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STUDIES OF THE ADIPOSE TISSUE TRANSCRIPTOME

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This thesis is dedicated to my wife Li Hao and my son Luc Haoran.

RÉSUMÉ

Les études disponibles suggèrent que l'expression des gènes et la régulation cellulaire du tissu adipeux pourraient jouer un rôle important dans le développement des complications reliées à l'obésité. L'identification et la caractérisation du transcriptome de tissu adipeux est susceptible d'aider à comprendre davantage la fonction endocrinienne de ce tissu et le rapport entre l'homéostasie de l'énergie et d'autres systèmes physiologiques. Les outils de la génomique fournissent l'avantage d'étudier simultanément l'expression d'un grand nombre de gènes possiblement impliqués dans les maladies associées à l'obésité. Dans cette thèse, nous avons principalement examiné le transcriptome du tissu adipeux dans diverses conditions physiologiques. La conversion de certains stéroïdes a également été étudiée. La méthodologie des micropuces d'expression a été utlisée, en plus des techniques usuelles de la biochimie des tissus adipeux. Nous avons étudié pour la première fois la variabilité d'expression des gènes dans le tissu adipeux sous-cutané et omental chez des hommes obèses. En second lieu, nous avons étudié la réponse du transcriptome de tissu adipeux à la dihydrotestostérone (DHT) chez les souris. Troisièmement, nous avons examiné le métabolisme de la progestérone dans les cellules adipeuses. Enfin, nous avons évalué l'impact de l'ablation des ovaires sur le métabolisme et le transcriptome de tissu adipeux de singe. En conclusion, dans nos études de transcriptomique examinant le profil d'expression des gènes dans des échantillons humains et d'animaux et dans différentes conditions, plusieurs gènes et voies moléculaires intéressantes ont été identifiés. Ces études peuvent fournir des informations sur les voies métaboliques et de signalisation étant à la base de l'étiologie de l'obésité et de ses complications.

ABSTRACT

Available evidence suggests that gene expression and cellular regulation of adipose tissue could play an important role in the development of obesity-related complications. Identification and characterization of the adipose tissue transcriptome is likely to reveal further insight into the endocrine function of adipose tissue and the relationship between energy homeostasis and other physiological systems. The latest genomic tools provide the advantage of simultaneously studying a large number of genes possibly involved in obesity-associated diseases. In my Ph.D. studies, we mainly examined the transcriptome of adipose tissue in various physiological conditions. We aimed at gaining insight into the molecular events that are critical for the development of obesity and other related diseases. Genomic methodology, such as microarrays have been used. We first studied gene expression variability in subcutaneous and omental adipose tissue of obese men. Second, we investigated the dihydrotestosterone response of the adipose tissue transcriptome in mice. Third, we examined progesterone metabolism in adipose cells. Then, we assessed the impact of hormonal treatment on the monkey adipose tissue metabolism and transcriptome. In conclusion, through the surveys of transcriptomics examining the gene expression profile in fat samples of humans and animals under different conditions, several interesting genes and molecular pathways were identified. These studies may provide information about metabolic and signaling pathways underlying body fat patterning or linking regional adiposity to obesity-related complications.

FOREWORD

I initiated my Ph.D. studies in the program of Physiologie-Endocrinologie under supervision of Drs. André Tchernof and Fernand Labrie. I am currently in my 10th semester. I have taken the following classes: The responsible conduct of research: Normative frameworks (ETH-64841, Fall 06 semester); Endocrinologie moléculaire (PHS-64381, Winter 07 semester), and Maladies humaines et anomalies du métabolisme (PHS-67244, Summer 07 semester). My research project has progressed as expected, with three original manuscripts that have been published, and two others to be submitted soon. For all the original studies in this thesis, I was principal author. I am involved in some of the data collection. I performed. The analytical work and I generated the text of all the manuscripts. Dr. André Tchernof was the supervisor and mentor for each of my original studies. Other co-authors of each manuscript have offered relevant suggestions for various aspects of studies.

This thesis entitled "Studies of the adipose tissue transcriptome" is presented to the "Faculté des études supérieures de l'Université Laval" for obtaining the degree "Doctor of Philosophy" (Ph.D.). The language used is English.

I would like to thank Dr. André Tchernof, my supervisor, for his acceptance and supervision, which made my Ph.D. studies as well as many achievements possible. I really appreciated his careful and patient supervision. He has given me guidance in my technical experiences, writing skills, and scientific thinking. I believe that my Ph.D. studies in this group represent a strong basis for my scientific career and will provide many benefits in the future.

I really appreciated for the co-supervision of Dr. Fernand Labrie. I thank him for accepting me and for the financial support. Without assistance from Dr. Labrie, it would have been impossible for me to complete Ph.D. studies in Canada. Thus, I express my sincere thanks to Dr. Fernand Labrie. Meanwhile, I also would like to thank to him on behalf of my wife. Without Dr. Labrie's support letter, I could not have invited her to come to Quebec city and live with me. I am very much indebted to him.

