the 3' restriction

fragments were isolated with streptavidin-coated magnetic beads (DynaL) and separated into two populations. Each population was ligated to one of the two annealed linker pairs and extensively washed to remove unligated linkers. The tag beside the most 3' NlaIII restriction site (CATG) of each transcript was released by digestion with BsmFI (tagging enzyme). The blunting kit from Takara Co. (Otsu, Japan) was used for the blunting and ligation of the two tag populations. The resulting ligation products containing the ditags were amplified by PCR with an initial denaturation step of 1 min at 95 °C, followed by 28 cycles of 20 s at 94 °C, 20 s at 60 °C and 2 s at 72 °C with 27 bp primers (St-Amand *et al.* 2001). Due to the low mRNA content of adipose tissue, a second PCR amplification was performed on the ditags for 14 cycles in order to enhance the size of the SAGE library without affecting the quantitative information required for group comparisons (Virlon *et al.* 1999). Each amplification was followed by acrylamide gel purification of the ditags. Finally, large-scale PCR was performed for eight cycles. The PCR product was digested with NlaIII, and the band containing the ditags was extracted from the acrylamide gel. The purified ditags were self-ligated to form concatemers. The concatemers of 500–1800 bp were isolated by agarose gel. The resulting DNA fragments were ligated into the SphI site of pUC19 and cloned into UltraMAX DH5 α FT (Invitrogen). White colonies were screened by PCR to select long inserts for automated sequencing.

Bioinformatic analysis

All SAGE tag sequences were deposited in the GEO database at the National Centre for Biotechnology Information (NCBI). Sequence files were analyzed by the SAGEana program, a modification of SAGEparser (<ftp://ftp.pbrc.edu/public/eesnyder/SAGE/>). Tags corresponding to linker sequences were discarded, and duplicate concatemers were counted only once. Identification of the transcripts was obtained by matching the 15 bp (CATG+11 bp tags) with the UniGene and GenBank databases. The matching procedure used was very restrictive since, in order to avoid the possibility of sequencing errors in the expressed sequence tags (EST) database, we did not consider the matches that were identified only once among

Table 1 General descriptive information about the four SAGE libraries analyzed

| | Intact | GDX | GDX+DHT | | Total |
|---|------------|------------|------------|------------|------------|
| | | | 3 h | 24 h | |
| Tags sequenced | 45996 | 45521 | 51658 | 49256 | 192431 |
| Transcript species | 17660 | 19768 | 19207 | 24553 | 61931 |
| Well-characterized transcripts (%) | 3401 (19) | 3388 (17) | 4011 (21) | 4985 (20) | 8274 (13) |
| Partially characterized transcripts (%) | 1872 (11) | 1859 (10) | 2005 (11) | 2642 (11) | 5472 (9) |
| Novel transcripts (%) | 11783 (67) | 13887 (70) | 12542 (65) | 16006 (65) | 49798 (76) |
| Multiple matches (%) | 604 (3) | 634 (3) | 649 (3) | 920 (4) | 1387 (2) |
| Tags detected more than once | 4332 | 4127 | 5143 | 5003 | 10136 |
| Well-characterized transcripts (%) | 1685 (39) | 1580 (38) | 2252 (44) | 2301 (46) | 3919 (39) |
| Partially characterized transcripts (%) | 656 (15) | 600 (15) | 715 (14) | 732 (15) | 1545 (15) |
| Novel transcripts (%) | 1659 (38) | 1618 (39) | 1805 (35) | 1503 (30) | 3947 (39) |
| Multiple matches (%) | 332 (8) | 329 (8) | 371 (7) | 467 (9) | 725 (7) |
| Transcript species expressed >0.1% | 90 | 72 | 100 | 55 | 144 |
| Well-characterized transcripts (%) | 57 (63) | 43 (60) | 65 (65) | 33 (60) | 91 (63) |
| Partially characterized transcripts (%) | 10 (11) | 8 (11) | 10 (10) | 5 (9) | 14 (10) |
| Novel transcripts (%) | 10 (11) | 12 (17) | 9 (9) | 7 (13) | 20 (14) |
| Multiple matches (%) | 13 (15) | 9 (12) | 16 (16) | 10 (18) | 19 (13) |

the numerous sequences of an UniGene cluster. Indeed, the chance of matches with EST containing sequencing errors drops dramatically when at least two EST are identified in a UniGene cluster for a given tag sequence. A minimum of one EST with a known polyA tail had to be in the UniGene cluster to identify the last NlaIII site on the corresponding cDNA. Classification of the genes was based upon the updated information of the genome directory (Adams *et al.* 1995) found at the TIGR website (www.tigr.org/). To analyze the promoter sequences for the presence of hormone-responsive elements (HRE), the 2 kb upstream regions of the annotated transcription start of the differentially expressed transcripts were extracted from the mouse genome at NCBI (build 32, version 1). With a Perl script, the promoter sequences were parsed to find the occurrence and positions of the sequences TGTTCT and AGAACA, which are present in more than one natural androgen-responsive elements (Nelson *et al.* 1999). When the genes were on the minus strand of the mouse genome, the downloaded sequences were transformed into their reverse-complement before the parsing procedure.

Statistical analysis

We used the comparative count display (CCD) test to identify the transcripts that were

differentially expressed significantly ($P \leq 0.05$) between the groups with more than a twofold change. The CCD test makes a key-by-key comparison of two key-count distributions by generating a probability that the frequency of any key in the distribution differs by more than a given fold factor from the other distribution. This statistical test has been described elsewhere (Lash *et al.* 2000). The data are normalized to 50 000 tags in order to facilitate visual comparison in the tables.

Results

Four libraries (intact, GDX, GDX+DHT3h and GDX+DHT24h) were generated to characterize the effects of castration and DHT on adipose tissue transcriptome. Approximately 50 000 tags were sequenced in each group for a total of 192 431 tags. Thus, 61 931 different transcript species were detected. The majority of the tags sequenced represent novel transcripts. However, most of the transcript species expressed at high levels correspond to transcripts of known genes (Table 1). The 196 well-characterized transcripts differentially expressed at a significant level ($P \leq 0.05$) are presented in Tables 2–8, according to their functions. There were 117 transcripts upregulated

Table 2 Differentially expressed transcripts involved in sugar and lipid metabolism