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LIST OF ABBREVIATIONS

AKR1C	Aldo-keto reductase 1 C
AR	Androgen receptor
BAT	Brown adipose tissue
BMI	Body mass index
cDNA	complementary DNA
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CT	Computed tomography
CV	Coefficient of variation
DHEA-S	Dehydroepiandrosterone sulfate
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
ER	Estrogen receptor
E2	17β-estradiol
FDR	False discovery rate
FTO	Fat mass and obesity-associated gene
GDX	Gonadectomized
HSD	Hydroxy steroid dehydrogenase
HSL	Hormone-sensitive lipase
LPL	Lipoprotein lipase
MRI	Magnetic resonance imaging
NEFAs	Nonesterified fatty acids
OVX	Ovariectomized
PPARγ	Peroxisome proliferator-activated receptor γ
PR	Progesterone receptor
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase reaction
SAGE	Serial analysis of gene expression
SC	Subcutaneous

OM	Omental
RMA	Robust multiarray analysis
SHBG	Sex hormone binding globulin
TAG	Triacylglycerol
WAT	White adipose tissue

INTRODUCTION

1. Obesity

Obesity is now recognized as a common metabolic disease (International Classification of Disease code E66) (Formiguera and Canton, 2004; World Health Organization, 1997), and the prevention of obesity has become one of the leading priorities for the World Health Organization (WHO) (Lean et al., 2006). Body mass index (BMI, kg/m^2), which is the ratio of weight in kilograms divided by the height in meters squared is highly correlated with body fat and is therefore used to categorize obesity in clinical assessment and epidemiological studies (Formiguera and Canton, 2004). A BMI of 18.5-24.9 kg/m^2 is considered normal, overweight is defined as a BMI between 25.0 and 29.9 kg/m^2, and a BMI of $\geq$ 30 kg/m^2 defines obesity (National Institutes of Health, 2008). WHO defines obesity as an epidemic and the abnormal or excessive fat accumulation that may impair health (World Health Organization, 2006). Increases in adipocyte number and/or size are considered to be distinctive characteristics of obesity (Coppack et al., 1990). Obesity is studied in a broad range of disciplines from molecular biology to epidemiology.

1.1 Epidemiology

During the last decades, the prevalence of overweight and obesity has been dramatically increasing worldwide (Ogden et al., 2007). In 2004, approximately 59% of the Canadian adults were overweight and about 23.4% were obese (Tjepkema, 2006). The prevalence of obesity among boys and girls increased from 3% in 1978/79 to 8% using BMI as an indicator (Shields, 2006). Ogden et al (2007) also reported that in 2004, 32.9% of adults (20-74 years old) were obese and more than 17% of teenagers (12-19 years old) were overweight in the United-States, representing 30 million men and 36 million women, 2.3 million boys and 1.9 million girls in the 6 to 11-year-old range. A total of 3.1 million boys and 2.6 million girls were overweight among American adolescents 12 to 19 years of age. The prevalence of adult obesity has exceeded 20% in most of Europe (5-23% in men, 7-36% in women), is 40-70% in the Gulf states and Polynesian islands, and in most countries, the prevalence of obesity now exceeds 15% (Lean et al., 2006).

In developing countries, the prevalence rates are also rapidly catching up as their populations increasingly adopt a westernized diet and lifestyle (Ogden et al., 2007). In China, India, some of the Polynesian islands, the prevalence of obesity ranges from less 5% to more than 75% (McClean et al., 2008). Chinese first nutrition and health survey (Wu et al., 2005) reported that 12% of adults and 8% of children are obese in urban regions, and approximately more than 60 million people became obese between 1992 and 2002. Evidence from a number of studies (Ge, 2006; Levine, 2008; Liu et al., 2004; Reynolds et al., 2007; Wang et al., 2007a; Wang et al., 2007d; Wu, 2006) suggest that assuming increased trends continue, Chinese obesity rates may be predicted to exceed the prevalence of obesity in the United-State by 2020. Thus, great challenges are raised in the Middle East, Pacific Islands, Southeast Asia, and China (Hossain et al., 2007).

These numbers suggest that obesity can now be viewed as a global pandemic, and the continued increase in its prevalence is predicted to surpass smoking as the leading risk factor for mortality in the next few years (Mokdad et al., 2004). The WHO predicts that around 2.3 billion adults will be overweight and more than 700 million adults will be obese (at least 10% of the projected global population) by 2015 (World Health Organization, 2006). The latest data (Ogden et al., 2006) indicate that there has been a plateau in the prevalence of obesity over the last several years in the United-States and suggest that either public health control efforts start to generate effects or that the obese phenotype is already present in the subset of population that is susceptible to obesity (Bessesen, 2008).

Almost all of the organs and tissues in the body might be affected by obesity. The world-wide increases in the prevalence of obesity bring serious concerns about the future with increasing rates of obesity-related complications and metabolic disturbances around the globe, including cardiovascular disease, non-insulin dependent diabetes, ischaemic heart disease, stroke and dyslipidaemia, etc. In United-States, the prevalence of diagnosed type 2 diabetes continued to increase with the concomitant trend of increases in obesity (Gregg et al., 2005). Pediatric obesity is found to achieve a much greater risk of premature death and disability in adulthood (Sethi and Vidal-Puig, 2007). Among children, hypertension and higher levels of serum lipids are associated with higher BMI, and higher BMI generates a

greater risk of cardiovascular diseases (Freedman et al., 2006). Obesity also produces greater risk of accidents, psychosocial problems, hypercoagulability, gallstones, osteoarthritis, back pain, complications of pregnancy, chronic obstructive pulmonary disease (COPD), asthma, obesity hypoventilation syndrome, pulmonary embolism, aspiration pneumonia and obstructive sleep apnoea, as well as cancers of endometrial, ovarian, breast, cervical, prostate, colorectal, gallbladder, pancreatic, hepatic and renal (Gonzalez, 2006; Koenig, 2001; Stunkard, 1996; Tsai et al., 2006). In addition to producing illness, Alley and Chang (2007) reported that 42.2% of obese individuals manifested some degree of reducing functional capacity compared with 26.6% in normal-weight individuals, and suggested that obesity might be associated with disability and impaired activities of daily living.

Obesity is also considered as a chronic disease and associated with increased mortality (Ogden et al., 2007). In Canada, overweight and obesity might result in 1 in 10 of premature deaths among 20 to 64 year-old adults (Katzmarzyt and Ardern, 2004). The investigators of the Framingham Heart Study (Peeters et al., 2003) found that male nonsmokers lost 5.8 years of life expectancy due to obesity. Adams et al. examined 10-yr mortality rates in more than 500,000 Americans and demonstrated a 20% to 40% increase of mortality in either men or women who were overweight in midlife, and found a 2- to 3-fold increase in the risk of mortality in the obese population.

On the other hand, by being a risk factor of morbidity, disability, as well as increased rates of mortality (Bessesen, 2008), obesity is recognized as a significant health and economic burden on the health care system (Birmingham et al., 1999; Katzmarzyt and Janssen, 2004), and may consume up to 9% of a country's health service and be responsible for a large percentage of healthcare budgets (Lean et al., 2006). In Canada, it has been estimated that $1.6 billion per year are directly costed health care of obesity, and another $2.7 billion are indirectly spended on illness, injury-related work disability and premature death which are due to obesity (Katzmarzyt et al., 2003; Katzmarzyt and Janssen, 2004). Clearly, given the health risks related to obesity and overweight, its high prevalence rates are translated into an excessive public health and economic burden.

1.2 Environmental and genetic causes of obesity

Obesity is a complex and multifactorial disorder influenced by a mixture of genetic and environmental factors, including increased of appetite, reduced opportunities for energy expenditure, sedentary lifestyles, and constant availability of inexpensive energy-dense food. Better understanding of the mechanisms initiating obesity could help in identifying effective prevention strategies and successful therapeutic approaches (Romao and Roth, 2008). A series of studies (Jolliffe, 2004; Romon et al., 2005; Warldle and Boniface, 2008) examining the response to the "obesogenic" environment indicated that the variation of appetite and eating behaviour contributes to this interaction. Human appetite is a complex interaction of biological signals associated with neuroanatomical (Broberger, 2005), genetic (de Castro, 2004), pathophysiological (Nilsson-Ehle, 1981), nutritional (French and Robinson, 2003), physical (Blundell and King, 1999), psychological (Kishi and Elmquist, 2005), endocrinological (Meier and Gressner, 2004), and social-environmental factors (Christakis and Fowler, 2007).

The hypothalamus (e.g. arcuate nucleus, paraventricular nucleus, lateral hypothalamic area), gastrointestinal tract and peripheral hormone secreted cells in the liver, pancreas, muscle, and adipose tissue form the basis of the control network of appetite (Hillebrand et al., 2002; Leibowitz and Wortley, 2004; Peelman et al., 2004; Small and Bloom, 2004). Case-control studies (Meyer and Pudel, 1972; Schachter et al., 1968; Stunkard and Kaplan, 1977; Stunkard and Fox, 1971) in comparing obese and normal-weight adults demonstrated that a impaired satiety responsiveness was detected among obese subjects. Carnell and Wardle (2008) support the notion that early expression of appetitive traits, namely reducing responsiveness to internal satiety signals, increasing responsiveness to external food cues, and high preference for energy-dense foods might provide plausible mechanism for childhood obesity.

Several cross-sectional and longitudinal studies (Berkey et al., 2000; Eisenmann et al., 2002; Ekelund et al., 2002; Kawabe et al., 2000; Lazzer et al., 2003; Macek et al., 1989; Moore et al., 2003; Proctor et al., 2003; Raitakari et al., 1997; Rowlands et al., 1999;

Schmitz et al., 2002; Suter and Hawes, 1993) revealed that children and adolescents with high levels of physical activities appear to have lower adiposity than those with lower activity levels. A strong association between excessive TV viewing and pediatric obesity was observed (Eisenmann et al., 2002; Lazzer et al., 2003; Proctor et al., 2003; Tremblay and Willms, 2003). Children have been found to have poor eating habits and not consume healthy diets, and being physically inactive is a common phenomenon in many adolescents (Ogden et al., 2007). Eaton et al. (Eaton et al., 2006) reported that in 2005, almost 40% of American adolescents spent 3 or more hours per day watching television and only 33% had daily physical education in school.