| Tags | I | G | 3 h | 24 h | Description (UniGene, Genbank) ^{TGTTCT/AGAACA} |
|--------------|-----|-----|------|------|---|
| Sugar | | | | | |
| CCTACTAACCA | 61 | 48 | 228* | 45 | ↑ aldolase 1, A isoform (Mm. 16763, BC043026) ^{-179/} |
| CAAAAATAAAA | 0 | 2 | 1 | 25* | ↑ enolase 1, alpha non-neuron (Mm. 90587, BC010685) ^{-1832,-948} |
| GCCTCCAAGGA | 37 | 22 | 101* | 53 | ↑ glyceraldehyde-3-phosphate dehydrogenase (Mm. 5289, AK081405) ^{-1086/} |
| TTGCTTTGTTG | 5 | 9 | 6 | 47* | ↑ phosphoenolpyruvate carboxykinase 1, cytosolic (Mm. 42246, BC037629) ⁻⁶⁰⁷ |
| GGACAGCACAC | 1 | 9 | 73* | 37 | ↑ pyruvate carboxylase (Mm. 1845, M97957) ^{-1894,-1843,-504/} |
| GCTTGTGACGA | 0 | 0 | 0 | 17* | ↑ transaldolase 1 (Mm. 29182, BC04754) |
| GGATGCTGGGT | 24 | 18 | 48 | 94* | ↑ transketolase (Mm. 154387, AK012794) ^{-1325,-1249} |
| TAAGGGAAATA | 3 | 0 | 0 | 22* | ↑ triosephosphate isomerase (Mm. 4222, X53333) |
| CCAAATAAAAC | 12 | 19 | 2* | 49 | ↓ lactate dehydrogenase 1, A chain (Mm. 141443, U13687) |
| TTCCAGCTGCT | 59 | 63 | 23 | 9* | ↓ phosphoglycerate mutase 1 (Mm. 16783, BC002241) ^{-1121,-469/-476} |
| Lipid | | | | | |
| TGCCTTCTCTG | 0 | 0 | 0 | 12* | ↑ acyl-coenzyme A dehydrogenase, very long chain (Mm. 18630, BC026559) ^{-713/} |
| GAACAGTCGAC | 15 | 13 | 12 | 99* | ↑ adipocyte complement-related protein (Mm. 3969, BC028770) ^{-836/-865} |
| CATCGCCAGTG | 118 | 90 | 398* | 118 | ↑ apolipoprotein E (Mm. 156335, BC028816) ^{-382,-128} |
| TTTGCTTTAAA | 8 | 11 | 6 | 47* | ↑ ATP citrate lyase; RIKEN (Mm. 25316, BC021502; Mm. 45765, BC021413) ^{-633,-391/-575} |
| GAACATTTTCAG | 1 | 0 | 6 | 31* | ↑ EST ATP citrate lyase (Mm. 25316, BY687740) ^{-633,-391/-575} |
| TTGAGCTCTGA | 3 | 2 | 1 | 22* | ↑ carboxylesterase 3 (Mm. 120807, BC019198) ⁻³⁴⁵ |
| CTGCATAGCTC | 3 | 1 | 1 | 19* | ↑ CD36 antigen (Mm. 18628, AK052825) ^{-926,-551,-51} |
| TATGTCCACGA | 0 | 1 | 15* | 12 | ↑ diacylglycerol O-acyltransferase 1 (Mm. 22633, BC003717) |
| TGGGTGTCCAG | 1 | 2 | 5 | 37* | ↑ fatty acid coenzyme A ligase, long chain 2 (Mm. 28962, AK004897) ^{-1571,-429/-536} |
| ATGCAGGGCCA | 43 | 66 | 520* | 126 | ↑ fatty acid synthase (Mm. 3760, AF127033) |
| ACTCAATTCAG | 22 | 26 | 131* | 30 | ↑ glycerol-3-phosphate dehydrogenase 1 (soluble) (Mm. 10669, BC019391) ^{-156/} |
| GCTTCCTGAGC | 3 | 0 | 10 | 16* | ↑ hormone-sensitive lipase (Mm. 1721, BC021642) ⁻⁵¹⁶ |
| GTCTAAAATTA | 8 | 3 | 4 | 29* | ↑ lipoprotein lipase (Mm. 1514, AK002645) |
| ACAAGTCTCTG | 0 | 1 | 1 | 37* | ↑ EST lipoprotein lipase (Mm. 1514, AA537700) |
| CAAAGCCCCAC | 12 | 38 | 8* | 24 | ↓ EST lipoprotein lipase (Mm. 1514, BE625478) |
| TGTAACAAATG | 2 | 0 | 9 | 12* | ↑ long chain fatty acyl elongase (Mm. 26171, AK029029) |
| CTCAGTATCCC | 3 | 2 | 19* | 6 | ↑ low-density lipoprotein receptor-related protein 1 (Mm. 7221, X67469) |
| TTGTCAGGTAG | 4 | 14 | 2 | 54* | ↑ malic enzyme, supernatant (Mm. 148155, J02652) ^{-1545,-1277,-687/-1374} |
| TGAGCATCGGG | 15 | 16 | 75* | 41 | ↑ monoglyceride lipase (Mm. 194795, AJ316580) ^{-1068,-594/} |
| GTCTGGGGGGGA | 167 | 91 | 124 | 27* | ↓ EST monoglyceride lipase (Mm. 194795, BE651758) ^{-1068,-594/} |
| GGCAAGTGCTA | 1 | 5 | 19 | 35* | ↑ stearoyl-coenzyme A desaturase 1 (Mm. 140785, AF509570) |
| AAAACCATTGC | 29* | 89 | 78 | 358* | — stearoyl-coenzyme A desaturase 1 (Mm. 140785, BC007474) |
| GCTGCCCTGGG | 1 | 0 | 18* | 11 | ↑ EST stearoyl-coenzyme A desaturase 1 (Mm. 140785, AA415297) |
| AGATAGATTTG | 12 | 4 | 30* | 17 | ↑ sterol carrier protein 2, liver (Mm. 1779, BC034613) ^{-1000/-1479,-1136,-721,-656} |
| TGGATGCCTTC | 36 | 22 | 21 | 1* | ↓ alcohol dehydrogenase 1, class I (Mm. 2409, BC013477) ^{-1001,-295/-1938,-1895} |
| GACACCAGAGC | 4 | 12 | 1 | 0* | ↓ brain acyl-CoA hydrolase (Mm. 197523, BC013507) ^{-1466,-1012} |
| AAGACCTATGT | 519 | 265 | 95* | 65* | ↓ diazepam binding inhibitor (Mm. 2785, BC028874) ^{-1234/} |
| AGCCAAAGGAA | 276 | 380 | 73* | 273 | ↓ fatty acid binding protein 4, adipocyte (Mm. 582, BC002148) ^{-415/-1542,-1492} |

I, intact; G, GDX; 3 h, DHT3h; 24 h, DHT24h.

*Significantly different ($P < 0.05$) from G. Arrows are used to show a simplified representation of the effects of DHT. When castration induces a significant change in expression level, a dash is used instead of an arrow.

Table 3 Differentially expressed transcripts involved in energy metabolism

| Tags | I | G | 3 h | 24 h | Description (UniGene, Genbank) ^{TGTTCT/AGAACA} |
|--------------|------|-----|------|------|--|
| CAACTGTATTT | 1 | 1 | 2 | 20* | ↑ aconitase 2, mitochondrial (Mm. 154581, BC004645) ^{-390/} |
| CATCTTCAGCC | 20 | 13 | 49* | 16 | ↑ ATPase, Ca ⁺⁺ transporting, cardiac muscle, fast twitch 1 (Mm. 35134, AY081946) |
| CGGGAGATGCT | 2 | 1 | 1 | 16* | ↑ ATP synthase, H ⁺ transporting, mitochondrial F1 complex, O subunit (Mm. 41, BC012241) |
| CAGGCCACACA | 1 | 5 | 0 | 45* | ↑ ATP synthase, H ⁺ transporting mitochondrial F1 complex, β subunit (Mm. 103838, AK010314) |
| ATAATACATAA | 190 | 139 | 102 | 346* | ↑ ATP synthase F0 subunit 6 (8593–8607)** |
| TAGATATAGGC | 99 | 116 | 28* | 38* | ↓ ATP synthase F0 subunit 6 (8596–8582) |
| TTGATGTATCT | 0 | 0 | 0 | 17* | ↓ ATP synthase F0 subunit 8 (7791–7777) |
| AGGACAAATAT | 25 | 36 | 224* | 138* | ↑ cytochrome b (14539–14553) |
| AATATGTGTGG | 5 | 3 | 1 | 29* | ↑ cytochrome c oxidase, subunit VIc (Mm. 548, BC024666) |
| TATTGGCTCTG | 0 | 0 | 1 | 36* | ↑ cytochrome c oxidase, subunit VIIIa (Mm. 14022, AK002218) ^{-1524/-703,-641} |
| AGGAGGACTTA | 2 | 7 | 17 | 48* | ↑ NADH dehydrogenase subunit 2 (4410–4424) |
| GCTGCCCTCCA | 732 | 568 | 131* | 330 | ↓ cytochrome c oxidase subunit 1 (6813–6827) |
| TGGTGTAAAGCA | 93 | 98 | 12* | 4* | ↓ cytochrome c oxidase subunit 1 (6676–6662) |
| ATGAGAACAGC | 16 | 25 | 1* | 1* | ↓ cytochrome c oxidase subunit 1 (6731–6717) |
| AAGTCATTCTA | 23 | 68 | 1* | 7* | ↓ cytochrome c oxidase subunit 1 (6816–6802) |
| GGCAGTTACGA | 1 | 7 | 45* | 17 | ↑ cytochrome c oxidase subunit 1 (5511–5497) |
| TAGTTACTTAC | 9 | 19 | 13 | 127* | ↑ cytochrome c oxidase subunit 1 (6093–6107); NEW1 domain containing protein (Mm. 22338, AK046719) |
| AGCAGTCCCCT | 625 | 550 | 336 | 82* | ↓ cytochrome c oxidase subunit 2 (7497–7511) |
| AGTGGAGGACG | 132 | 91 | 40 | 26* | ↓ cytochrome c oxidase subunit 2 (7500–7486) |
| CTGCGGCTTCA | 42 | 41 | 6* | 5* | ↓ cytochrome c oxidase subunit 3 (9325–9311) |
| AGCAATTCAAA | 7 | 24 | 2* | 27 | ↓ NADH dehydrogenase subunit 3 (9682–9696) |
| GTAGTGAAGT | 45 | 110 | 9* | 26* | ↓ NADH dehydrogenase subunit 3 (9685–9671) |
| ATGACTGATAG | 259* | 696 | 179* | 162* | — NADH dehydrogenase subunit 4 (11230–11244) |
| GAGTTTGGATT | 12 | 25 | 5 | 4* | ↓ NADH dehydrogenase subunit 4 (11080–11066) |
| ATTATAGTACG | 9 | 19 | 3 | 0* | ↓ NADH dehydrogenase subunit 4 (11192–11178) |
| GTTTTGGATTA | 2 | 2 | 15 | 44* | ↑ NADH dehydrogenase subunit 4 (10671–10657) |

I, intact; G, GDx; 3 h, DHT3h; 24 h, DHT24h.

*Significantly different ($P < 0.05$) from G. Arrows are used to show a simplified representation of the effects of DHT. When castration induces a significant change in expression level, a dash is used instead of an arrow.