The basal rate of energy expenditure and the energy consumed with fidgeting or nonactivity thermogenesis are two regulators in energy utilization (Levine et al., 2000; Levine, 2008). All of these factors are found to show definite familial clustering with very wide variation among families and indicate a pronounced impact of genetic origin (Romao and Roth, 2008). Neel (1962) put forward the thrifty gene hypothesis, according to which heritable factors are involved in maximizing metabolic efficiency to store energy under starvation, but increasing intake and weight gain are induced in times of abundance. Morbid obesity, which is induced in the leptin-deficient (ob/ob) mouse model provides evidence to support this theory (Romao and Roth, 2008). The homozygous ob/ob genotype may effectively protect from starvation. Previous studies (Loos and Bouchard, 2008) reported that the fat mass and obesity-associated (FTO) gene had several variant alleles in the first intron, generating a significant association with obesity-related phenotypes.

However, the genetics of obesity cannot be considered as the single rational explanation to the world-wide epidemic prevalence in obesity (Hill and Peters, 1998). The current environment of overabundance of food and significant decrease in physical activity produces a positive energy imbalance between energy intake and energy expenditure, and promotes excessive energy storage and therefore results in the development of obesity. Long-term studies of residents of Framingham (Christakis and Fowler, 2007) described a network phenomenon of "friends make friends fat" and suggested that social influences can be considered to play a critical role in the development of obesity. A number of studies

(Patel and Srinivasan, 2002; Romao and Roth, 2008) reported that the gestational environment during fetal development may produce long-lasting impacts on initiating obesity and metabolic disorders. Generally, interaction of multiple genes that predispose to obesity with exposure to many environmental risks in genetically susceptible individuals might contribute to the development of obesity and the growing in prevalence of obesity (Ogden et al., 2007).

1.3 Management of obesity

As a chronic medical condition, obesity needs long-term intervention in lifestyle modification (changed diet and physical activity behaviours), weight loss medication and surgery. Standard behaviour modification techniques are used in the management of obesity, including self-monitoring and goal-setting, modifying specific eating behaviours (e.g., slowing the rate of eating, controlling where eating occurs, delaying gratification), stimulus control and reinforcement management (Wadden, 1993). The goals for change in obese patients are determined according to detailed records of unhealthy behaviours, the precipitants, consequences and moderating factors. In order to eliminate unhealthy behaviour, stimuli (e.g., situations, times, people, emotions) should be identified and thereafter altered (e.g., reducing the availability of tempting high-fat, energy-dense foods in a person's home, buying only small amounts of a tempting food, avoiding certain social situations that elicit excess eating). Reinforcement management is performed to reward someone who completes a specific behaviour.

Currently, dietary modification is the most effective intervention in any weight loss strategy (Bessesen, 2008). In a series of studies of combining diet and exercise therapy for the treatment of overweight and obesity in adults, a significant weight loss was found after treatment, but no significant difference in the effects over a 1 year follow-up period was detected comparatively to those achieved by diet alone (Foreyt et al., 1993; Miller et al., 2004; Sikand et al., 2008; Wadden et al., 1997). A minimum of 150 to 420 minutes of exercise per week is recommended to reduce risk of chronic disease by the US Centers of Sport Medicine and the Institute of Medicine (Food and Nutrition Board, 2002; Pate et al., 1995). A number of studies (Ballor and Keesey, 1991; Garrow and Summerbell, 1995;

Miller et al., 1997; Wing, 1999) revealed that physical activity combined with dietary control could play an important role in behavioural interventions and generate a short-term weight reduction among overweight and obese adults. In a study of dose-response relationship between exercise and fitness including 464 sedentary obese/overweight postmenopause (Church et al., 2007), a linear dose response with measurable improvements in fitness was found and gave the evidence to support the notion that physical activity may bring measurable advantages.

1.4 Prevention of obesity in adults and children

Obesity prevention may play an important role in decreasing morbidity and mortality due to the chronic effects of excess fat accumulation (Seidell et al., 2005; World Health Organization, 2000). The fundamental goal in obesity prevention is to prevent the onset of obesity in those adults who have normal body weight (Kumanyika et al., 2002). In the pediatric age, the major emphasis should be on obesity prevention to treat obese children and promote weight loss as well as delayed obesity development into adulthood (Kumanyika et al., 2008).

The public Health Approaches to the Prevention of Obesity Working Group of the International Obesity Task Force (Kumanyika et al., 2002; Kumanyika et al., 2008) defines a comprehensive approach to obesity prevention. Such approach should address both dietary habits and physical activity patterns of the population at the societal and individual level as well as immediate and distant causes. It should also have multiple focal points and levels of intervention (e.g. at national, regional, community and individual levels), and should include both policies and programs and build links between sectors that may otherwise be viewed as independent.