**Tags matching the mitochondrial genome (Genbank accession no. NC_001569); values listed for these tags indicate the locus within the mitochondrial genome.

by DHT, 69 transcripts downregulated by DHT and 10 transcripts regulated by castration.

Genes involved in the glycolysis pathway, such as the aldolase 1 A isoform, enolase 1 alpha non-neuron and glyceraldehyde-3-phosphate dehydrogenase (Table 2), as well as transcripts implicated in *de novo* fatty acid synthesis, such as ATP citrate lyase and fatty acid synthase, were upregulated by DHT (Table 2). Furthermore, two transcripts of the triacylglycerol synthesis pathway, namely, glycerol-3-phosphate dehydrogenase 1 (soluble) (GPD1) and diacylglycerol *O*-acyltransferase (DGAT) 1, were upregulated (Table 2). Genes involved in lipolysis, such as hormone-sensitive lipase, were also upregulated by DHT. Apolipoprotein E and low-density

lipoprotein receptor-related protein 1 gene expression was increased by DHT, whereas a switch of transcript species of lipoprotein lipase (LPL) and monoglyceride lipase was observed (Table 2).

Transcripts implicated in energy metabolism (Table 3) as well as in amino-acid metabolism, nucleotide metabolism, transport metabolism, protein modification and general metabolism (Table 4), displayed numerous patterns of gene expression after androgen modulation. Cell division was also affected, since almost all the differentially expressed genes related to cell cycle, including cyclin I, and the genes associated with apoptosis, such as fat-specific gene 27, were upregulated by DHT (Table 5).

Table 4 Differentially expressed transcripts involved in other metabolism

| Tags | I | G | 3 h | 24 h | Description (UniGene, Genbank) ^{TGTTCT/AGAACA} |
|-----------------------------|-----|----|------|------|---|
| Amino acid | | | | | |
| GAAACTCTACT | 7 | 3 | 21 | 36* | ↑ cysteine dioxygenase 1, cytosolic (Mm. 29996, BC013638) ^{-1838/-1884} |
| TATAGTATGTT | 1 | 0 | 0 | 35* | ↑ glutamate-ammonia ligase (glutamine synthase) (Mm. 2338, AY044241) |
| Nucleotide | | | | | |
| TCCTTGGGGGT | 18 | 2 | 19* | 7 | ↑ histidine triad nucleotide binding protein (Mm. 425, AK012433) ^{-1300,-358/-1311,-384} |
| GTGCTGCCAGT | 17 | 45 | 8* | 1* | ↓ ectonucleotide pyrophosphatase/phosphodiesterase 2 (Mm. 28107, AF123542) ⁻¹⁵⁰⁸ |
| Transport | | | | | |
| GTCAATGACGT | 3 | 0 | 15* | 7 | ↑ aquaporin 1 (Mm. 18265, BC007125) |
| TCAGGCTGCCT | 15 | 11 | 4 | 72* | ↑ ferritin heavy chain (Mm. 1776, BC012314) ^{-1572,-599/-1289,-546} |
| CCCTGGGTTCT | 10 | 24 | 149* | 58 | ↑ ferritin light chain 1; ESTs similar to ferritin L subunit 2; EST RIKEN (Mm. 7500, BC019840; Mm. 220829, BQ950380; Mm. 34374, BI789635) |
| CTTCTCATTTG | 2 | 1 | 3 | 18* | ↑ lysosomal-associated protein transmembrane 4A (Mm. 30071, AK084515) ^{-1464/-1610} |
| AATTTCTTCCT | 0 | 0 | 0 | 93* | ↑ major urinary protein 1 and 2 (Mm. 157893, BC012221; Mm. 4516, BC012259) |
| CAGAAGAAGCT | 0 | 0 | 0 | 29* | ↑ EST major urinary protein 1 (Mm. 157893, CA457333) |
| AGTCTCGAGGG | 1 | 5 | 1 | 38* | ↑ solute carrier family 1, member 7 (Mm. 1056, BC037462) ⁻¹²⁵² |
| GTCAGTCCACA | 3 | 1 | 15* | 16* | ↑ solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1 (Mm. 229291, BC037087) |
| Protein modification | | | | | |
| CAGGTGTCCAC | 14* | 0 | 3 | 1 | — EST protein tyrosine phosphatase 4a2; EST RIKEN (Mm. 193688, AV049645; Mm. 36280, AU024264) ^{-836/} |
| General | | | | | |
| TGGAGATAAGC | 2 | 3 | 26* | 23* | ↑ acid phosphatase 5, tartrate resistant (Mm. 46354, BC019160) ⁻²⁰⁴ |

I, intact; G, GDx; 3 h, DHT3h; 24 h, DHT24h.

*Significantly different ($P < 0.05$) from G. Arrows are used to show a simplified representation of the effects of DHT. When castration induces a significant change in expression level, a dash is used instead of an arrow.

Some transcripts involved in RNA synthesis function displayed downregulation, except for the DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 and the CCAAT/enhancer binding protein (C/EBP) alpha transcripts, which were upregulated by DHT (Table 5). Genes associated with cell signaling, such as resistin and growth hormone receptor, were upregulated by DHT, whereas others, such as calmodulin 4, were downregulated (Table 5). Keratins (K), which are implicated in the cytoskeleton, were all downregulated by DHT (Table 6). On the other hand, other cytoskeletal components, such as actin beta cytoplasmic and gelsolin, as well as transcripts related to extracellular matrix, such as various procollagen isoforms, secreted acidic cysteine-rich glycoprotein (SPARC) and matrix metalloproteinase 2 (MMP-2),

were upregulated by DHT (Table 6). Genes involved in cell and organism defense, such as superoxide dismutase 3 extracellular, glutathione peroxidases 3 and 4, and transcripts of the histocompatibility 2 complex, were upregulated by DHT (Table 7). In contrast, other transcripts, such as carbonic anhydrases, heat-shock proteins 1 and 4, adipsin (EST) and lysozyme, were downregulated. Finally, transcripts associated with protein synthesis were affected in different ways by the androgen (Table 8). In addition, many novel transcripts were significantly differentially expressed (Table 9). Remarkably, 24 h after DHT injection, the tag CATG TTTGACAATGA was increased 353 times, whereas the tag CATG TCCCTATAAGC was decreased by almost 300-fold.

Table 5 Differentially expressed transcripts involved in cell division, RNA synthesis and cell signalling

| Tags | I | G | 3 h | 24 h | Description (UniGene, Genbank) ^{TGTTCT/AGAACA} |
|-------------------------------|-----|----|------|------|---|
| Cell cycle | | | | | |
| TACTGCTGATA | 0 | 0 | 0 | 13* | ↑ cyclin I (Mm. 22711, BC003290) ⁻⁵⁰⁷ |
| CTGTTTCAAGG | 12 | 4 | 34* | 12 | ↑ G0/G1 switch gene 2 (Mm. 3283, AK003165) ^{-645/-1831,-595} |
| GCGGCGGATGG | 88 | 40 | 257* | 72 | ↑ lectin, galactose binding, soluble 1 (Mm. 43831, NM_008495) |
| Apoptosis | | | | | |
| GCTTATAGATC | 2 | 2 | 19* | 1 | ↑ B-cell receptor-associated protein 31 (Mm. 17, BC002106) |
| CAGCTGCCTCT | 4 | 0 | 3 | 76* | ↑ fat specific gene 27 (Mm. 10026, AK080133) ^{-575/-300} |
| TGGGTTGTCTA | 34 | 60 | 12* | 76 | ↓ tumor protein, translationally-controlled 1 (Mm. 254, X06407) ⁻⁵⁹⁵ |
| Chromosome structure | | | | | |
| TCGATGTCTGA | 23* | 0 | 0 | 0 | — protamine 2 (Mm. 541, AK005729) |
| Meiosis | | | | | |
| TCAACAAGCAC | 3 | 15 | 0* | 10 | ↓ EST synaptonemal complex protein 3 (Mm. 148209, AW490250) |
| RNA processing | | | | | |
| GCCTTCCAATA | 1 | 0 | 1 | 12* | ↑ DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 (Mm. 19101, BC009142) ⁻¹⁷⁰ |
| GGCAGCACAAA | 15 | 12 | 9 | 0* | ↓ EST heterogeneous nuclear ribonucleoprotein L (Mm. 9043, BB399813) ^{-1466/} |
| Transcription factors | | | | | |
| CTAGATGTCGT | 0 | 1 | 1 | 17* | ↑ C/EBP α (Mm. 34537, BC011118) |
| ACGCAGTGGGT | 9 | 14 | 7 | 0* | ↓ EST nuclear factor I/A (Mm. 4771, AK004196) ^{-1885,-1849} |
| General | | | | | |
| CACGTGCCTGA | 38 | 21 | 10 | 1* | ↓ zinc-finger homeobox 1b (Mm. 37676, AF033116) ⁻⁸⁵² |
| Cell adhesion | | | | | |
| TGAGCTTTGGG | 9 | 0 | 26* | 8 | ↑ melanoma cell adhesion molecule (Mm. 39103, BC026985) ^{-1377,-1151} |
| CAATGTGGGTT | 0 | 0 | 0 | 20* | ↑ osteoblast specific factor 2 (fasciclin I-like) (Mm. 10681, BC031449) |
| TGCAGGTGCAC | 22 | 21 | 19 | 3* | ↓ EST integrin β 1 (fibronectin receptor β) (Mm. 4712, BB109045) ^{-634,-39} |
| Effector modulator | | | | | |
| ACTCGGAGCCA | 60 | 42 | 21 | 7* | ↓ calmodulin 1 (Mm. 34246, AK004673) ^{-1928/-1360,-965} |
| AGTGAGGAAGA | 14 | 15 | 0* | 1* | ↓ calmodulin 4 (Mm. 21075, AK009664) ^{-1901,-829} |
| TTTTTGAACAA | 26 | 34 | 13 | 3* | ↓ catenin beta (Mm. 3476, BC006739) |
| TGCACACAAC | 24 | 29 | 1* | 0* | ↓ S100 Ca binding protein A3 (Mm. 703, AF004941) ^{-1160/-1518,-668,-525} |
| TATCCCACGCC | 1 | 3 | 0 | 24* | ↑ S100 Ca binding protein A11 (calizzarin) (Mm. 175848, BC021916) ^{-1504,-872/-1869} |
| Hormone/growth factors | | | | | |
| CCACTGTGTCC | 9 | 7 | 70* | 26 | ↑ resistin (Mm. 1181, AF290870) ^{-1877/} |
| Receptors | | | | | |
| CATACGCATAA | 3 | 0 | 0 | 18* | ↑ growth hormone receptor (Mm. 3986, BC024375) ^{-411,-366,-292,-231,-222/-1064} |
| GTCTGCTGATG | 77* | 23 | 53 | 23 | — guanine nucleotide binding protein, beta 2, related sequence 1 (Mm. 5305, X75313) |
| GAATATGCAGC | 4 | 19 | 0* | 0* | ↓ kinase insert domain protein receptor (Mm. 285, AK031739) ⁻¹⁵⁵⁴ |
| CAAACCTTTATA | 8* | 37 | 5* | 2* | — pheromone receptor V3R4 (Mm. 160379, AF324869) ^{-1599,-1094/} |