2. Body fat distribution

There is a striking sex difference in body fat distribution between men and women (Figure 1). Jean Vague (1947; 1956) firstly recognized that men tended to accumulate adipose tissue in the abdomen (android fat distribution) while women tended to accumulate fat in the gluteo-femoral region (gynoid fat distribution). Abdominal adipose tissue areas measured by imaging techniques such as computed tomography (CT) or magnetic resonance imaging (MRI) later showed a clear sex-related difference in visceral fat accumulation. In men, abdominal adipose tissue tends to accumulate in the visceral area to a greater extent than in women (Kuk et al., 2005; Ross et al., 1994), and for a similar fat mass, men have on average a two-fold higher visceral adipose tissue accumulation compared to women (Lemieux et al., 1993). Pond (1992) suggested that these differences are likely due to intrinsic features of each adipose tissue compartment. The differences in body fat distribution between men and women may also be due to the regulation by sex steroid hormones (Mayes and Watson, 2004; Tchernof and Despres, 2000).

The varying degree of fat accumulation in different depots leads to a heterogeneous link between obesity and metabolic consequences. Intraabdominal (visceral) fat accumulation is related to a substantially greater risk of diabetes, insulin resistance, hyperinsulinemia, dyslipidemia, proinfammatory alteration, and accelerated atherosclerosis than subcutaneous (SC) fat (peripheral) (Despres et al., 1990; Juhan-Vague et al., 2002; Lemieux et al., 2001). The worldwide INTERHEART study (Yusuf et al., 2004) indicated that abdominal obesity might be considered as an independent risk factor for myocardial infarction. Using CT or MRI, previous studies (Despres and Lemieux, 2006) demonstrated that excess fat accumulation located inside the abdominal cavity on anatomical structures such as the greater omentum, mesentery, and retroperitoneal space is now recognized as the most common feature among patients with the metabolic syndrome, referring to a combination of medical disorders that increase the risk of developing cardiovascular disease and diabetes (Despres and Lemieux, 2006).

The lipolytic responsiveness in adipose tissue taken from SC sites is lower than that of adipose tissue from visceral (omental, OM) sites (Tchernof et al., 2006). Some distinct biological characteristics of visceral adipose tissue may contribute to the link between intraabdominal fat accumulation and metabolic complications. In women covering the spectrum from a normal body weight to obesity, adipocyte size is 20 to 30% smaller in the OM compartment than in the SC fat depot (Boivin et al., 2007; Tchernof et al., 2006). Adipose cell size is found to play an important role in the modulation of adipocyte function (Farnier et al., 2003) such as adipoctye lipolysis, lipid synthesis and glucose uptake (Farnier et al., 2003; Franck et al., 2007; Zinder and Shapiro, 1971), as well as gene expression profiles (Jernas et al., 2006). Larger fat cell size seems to be associated with increased lipid synthesis and lipolysis, which results in an enhanced fatty acid flux into the circulation (Smith et al., 2006a). Although visceral adipose tissue cannot be viewed as a major source for serum free fatty acids in women at the whole-body level (Nielsen et al., 2004), adipocytes in the visceral fat depots have been found to be sensitive to lipolytic stimuli (β-adrenergic agonist stimulation) (Edens et al., 1993; Reynisdottir et al., 1997; Richelsen et al., 1991; Tchernof et al., 2006), and resistant to lipolytic suppression by insulin (Zierath et al., 1998). Evidence that splanchnic lipolysis correlates with visceral fat mass (Nielsen et al., 2004) may indicate that visceral obesity is associated with increased delivery of fatty acids to the liver. This may provide a rational explanation as to why visceral fat accumulation in men produces a greater risk of cardiovascular disease (Boivin et al., 2007).

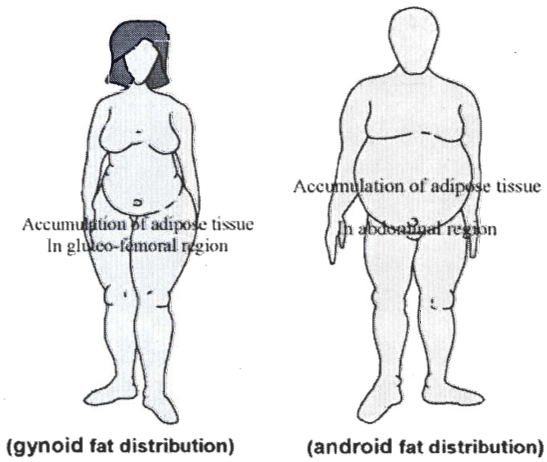


Figure 1: Body fat distribution paterns in humans

Adipose tissue is involved in the uptake, storage and release of lipids (Pond, 1992). However, adipose tissue can also be considered as an important site of steroid biosynthesis and production of various secreted proteins (Trayhurn and Beattie, 2001) (Figure 2). Adipose tissue is in fact regarded as an active secretory organ, sending out and responding to signals that modulate appetite, energy expenditure, insulin sensitivity, endocrine and reproductive systems, bone metabolism, inflammation and immunity. Additionally, adipose tissue contains connective tissue matrix, nerve tissue, stromovascular cells, and immune cells (Frayn et al., 2003).

Adipose tissue secretes a variety of biologically active molecules, namely adipocytokines/adipokines (Friedman and Halaas, 1998; Hotamisligil and Spiegelman, 1994; Saltiel and Kahn, 2001; Shimomura et al., 1996; Spiegelman and Flier, 1996). In adipose tissue, only leptin and adiponectin (and possibly resistin, adipsin, and visfatin) are primarily produced by adipocytes and can therefore be properly classified as adipokines. In general, with the biological functions of storing and releasing energy, adipose tissue also contains the metabolic machinery to communicate with distant organs including the central nervous system (CNS) (Kershaw and Flier, 2004).

Thus, as an extremely complex and highly active metabolic and endocrine organ, adipose tissue is integrally involved in coordinating several biological processes including energy metabolism, neuroendocrine function, and immune function (Kershaw and Flier, 2004). It is currently believed that in addition to the release of free fatty acids, altered adipokine function may mediate part of the preferential link between visceral obesity and metabolic disease (Tchernof, 2008).

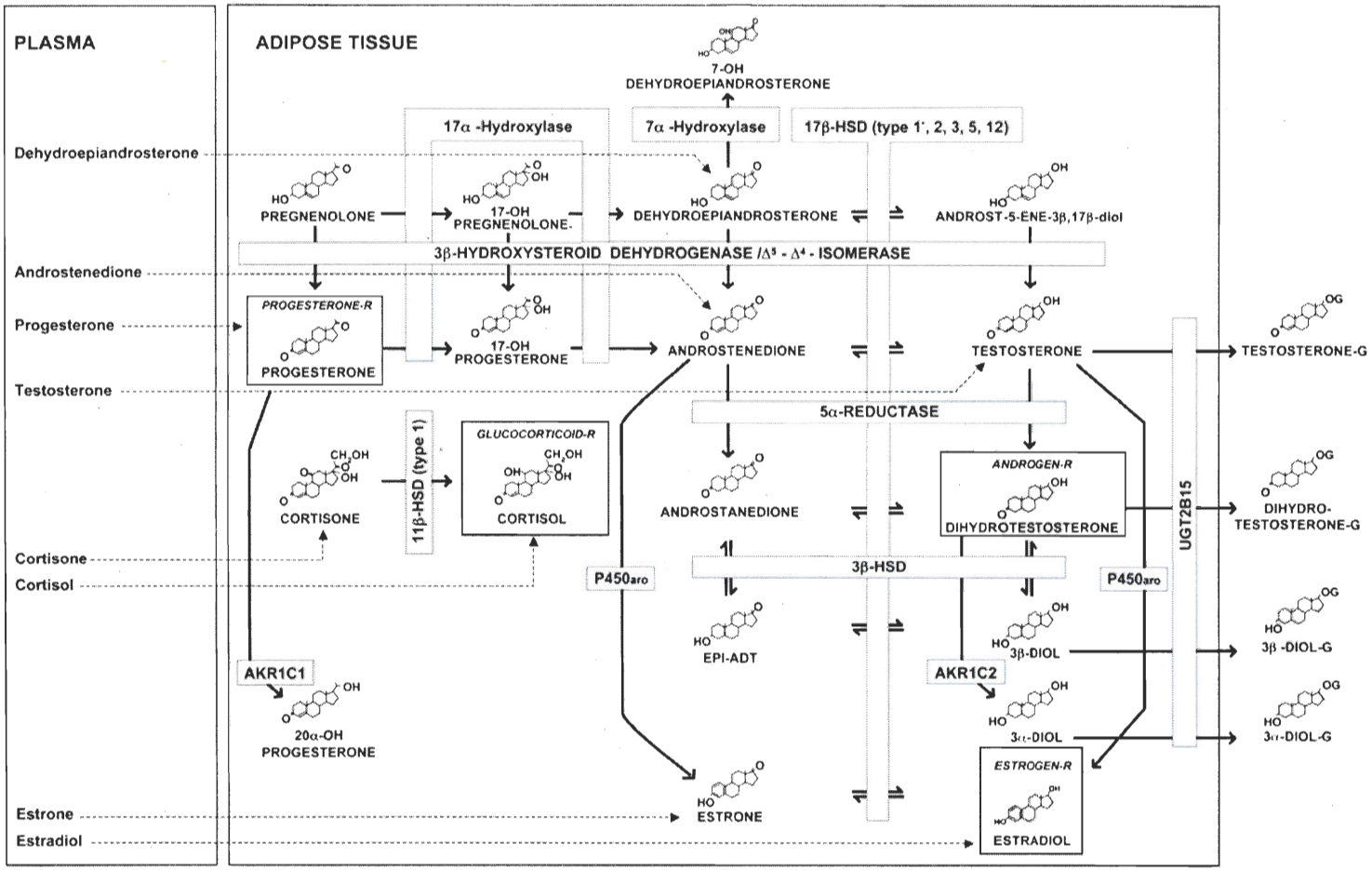


Figure 2. Schematic respresentation of steroid conversions and steroidogenic enzymes present in adipose tissue.