I, intact; G, GDX; 3 h, DHT3h; 24 h, DHT24h.

*Significantly different ($P < 0.05$) from G. Arrows are used to show a simplified representation of the effects of DHT. When castration induces a significant change in expression level, a dash is used instead of an arrow.

Table 6 Differentially expressed transcripts involved in cytoskeletal and extracellular matrix

| Tags | I | G | 3 h | 24 h | Description (UniGene, Genbank) ^{TGTTCT/AGAACA} |
|-----------------------------|------|----|------|------|---|
| Cytoskeletal | | | | | |
| CCCTGAGTCCA | 39 | 30 | 117* | 33 | ↑ actin, beta, cytoplasmic (Mm. 297, X03672) ^{-778/-1950,-1350,-1078} |
| GATACTTGAA | 14 | 20 | 92* | 68* | ↑ EST actin, beta, cytoplasmic (Mm. 297, BY464177) ^{-778/-1950,-1350,-1078} |
| CTCCTGGACAC | 15 | 26 | 258* | 128* | ↑ gelsolin (Mm. 21109, NM_146120) ^{-922,-857,-848/-395} |
| GAGCAGACCGT | 38 | 25 | 89* | 19 | ↑ myosin, heavy polypeptide 4, skeletal muscle (Mm. 35531, AJ278733) ⁻¹⁰⁴⁵ |
| GTGATTGCTAAG | 204* | 49 | 74 | 21 | — EST myosin light chain, phosphorylatable, fast skeletal muscle (Mm. 252182, AV082184; Mm. 249289, AV214319; Mm. 14526, AK010483; Mm. 251434, AV148670) ^{-1509/-857,-124} |
| ACCTCTCAGAT | 0 | 0 | 13* | 9* | ↑ pericentrin 2; ferritin light chain 1; EST ferritin light chain 2 (Mm. 4379, U05823; Mm. 7500, AK002547; Mm. 30357, NM_008049) |
| GGCTGGGGGCT | 7 | 9 | 38* | 11 | ↑ profilin 1 (Mm. 2647, BC002080) |
| TTGGTGAAGGA | 11 | 0 | 7 | 33* | ↑ thymosin, beta 4, X chromosome (Mm. 142729, BC018286) ^{-1449,-657/-291} |
| ATGTCTCAAAG | 7 | 5 | 1 | 44* | ↑ EST tubulin, alpha 1; 2; 6 (Mm. 196396, BC008117; Mm. 197515, AK075955; Mm. 88212, BB001495) |
| CTGCTCAGGCT | 41 | 47 | 1* | 0* | ↓ keratin complex 1, acidic, gene 14 (Mm. 6974, BC011074) ^{-1160/-1296} |
| CCCAGAGCACT | 14 | 18 | 1* | 0* | ↓ keratin complex 2, basic, gene 1 (Mm. 18137, AK019521) ^{-1937/} |
| TTCTTTGGTGA | 39 | 48 | 6* | 0* | ↓ keratin complex 2, basic, gene 5 (Mm. 22657, BC006780) |
| TGGTGCACCTC | 25 | 32 | 0* | 0* | ↓ keratin complex 2, basic, gene 6g (Mm. 89769, AB033744) |
| GAGGGCCGAA | 141 | 59 | 66 | 13* | ↓ troponin I, skeletal, fast 2 (Mm. 39469, BC028515) ⁻¹⁰²² |
| Extracellular matrix | | | | | |
| GCATTGAAAG | 0 | 4 | 0 | 27* | ↑ dermatopontin (Mm. 28935, AF143374) ^{-1726/-526,-399} |
| TGCCGGATGAC | 21* | 2 | 13 | 4 | — dermatopontin (Mm. 28935, AK019890) ^{-1726/-526,-399} |
| GGAAATGGCAA | 0 | 0 | 0 | 14* | ↑ matrix metalloproteinase 2 (Mm. 29564, M84324) ^{-1957,-965,-884,-767/} |
| GTTCCAAAGAA | 1 | 0 | 0 | 14* | ↑ EST procollagen, type I, alpha 2 (Mm. 4482, AK075707) ^{-1157/} |
| TCTTCTATGCA | 38 | 49 | 33 | 11* | ↓ EST procollagen, type I, alpha 2 (Mm. 4482, BQ126567) ^{-1157/} |
| TGTTCACTTG | 5 | 4 | 3 | 74* | ↑ procollagen, type III, alpha 1 (Mm. 147387, AK041115) ^{-708/} |
| GTGTCTGATAA | 3 | 8 | 36* | 10 | ↑ procollagen, type IV, alpha 1 (Mm. 738, J04694) ^{-1586,-1058/} |
| GTGCTGCCCTG | 2 | 1 | 19* | 3 | ↑ procollagen, type V, alpha 3 (Mm. 30477, AF176645) ⁻⁴⁰⁰ |
| GCTCCCCACA | 1 | 0 | 0 | 17* | ↑ procollagen, type VI, alpha 1 (Mm. 2509, X66405) ^{-955/-1538,-1162} |
| CAAACCTCTCAC | 22 | 20 | 5 | 100* | ↑ secreted acidic cysteine rich glycoprotein (Mm. 35439, AK014286) ⁻⁴²⁴ |
| GAACATTGCAC | 32 | 45 | 135* | 61 | ↑ secreted acidic cysteine rich glycoprotein (Mm. 35439, BC004638) ⁻⁴²⁴ |
| General | | | | | |
| TAAGTAGCAAA | 1 | 1 | 1 | 48* | ↑ integral membrane protein 2B (Mm. 4266, BC021786) ^{-1449/} |
| TTTCCTTCAAC | 50 | 49 | 17 | 6* | ↓ EST clathrin, light polypeptide (Lca) (Mm. 198817, B1736877) ^{-75/} |
| GGGTTGGCCCA | 14 | 18 | 5 | 0* | ↓ nidogen 2 (Mm. 20348, AB017202) |

I, intact; G, GDx; 3 h, DHT3h; 24 h, DHT24h.

*Significantly different ($P < 0.05$) from G. Arrows are used to show a simplified representation of the effects of DHT. When castration induces a significant change in expression level, a dash is used instead of an arrow.

Among the 176 differentially expressed transcripts presented in Tables 2–8, excluding the transcripts from mitochondrial genome, 118 possessed one or more potential HRE in there

promoter sequence. Moreover, 29 of these transcripts had one or more potential HRE 500 bp upstream of the transcription initiation start.

Table 7 Differentially expressed transcripts involved in general homeostasis, stress response and immunity

| Tags | I | G | 3 h | 24 h | Description (UniGene, Genbank) ^{TGTTCT/AGAACA} |
|------------------------|-----|------|------|------|---|
| General | | | | | |
| CCCTGCCTTAA | 0 | 2 | 4 | 19* | ↑ creatine kinase, muscle (Mm. 2375, AK009950) ⁻⁷⁴⁵ |
| GAAAAGTGGAT | 2 | 0 | 16* | 6 | ↑ epoxide hydrolase 2, cytoplasmic (Mm. 15295, BC015087) ^{-936/} |
| CTATCCTCTCA | 22 | 19 | 12 | 93* | ↑ glutathione peroxidase 3 (Mm. 7156, AK002219) ^{-229/-1647} |
| AAGGTCTGCCT | 13 | 19 | 98* | 33 | ↑ glutathione peroxidase 4 (Mm. 2400, D87896) |
| GAAGAGGGGGGA | 74* | 15 | 28 | 71* | — haptoglobin (Mm. 26730, M96827) ^{-1792,-1191/-820,-545} |
| GGGGGAGTGGA | 2 | 0 | 24* | 4 | ↑ neuronatin (Mm. 140956, BC036984) ⁻¹⁵¹ |
| TTTCCAGGTGT | 0 | 0 | 2 | 24* | ↑ selenoprotein W, muscle 1 (Mm. 42829, NM_009156) |
| TATCTGTGCAT | 29 | 65 | 14* | 13* | ↓ selnoprotein P, plasma, 1 (Mm. 22699, X99807) ⁻⁵⁵⁶ |
| TTCCCAGTACAC | 8 | 4 | 34* | 10 | ↑ superoxide dismutase 3, extracellular (Mm. 2407, BC010975) ⁻¹²⁶⁷ |
| CTACGTTCTCT | 1 | 0 | 0 | 12* | ↑ thioredoxin-like 2 (Mm. 29675, AK010354) ^{-846,-313/-1003} |
| CCCTGAGGGGT | 89 | 51 | 279* | 79 | ↑ transferrin (Mm. 37214, BC012313) ^{-1703,-673/-1061} |
| AGCAAGATGGT | 21 | 46 | 12* | 2* | ↓ aminolevulinic acid synthase 1 (Mm. 19143, BC022110) ^{-1396/} |
| CCTATTAATAAA | 987 | 1185 | 813 | 239* | ↓ carbonic anhydrase 3 (Mm. 300, BC011129) |
| AATTTACACACC | 90* | 232 | 142 | 451 | — carbonic anhydrase 3 (Mm. 300, M27796) |
| GGTGTGTTTTTA | 28 | 53 | 23 | 15* | ↓ EST carbonic anhydrase 3 (Mm. 300, AV291195) |
| GGAGGCAGAGG | 10 | 16 | 10 | 1* | ↓ carbonic anhydrase 5a, mitochondrial (Mm. 116761, BC030174) ^{-1582,-476} |
| TGAACCGTCCC | 33 | 27 | 4* | 8 | ↓ glutathione S-transferase, pi 2 (Mm. 426, BC002048) ^{-1262,-1185/} |
| Stress response | | | | | |
| TATTAGTCTTA | 30 | 27 | 13 | 5* | ↓ heat-shock protein, 1 (Mm. 1843, AK004658) ⁻⁶²⁹ |
| CTGAGCAGAAT | 10 | 25 | 4* | 1* | ↓ heat-shock protein 4 (Mm. 1032, BC003770) ^{-1383,-996/-1768,-1341} |
| GAATAATAAAA | 3 | 1 | 2 | 18* | ↑ heat-shock protein 8; EST heat shock cognate hsc 73; ESTs (Mm. 197551, BC006722; Mm. 258783, BY761643; Mm. 247242, BY415840) |
| Immunity | | | | | |
| GAGTGGATTCT | 1 | 0 | 0 | 14* | ↑ Cd63 antigen (Mm. 4426, BC012212) |
| GTTGTTTTCCA | 2 | 0 | 0 | 12* | ↑ Fc receptor, IgG, alpha chain transporter (Mm. 3303, BC003786) |
| GATTGAGAATG | 11 | 14 | 5 | 55* | ↑ EST histocompatibility 2, D region locus I; L region; histocompatibility 2, K region (Mm. 33263, AW 741231; Mm. 196214, BY576457; Mm. 16771, BC011306) |
| TCACACATTGC | 0 | 0 | 14* | 4 | ↑ histocompatibility 2, Q region locus 10 (Mm. 88795, K00614) ⁻⁶²⁷ |
| GTTCAAGTGAC | 5 | 5 | 1 | 50* | ↑ Ia-associated invariant chain (Mm. 258212, BC003476) ^{-1765/} |
| CTAATATTTGC | 0 | 0 | 0 | 12* | ↑ immunoglobulin kappa chain, variable 8; 28; constant region; EST RIKEN (Mm. 104747, BC028925; Mm. 220176, BC021781; Mm. 222734, X02816; Mm. 255225, AK008450) |
| GAGGACTGCCA | 28 | 16 | 57* | 14 | ↑ lymphocyte antigen 6 complex, locus E (Mm. 788, BC019113) |
| CATCTGAAAAA | 215 | 411 | 75* | 704 | ↓ EST adipsin (Mm. 4407, AW215391) |
| TGTCAGTCTGT | 122 | 83 | 92 | 26* | ↓ lysozyme (Mm. 45436, BC002069) ^{-581/} |

I, intact; G, GDx; 3 h, DHT3h; 24 h, DHT24h.

*Significantly different ($P < 0.05$) from G. Arrows are used to show a simplified representation of the effects of DHT. When castration induces a significant change in expression level, a dash is used instead of an arrow.

Discussion

There is much evidence that sex hormones mediate changes in adipose tissue distribution, the main observations indicating that there is a negative

correlation between circulating testosterone levels and intra-abdominal fat mass and increased prognostic factors for atherosclerosis, risk of cardiovascular disease and diabetes (Seidell *et al.* 1990, Zumoff *et al.* 1990, Tsai *et al.* 2000). Through