Sources of steroid precursors in plasma are indicated on the left. Active steroids at the receptor level are boxed. Glucuronide conjugates potentially produced by adipose tissue are indicated on the right (-G). This is an updated version of the figure in previous review article (Belanger et al., 2002).

2.1 Development of adipose cells

The development of adipose tissue or preadipocyte differentiation into mature adipocytes is also called adipogenesis. Like muscle, cartilage, and bone, adipose tissue is generally regarded as deriving from multipotent mesodermal progenitor cells. Most researchers describe two phases of adipogenesis in the intermediates between multipotent mesenchymal stem cells and mature adipocytes, including determination and terminal differentiation (Rosen and MacDougald, 2006). The conversion of a pluripotent stem cell to a preadipocyte is defined as determination, and preadipocytes cannot be identified from its morphology but by the loss the potential to develop into other cells types.

The process of preadipocytes achieving mature adipocyte properties is called terminal differentiation which recruits mechanism related to lipid transport and synthesis, insulin sensitivity, and the secretion of adipocyte-specific protein. What are the differences between preadipocytes and mature adipocytes? Preadipocytes possess fibroblast-like morphological characteristics and has distinguishable markers, including preadipocyte factor 1 (Pref-1) or Drosophila Homolog-like 1 (DLK-1) (Villena et al., 2002), type VI collagen alpha 2 chain (COL6A2) (Ibrahimi et al., 1993), and FRP2/SFRP2 which is a secretory protein related to the Wnt antagonist (Hu et al., 1998). Mature adipocytes contain a large unilocular or multilocular lipid droplets surrounded by perilipin protein (Greenberg et al., 1991), and are characterized to produce PPARγ2, markers of terminal differentiation (Glut4 and fatty-acid synthase), leptin (white adipose tissue, WAT) or UCP-1 (brown adipose tissue, BAT), and perform insulin-regulated glucose uptake and metabolism (Rosen and MacDougald, 2006).

The various steps of adipogenesis made it difficult to examine adipocyte recruitment and differentiation in vivo (Sethi and Vidal-Puig, 2007). Many strategies have established immortalized or primary cell lines that can differentiate in vitro, such as mouse 3T3-L1 and 3T3-F442A cell lines as well as immortalized brown preadipocyte cell lines (Rosen and Spiegelman, 2000). All of these approaches make it possible to discern and perceive the process associated with adipogenesis. Primary cultures derived from human samples can

also be used (Hauner et al., 2001) to study human adipogenesis in vivo. This is the approach that was privileged in the present thesis.

Preadipocytes are induced to differentiate into mature adipocytes through growth arrest, clonal expansion, early differentiation, and terminal differentiation (Farmer, 2006). Adipogenesis has been found to be regulated by a cascade of environmental, genetic, epigenetic, and pharmacological factors under the stimulation of positive caloric balance (increasing appetite and/or decreasing fuel dissipation) (Sethi and Vidal-Puig, 2007). Among these factors, peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα) coordinating the expression of adipogenic genes are indispensable and critical regulators for adipocyte development and formation (Jitrapakdee et al., 2005). PPARγ is considered to have the function to induce expression of a population of target genes associated with lipid and glucose metabolism, mitochondrial biogenesis, and secretion of adipokines. The function of C/EBPα is to maintain PPARγ production and regulate the sequential cascade of adipocyte genes involved in adipokine secretion as well as insulin-dependent glucose uptake.

PPARγ is considered to play a crucial role for fat cell development and maintenance of the differentiation of adipocytes (Rosen and MacDougald, 2006). Without it, preadipocytes cannot be induced to express any phase of the adipocyte phenotype and differentiate into mature adipocytes (Rosen et al., 2002). PPARγ is expressed as two isoforms, namely PPARγ1 and PPARγ2 generated by alternative splicing and promoter usage of the Pparg gene (Farmer, 2006; Gesta et al., 2007). C/EBPα, C/EBPβ, C/EBPγ, C/EBPδ, and CHOP are five member of the C/EBP family and can be viewed as components of the basic-leucine zipper class of transcription factors to act as homo- or heterodimers (Graves et al., 1986; Landschulz et al., 1988; Lekstrom-Himes and Xanthopoulos, 1998). Farmer (2006) demonstrated that the early expression of C/EBPβ and C/EBPδ stimulates the expression of C/EBPα and PPARγ during adipocyte differentiation. Mice with adipocyte-specific knockout of C/EBPα exhibit failure of lipid accumulation in adipocytes and reduced adipose mass (Wang et al., 1995). The metabolic effects of C/EBPα in adipose tissue are not well documented, although several C/EBPα target genes have been identified, including adiponectin (ADPOQ), 11-beta hydroxysteroid dehydrogenase (HSD11B1), diacylglycerol

acyltransferase 2 (DGAT2), beta(3)-adrenergic receptor (ADRB3), glucose transporter 4 (GLUT4), leptin (LEP), and growth arrest associated gene 45 (GADD45) (Constance et al., 1996; Dixon et al., 2001; Gout et al., 2006; Hemati et al., 1997; Hollenberg et al., 1997; Hwang et al., 1996; Payne et al., 2007; Qiao et al., 2005). C/EBPα deficiency also results in insulin resistance cell culture models and an inhibition of differentiation of WAT in vivo (El-Jack et al., 1999; Linhart et al., 2001; Wu et al., 1999), and these observations suggest that C/EBPα may play an important role in the termination of adipogenesis.