Table 8 Differentially expressed transcripts involved in protein synthesis

| Tags | I | G | 3 h | 24 h | Description (UniGene, Genbank) ^{TGTTCT/AGAACA} |
|--|-----|-----|------|------|--|
| Post-translational modification/targeting | | | | | |
| TGAACACTGAA | 1 | 1 | 1 | 18* | ↑ transglutaminase 2, C polypeptide (Mm. 18843, BC016492) ^{-1456,-199/} |
| TAGCTTCCTCT | 0 | 0 | 1 | 12* | ↑ sequestosome 1; EST RIKEN (Mm. 200125, BC006019; Mm. 41784, BM207023) |
| Protein turnover | | | | | |
| CAGATCTTTGT | 29 | 27 | 110* | 43 | ↑ ubiquitin C; EST RIKEN (Mm. 331, BC021837; Mm. 41423, BB476893) ^{-513/} |
| GTAAGCATAAA | 1 | 2 | 0 | 19* | ↑ EST ubiquitin B (Mm. 235, BU529368) |
| TGACCCCGGGA | 1 | 2 | 1 | 24* | ↑ ubiquitin A-52 residue ribosomal protein fusion product 1 (Mm. 43005, BC014772) |
| GTGGAGGCGCC | 99 | 88 | 2* | 3* | ↓ cystatin E/M (Mm. 36816, NM_028623) ^{-782/-1417,-1261} |
| GAGTAAGGACA | 9 | 12 | 0* | 1 | ↓ kallikrein 7 (chymotryptic, stratum coneum) (Mm. 34974, BC027823) ⁻⁵⁶¹ |
| Translation factors | | | | | |
| GAGCTCCAGCG | 18 | 3 | 35* | 4 | ↑ eukaryotic translation initiation factor 4E binding protein 1 (Mm. 6700, BC002045) ^{-1790,-846/-1808,-920,-816} |
| TGCAATATGGC | 22 | 30 | 7 | 2* | ↓ EST eukaryotic translation initiation factor 4A2 (Mm. 16323, BY674584) |
| Ribosomal proteins | | | | | |
| GGATTTGGCTT | 11 | 8 | 2 | 44* | ↑ ribosomal protein, large P2 (Mm. 14245, BC012413) ^{-859/-463} |
| AACAATTTGGG | 3 | 1 | 0 | 37* | ↑ ribosomal protein L9 (Mm. 14244, BC013165) |
| TGGTCAGGATC | 17 | 12 | 2 | 0* | ↓ EST ribosomal protein L9 (Mm. 14244, AV290443) |
| ACATCATAGAT | 0 | 0 | 0 | 16* | ↑ ribosomal protein L12 (Mm. 70127, BC018321) ^{-1781/-1911} |
| TGGATCAGTCT | 3 | 2 | 2 | 50* | ↑ ribosomal protein L19 (Mm. 30806, BC010710) ^{-1598,-374/-1653} |
| CCAGAACAGAC | 8 | 5 | 1 | 32* | ↑ ribosomal protein L30 (Mm. 3487, BC002060) ^{-1200,-93/-204} |
| GTGAAACTAAA | 5 | 4 | 2 | 32* | ↑ ribosomal protein S4, X-linked (Mm. 66, BC009100) ^{-1468,-380/-1558} |
| GACCTGGAGCC | 33 | 13 | 83* | 24 | ↑ EST ribosomal protein S14 (Mm. 43778, AV094184) ^{-1809,-221/} |
| AATTTCAAAC | 3 | 1 | 0 | 28* | ↑ ribosomal protein S17 (Mm. 42767, BC002044) ⁻⁸⁶² |
| CTGTAGGTGAT | 0 | 0 | 1 | 23* | ↑ ribosomal protein S23 (Mm. 30011, BC002145) |
| TAAAGAGGCCG | 3 | 0 | 0 | 28* | ↑ ribosomal protein S26 (Mm. 372, BC036987) ⁻³⁷⁴ |
| GGCTTCGGTCT | 16 | 3 | 1 | 45* | ↓ ribosomal protein, large, P1; EST RIKEN (Mm. 3158, AK010656; Mm. 233844, AI529467) ^{-1798/} |
| GTTGCTGAGAA | 227 | 145 | 75 | 29* | ↓ ribosomal protein 10 (Mm. 100113, BC024901) ^{-939,-660} |
| GAGGAGAAGAA | 172 | 145 | 32* | 18* | ↓ ribosomal protein L3 (Mm. 3486, BC009655) ^{-1476/-1678,-1352,-1158} |
| CTGCTATCCGA | 141 | 112 | 61 | 10* | ↓ ribosomal protein L5 (Mm. 4419, BC026934) ^{-1900,-1022/} |
| AATCCTGTGGA | 68 | 44 | 46 | 9* | ↓ ribosomal protein L8 (Mm. 30066, U67771) ^{-1256,-704,-628/} |
| CCCACAAGGTA | 86 | 67 | 30 | 14* | ↓ ribosomal protein L27 (Mm. 28985, BC024366) ⁻²⁴⁴ |
| ATCCGAAAGAA | 28 | 45 | 40 | 9* | ↓ ribosomal protein L28 (Mm. 3111, BC024395) |
| AAAACAGTGGC | 80 | 75 | 68 | 20* | ↓ ribosomal protein L37a (Mm. 21529, NM_009084) ⁻⁸⁵ |
| GGGAAGGCGGC | 170 | 88 | 58 | 23* | ↓ ribosomal protein S3a (Mm. 6957, BC039659) ^{-1876/} |
| TATGTCAAAGCT | 211 | 100 | 84 | 25* | ↓ ribosomal protein S12 (Mm. 21289, BC018362) |
| CCTACCAAGAC | 171 | 118 | 68 | 28* | ↓ ribosomal protein S20 (Mm. 21938, BC011323) ^{-1469,-1419,-1160} |
| GCCTTTATGAG | 143 | 105 | 58 | 23* | ↓ ribosomal protein S24 (Mm. 16775, AK002986) ^{-589/} |
| GATGACACCAG | 138 | 66 | 81 | 13* | ↓ ribosomal protein S28 (Mm. 200920, BC010987) ^{-1690,-1556,-1375} |
| CTAGTCTTTGT | 13 | 22 | 2* | 42 | ↓ ribosomal protein S29 (Mm. 154915, BC024393) ^{-1560/} |
| TTCAGCCCGTA | 13 | 16 | 31 | 1* | ↓ EST ribosomal protein S29 (Mm. 154915, CA787284) ^{-1560/} |

I, intact; G, GDX; 3 h, DHT3h; 24 h, DHT24h.

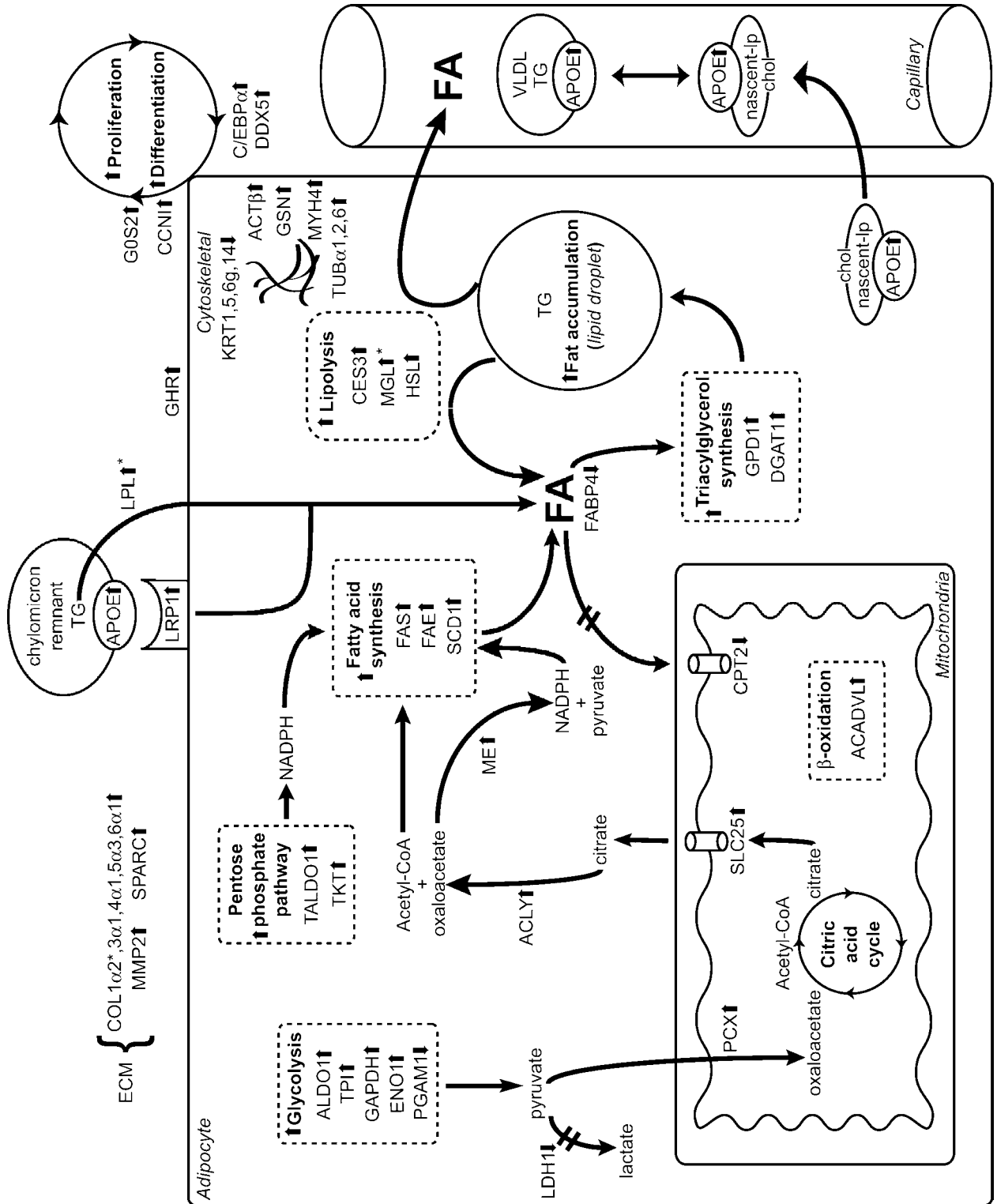
*Significantly different ($P < 0.05$) from G. Arrows are used to show a simplified representation of the effects of DHT.

Table 9 Novel transcripts differentially expressed

| Tags | I | G | 3 h | 24 h | | Tags | I | G | 3 h | 24 h | |
|--------------|-----|-----|------|------|---|--------------|-----|-----|-----|------|---|
| ATTTTCAGTTT | 7* | 136 | 7* | 115 | — | ATCTCACCCAG | 21 | 49 | 6* | 10* | ↓ |
| TCCTACAGTGG | 2* | 20 | 2* | 7 | — | TCCACGTACAT | 9 | 18 | 2* | 0* | ↓ |
| GAAAACGAGAA | 0* | 13 | 2 | 5 | — | GTGCAGGGGTG | 15 | 23 | 4* | 2* | ↓ |
| TTTGACAATGA | 0 | 1 | 85* | 353* | ↑ | GCTCTAGCTGC | 4 | 18 | 0* | 1* | ↓ |
| TTATAGACGGC | 1 | 11 | 58* | 74* | ↑ | AGTCAGATTTT | 7 | 14 | 0* | 0* | ↓ |
| GTCACCTTTTCG | 0 | 0 | 31* | 47* | ↑ | ACAGCAAGGGT | 5 | 14 | 0* | 0* | ↓ |
| GGCTGCGGCCT | 3 | 7 | 288* | 166* | ↑ | GATGGAGACGG | 5 | 22 | 3* | 3* | ↓ |
| CTTCCCCGGGA | 0 | 7 | 47* | 26 | ↑ | ATAGACTTTCA | 8 | 12 | 0* | 0* | ↓ |
| GCCTCCTGGGT | 2 | 0 | 40* | 18* | ↑ | ATCTCGAGAGG | 5 | 15 | 1* | 2 | ↓ |
| GATGGAGTGAC | 1 | 1 | 25* | 3 | ↑ | TCCCCGTACAA | 36 | 42 | 16 | 0* | ↓ |
| GGTGACCACAC | 14 | 12 | 125* | 28 | ↑ | TCCCCGTACAC | 62 | 78 | 26 | 0* | ↓ |
| CTGACGACTGA | 9 | 9 | 59* | 16 | ↑ | TCCCCGTACAG | 40 | 52 | 26 | 2* | ↓ |
| TTGAGTCCTCC | 2 | 0 | 31* | 10 | ↑ | CCGATGATCAG | 37 | 30 | 34 | 2* | ↓ |
| ATGGGTCAAAG | 0 | 2 | 27* | 11 | ↑ | CCCCTATTAAG | 30 | 25 | 21 | 1* | ↓ |
| TCGGTCCGAG | 2 | 1 | 27* | 8 | ↑ | CATCATAAAAC | 33 | 30 | 22 | 1* | ↓ |
| ACTGGGCAGGA | 0 | 0 | 15* | 9 | ↑ | CATCATAAAAG | 39 | 30 | 17 | 2* | ↓ |
| TTCCAAAGCAA | 0 | 1 | 13 | 83* | ↑ | TCCTATTAAGC | 53 | 90 | 41 | 0* | ↓ |
| GCGGAGATGAG | 5 | 0 | 22* | 14* | ↑ | TCCCTATTAAC | 39 | 37 | 23 | 1* | ↓ |
| GGCGGGACCAC | 16 | 4 | 44* | 7 | ↑ | TCCCTATTAAC | 29 | 31 | 21 | 1* | ↓ |
| GATGCGCTTGT | 13 | 4 | 25* | 3 | ↑ | TCCCTATTAAT | 28 | 24 | 17 | 0* | ↓ |
| GCAGCCAGGGC | 4 | 2 | 23* | 3 | ↑ | CAACCATCATC | 99 | 51 | 80 | 6* | ↓ |
| GTGGCGGTGGC | 1 | 0 | 14* | 2 | ↑ | CAAAGATTAAC | 196 | 205 | 87 | 51* | ↓ |
| TCCGGAGAAAA | 0 | 0 | 1 | 42* | ↑ | ATGTTGGGCAG | 28 | 16 | 7 | 1* | ↓ |
| TCCGGAGAAAG | 0 | 0 | 5 | 49* | ↑ | GAAGCACACAG | 23 | 23 | 6 | 0* | ↓ |
| GCCTGAATCAG | 1 | 1 | 9 | 82* | ↑ | TCCCCATACAT | 12 | 20 | 3 | 0* | ↓ |
| GGACAATTGTG | 0 | 0 | 2 | 19* | ↑ | TCCCCTATTA | 47 | 43 | 24 | 7* | ↓ |
| TAGCTGTGTGG | 0 | 0 | 1 | 15* | ↑ | CATCATAAAAT | 32 | 32 | 10 | 1* | ↓ |
| CAAGTAGATGA | 0 | 0 | 1 | 15* | ↑ | GCCCTATTAAG | 15 | 19 | 9 | 0* | ↓ |
| CAGTCAGAAAG | 0 | 0 | 0 | 14* | ↑ | TCCATATTAAG | 16 | 22 | 5 | 1* | ↓ |
| CAGCTAGTTGC | 0 | 0 | 1 | 22* | ↑ | TCCGCGTACAT | 15 | 20 | 5 | 1* | ↓ |
| AAGGAATAAGC | 2 | 7 | 12 | 31* | ↑ | TCCCTATTAAG | 22 | 15 | 5 | 1* | ↓ |
| GGCTAGATTTT | 3 | 2 | 11 | 24* | ↑ | TACCCGTACAT | 13 | 14 | 4 | 0* | ↓ |
| TGTGATGTCAG | 0 | 0 | 2 | 14* | ↑ | TTCTGGTTTGT | 55 | 71 | 27 | 10* | ↓ |
| TAATCATCGAA | 8 | 3 | 13 | 28* | ↑ | TCACTATTAAG | 11 | 19 | 13 | 0* | ↓ |
| TGGGAGATGCT | 2 | 0 | 9 | 15* | ↑ | TACCTATTAAG | 10 | 19 | 9 | 0* | ↓ |
| CCCTATTAAGC | 55 | 54 | 15* | 0* | ↓ | TCCCCTACATC | 9 | 18 | 5 | 0* | ↓ |
| CATCATAAAAA | 437 | 337 | 123* | 5* | ↓ | TCCGTATTAAG | 18 | 26 | 11 | 3* | ↓ |
| GCAGTGGGTAG | 238 | 500 | 95* | 55* | ↓ | TGCCTATTAAG | 16 | 23 | 11 | 2* | ↓ |
| TCACCGTACAT | 22 | 22 | 3* | 0* | ↓ | TCCCATTAAGC | 10 | 23 | 9 | 1* | ↓ |
| GCGGAGAAGAA | 21 | 19 | 0* | 0* | ↓ | TCCCTACTAAG | 14 | 25 | 7 | 2* | ↓ |
| CCCTTTCATAA | 107 | 104 | 18* | 16* | ↓ | CTTAACCTGTC | 15 | 25 | 10 | 2* | ↓ |
| CAATGCTGCCT | 32 | 20 | 2* | 3 | ↓ | TCCCCCGTACA | 12 | 21 | 5 | 1* | ↓ |
| TAGAGACTGCC | 23 | 58 | 2* | 14* | ↓ | TCGCCGTACAT | 12 | 19 | 5 | 1* | ↓ |
| AAAAATCATCC | 41 | 49 | 4* | 48 | ↓ | TTCTATTAAG | 16 | 23 | 12 | 3* | ↓ |
| GTGACCACGGG | 61 | 40 | 9* | 30 | ↓ | TCCCCGTACATC | 10 | 13 | 2 | 0* | ↓ |
| TCCCTATAAGC | 199 | 298 | 115* | 0* | ↓ | TCCCTTTAAGC | 4 | 13 | 2 | 0* | ↓ |
| TGTCAGGTGTC | 18 | 43 | 10* | 0* | ↓ | CATCATACATC | 21 | 22 | 4 | 3* | ↓ |
| TCCCCGACATC | 17 | 48 | 8* | 1* | ↓ | TCCCTATAAGA | 8 | 12 | 6 | 0* | ↓ |
| TCTCCGTACAT | 36 | 63 | 12* | 6* | ↓ | TCGCTATTAAG | 15 | 19 | 11 | 2* | ↓ |
| TCCCTGTTAAG | 12 | 18 | 2* | 0* | ↓ | CCTATTAAG | 14 | 19 | 11 | 2* | ↓ |

I, intact; G, GDx; 3 h, DHT3h; 24 h, DHT24h.

*Significantly different ($P < 0.05$) from G. Arrows are used to show a simplified representation of the effects of DHT. When castration induces a significant change in expression level, a dash is used instead of an arrow.



SAGE, we have characterized the effects of the nonaromatizable and most potent natural androgen, DHT, on the adipose tissue transcriptome. An overview of these data is presented by Fig. 1, which shows some of the changes mediated by the androgen on energy substrate pathways and adipocyte differentiation in male mice retroperitoneal adipose tissue.

The general upregulation of transcripts involved in glycolysis may lead to a greater production of pyruvate which does not seem to be directed to the production of ATP, since lactate dehydrogenase 1 A chain is downregulated. Thus, fuel for *de novo* fatty acid synthesis may be produced, and transcripts involved in *de novo* fatty acid synthesis machinery itself are upregulated. In fact, positive regulation by DHT of lipogenic enzymes such as ATP citrate lyase, malic enzyme and fatty acid synthase has already been observed in monkey prostates (Arunakaran *et al.* 1992). In addition, two transcripts coding for lipoprotein lipase (LPL) are upregulated (except one EST), as previously reported (Anderson *et al.* 2002). It should be mentioned that even if there is fatty acid synthesis, if the fat is not stored in the form of triacylglycerol, and if fat oxidation is stimulated, fat mass may decrease. In fact, there is an upregulation by DHT of GPD1 and DGAT1, which are involved in triacylglycerol synthesis.

The upregulation of transcripts involved in *de novo* fatty acid and triacylglycerol synthesis could reflect the differentiation of preadipocytes into adipocytes, since many of these genes are stimulated in the adipocyte-differentiation process (Mackall *et al.* 1976, Coleman *et al.* 1978). In

addition, C/EBP alpha, which is a major factor involved in adipocyte differentiation and in the expression of adipocyte-specific genes (Gregoire *et al.* 1998), is upregulated. Moreover, the present data also show an upregulation of DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5 by DHT. The expression of this gene has been associated with adipogenesis (Kitamura *et al.* 2001), and it has been identified among the transcripts associated with adipose tissue fattening in the cow (Oishi *et al.* 2000). Cell structure reorganization is necessary for the differentiation process (Croissandeau *et al.* 2002). Keratin (K) proteins gather in pairs of acidic and basic keratins to form intermediate filaments. For example, in the skin, K5 and K14 form heterodimers, and alteration of one of these two molecules leads to skin fragility (Schuilenga-Hut *et al.* 2003). The downregulation of these and other keratins in this study may affect adipocyte cell shape. Extracellular matrix (ECM) components, such as collagen types I, III, IV, V and VI, increase three- to sixfold under adipogenic conditions (Nakajima *et al.* 2002). Moreover, MMP-2 is also involved in adipocyte development, since it regulates the balance between ECM deposition and degradation. In fact, inhibition of MMP can block the adipocyte differentiation process (Croissandeau *et al.* 2002). Transcripts related to all these ECM components are upregulated by DHT in the present study. All these mechanisms can promote the adipocyte differentiation process. It should be mentioned that the stimulation by DHT of collagen type 1 in osteoblastic cells (Kasperk *et al.* 1996) and of MMP-2 in human prostate cancer cells (Liao *et al.* 2003) has already been observed.

Figure 1 Overview of the effects of dihydrotestosterone on energy substrate pathways and adipocyte differentiation in retroperitoneal adipose tissue. Abbreviations: ACADVL, acyl-coenzyme A dehydrogenase very long chain; ACLY, ATP citrate lyase; ACT β , actin beta cytoplasmic; ALDO1, aldolase 1 A isoform; APOE, apolipoprotein E; CCNI, cyclin I; C/EBP α , CCAAT/enhancer binding protein alpha; CES3, carboxylesterase 3; chol, cholesterol; COL1 α 2,3 α 1,4 α 1,5 α 3,6 α 1, procollagen type I alpha 2, type III alpha 1, type IV alpha 1, type V alpha 3, type VI alpha 1; CPT2, carnitine palmitoyltransferase 2; DDX5, DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 5; DGAT1, diacylglycerol O-acyltransferase 1; ENO1, enolase 1 alpha nonneuron; ECM, extracellular matrix; FA, fatty acids; FABP4, adipocyte fatty acid-binding protein 4; FAE, fatty acyl elongase; FAS, fatty acid synthase; G0S2, G0/G1 switch gene 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GHR, growth hormone receptor; GPD1, glycerol-3-phosphate dehydrogenase 1 (soluble); GSN, gelsolin; HSL, hormone-sensitive lipase; KRT1,5,6 g,14, keratin genes 1, 5, 6 g and 14; LDH1, lactate dehydrogenase 1 A chain; LPL, lipoprotein lipase; LRP1, low-density lipoprotein receptor-related protein 1; ME, malic enzyme supernatant; MGL, monoglyceride lipase; MYH4, myosin heavy polypeptide 4 skeletal muscle; MMP2, matrix metalloproteinase 2; nascent-lp, nascent lipoprotein; PCX, pyruvate carboxylase; PGAM1, phosphoglycerate mutase 1; SCD1, stearoyl-coenzyme A desaturase 1; SLC25, solute carrier family 25 (mitochondrial carrier; citrate transporter) member 1; SPARC, secreted acidic cysteine-rich glycoprotein; TALDO1, transaldolase 1; TG, triglyceride; TKT, transketolase; TPI, triosephosphate isomerase; TUB α 1,2,6, EST tubulin alpha 1, 2 and 6; VLDL, very low-density lipoprotein. *Except one EST.

Besides lipogenesis, transcripts involved in the lipolysis process are also upregulated. In fact, hormone-sensitive lipase and carboxylesterase 3, a lipase participating in the mobilization of fatty acids from the triacylglycerol content of adipose tissue (Dolinsky *et al.* 2001), are upregulated by DHT, while monoglyceride lipase is also upregulated (except one EST). On the other hand, the adipocyte complement-related protein, also known as adiponectin, is upregulated. Knockout of this gene revealed an increased β -oxidation in muscle and liver of mice (Ma *et al.* 2002). Furthermore, knockout of the aP2 gene, encoding for adipocyte FABP4, has indicated a defect in basal and stimulated lipolysis (Coe *et al.* 1999). The present study shows that the expression of this gene is downregulated by DHT. Finally, fatty acid coenzyme A ligase long chain 2, which activates long-chain fatty acids for both lipid synthesis and degradation via β -oxidation (Weiner *et al.* 1991), is upregulated by DHT, the most potent androgen.

As presented in Tables 2–8, the changes in androgen state affected all cell functions at various levels. In our previous study on the adipose tissue transcriptome under intact animal condition, many genes involved in the cell and organism defense were among the most highly expressed (Bolduc 2004 *et al.*). The present study shows that carbonic anhydrase 3, the most highly expressed gene in adipose tissue, is downregulated by DHT. It is still unclear why this gene is so highly expressed and what role it has in adipose tissue. In addition, the isoform carbonic anhydrase 5a is also downregulated. Generally, we have observed an upregulation by DHT of antioxidant proteins such as glutathione peroxidase 3 and 4, as well as superoxide dismutase 3 extracellular. Tags matching for the same gene or UniGene cluster were frequently found in the present study, particularly for the most abundant transcripts. For example, this was found for carbonic anhydrase 3. This may be partly explained by alternative polyadenylation cleavage site selection (Pauws *et al.* 2001) and alternative splicing (Mironov *et al.* 1999).

Analysis of all the data shows that various pathways are regulated by DHT in adipose tissue, and the gene expression profile changes induced by DHT suggest a promotion of fatty acid and triacylglycerol production as well as lipolysis in retroperitoneal adipose tissue. The equilibrium between these processes may bend on one side or

the other, resulting in fat accumulation or fat loss. An *in vitro* study revealed that DHT could stimulate lipolysis through adenylate cyclase activation (Xu *et al.* 1990). However, it has been observed, in intact men, that DHT treatment increased visceral fat mass (Marin 1995). These findings on the acute effects of DHT seem to contradict the observations revealing a negative correlation between abdominal obesity and serum testosterone levels in men (Seidell *et al.* 1990, Zumoff *et al.* 1990, Tsai *et al.* 2000). In fact, testosterone treatment can reduce visceral fat mass and waist–hip ratio (WHR) in men (Marin *et al.* 1992, Marin 1995). Moreover, testosterone inhibited triacylglycerol uptake in abdominal adipose tissue of obese men (Marin *et al.* 1995). On the other hand, DHT had no significant effect on either WHR (Marin *et al.* 1995) or triacylglycerol uptake (Marin *et al.* 1995). Differential display PCR has already shown that testosterone and DHT have different effects on prostate gene expression (Avila *et al.* 1998). The different and sometimes opposite actions of testosterone and DHT may indicate that testosterone effects are mediated by a compound created via the aromatization process (Jensen 2000).

While DHT administration affected the expression of hundreds of genes, 7 days of gonadectomy affected only a few. In fact, only 13 classified transcripts were significantly differentially expressed between the intact and the GDX groups. Several of them, such as NADH dehydrogenase subunit 4, pheromone receptor V3R4 and three novel transcripts, showed an inverse pattern of expression in comparing the effect of castration and DHT injection. The tag CATG ATTTTCAGTTT, classified as a novel transcript, displayed a very sharp regulation by androgen modulation. The expression level of this tag changed from 7 in intact to 136 in GDX, falling back to 7 in DHT3h and rising to 115 in DHT24h. On the other hand, the expression of protamine 2, a transcript associated with chromatin condensation in sperm (Aoki & Carrell 2003), could never be restored after castration. This gene may be a target of testosterone, which may have different effects from DHT on gene expression.

Several HRE possibilities were found in the promoter of the significantly differentially expressed transcripts, many of them being included in the 500 bp upstream region of the transcription initiation start. Since the occurrence of a 6 bp

sequence by chance alone is equal to once each 4096 pb, this finding reinforces the idea that these genes are potentially regulated by DHT.

In conclusion, the present data suggest that the administration of DHT to GDX male mice promotes processes involved in glycolysis, fatty acid and triacylglycerol production, lipolysis and cell shape reorganization, as well as cell proliferation and differentiation in retroperitoneal adipose tissue. Moreover, the steroid hormone affected almost all aspects of cell function by modulating hundreds of transcripts. In addition, many of those correspond to novel transcripts.

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