2.2 Adipose tissue metabolism

Adipose tissue contains the enzymatic machinery involved in the synthesis of triacylglycerols (TAG) under conditions of abundant energy fluxes as well as lipid mobilization during energy deficiency. Lipogenesis and lipolysis are considered as the two major metabolic processes involved in these processes. Fat synthesis in adipose tissue is called lipogenesis, including fatty acid synthesis in animals but not humans, and downstream triglyceride synthesis (Kersten, 2001). Lipogenesis is different from adipogenesis, the latter which defines the development of preadipocytes into mature adipocytes (Rosen and Spiegelman, 2000) as mentioned. Lipoprotein lipase (LPL) in adipose tissue capillaries is a key enzyme involved in lipid uptake and storage. Lipogenesis is very responsive to nutritional regulation. Dietary polyunsaturated fatty acids decrease lipogenesis whereas a food enriched carbohydrates is capable of enhancing lipogenesis in adipose tissue and subsequently results in increased of postprandial triglyceride blood concentration (Jump et al., 1994). The fasting state can decrease lipogenesis and parallelly increase the rates of lipolysis in adipose tissue. As a substrate for lipogenesis, plasma glucose may promote lipogenesis by inducing the expression of lipogenic genes and stimulating the secretion of insulin as well as suppressing the efflux of gucagon in pancreas (Kersten, 2001).

In adipocytes, insulin is considered as a hormonal stimulator to increase the uptake of glucose as well as activate lipogenic and glycolytic enzymes. Insulin also has been reported to influence the expression of lipogenic genes through the transcription factor sterol regulatory element binding protein-1 (SREBP-1) (Foretz et al., 1999). In addition, lipogenesis is found to be affected by growth hormone (GH). Etherton (2000) reported that

significant reduction of lipogenesis in adipose tissue was the consequence of GH administration with simultaneous gain of muscle mass. Previous studies (Bai et al., 1996; Siegrist-Kaiser et al., 1997; Wang et al., 1999) reveal that leptin promotes the secretion of glycerol from adipose cells through its enhancing of fatty acid oxidation and suppressing of lipogenesis, suggesting that leptin probably inhibits fat accumulation via both processes of limiting food intake and influencing some specific metabolic processes in adipose tissue. Acylation stimulating protein (ASP) secreted in adipose tissue increases triglyceride storage in adipocytes (Cianflone et al., 2003; Sniderman et al., 2000).

TAG breakdown into nonesterified fatty acids (NEFAs) and glycerol is defined as lipolysis. Adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL) and monoacylglycerol lipase are critical adipocyte neutral lipases associated with the hydrolysis of intracellular TAGs (Langin and Arner, 2006). Perilipin which coordinates the action of HSL has been shown to be involved in the formation of lipid droplets in the hydrolytic process (Frayn et al., 2005). After TAG breakdown, NEFAs are released from adipocytes and delivered to the liver, skeletal muscle and heart, while glycerol is exclusively transported to the liver. In vitro, certain amount of NEFAs can be re-esterified by mature adipocytes. For this reason, measures of lipolysis, such as those performed in the present thesis, have used glycerol release as a lipolytic index (Tchernof et al., 2006). In humans, catecholamines and insulin play an important role in acute regulation of lipolysis and lipid utilization (horowitz, 2003; Langin and lafontan, 2004). Natriuretic peptides (NPs), cardiac hormones and adipokines have been reported to influence lipolysis and lipid mobilization in humans (Lafontan et al., 2008; Morisset et al., 2008).

As fat tissue is responsible for storing NEFA, adipose tissue can be considered as a metabolic buffer to control daily lipid fluxes in the body (Frayn, 2002). Much like the liver buffers the influx of glucose in the postprandial period, adipose tissue appears to produce a rapid response to food intake, taking up fatty acids after meals and liberating them later when appropriate. The amount of serum triacylglycerol is around 3 g (1 mmol/L), and the influx of triacylglycerol should potentially increase the plasma triacylglycerol concentration up to tenfold when a meal containing 33 g fat is ingested. However, this does

not happen. The circulating triacylglycerol levels have been found to increase by less than 100% in normal individuals (Coppack et al., 1990). These data suggest that adipocytes are able to uptake fatty acids and exert a protective action against the lipotoxic damage to other tissues. Frayn (2002) reaches the conclusion that adipose tissue inhibits efflux of NEFAs from fat cells through increasing triacylglycerol clearance from circulation.

Previous studies (Frayn et al., 2005) also reported that adipose tissue has site-specific properties. As mentioned, lower-body fat accumulation has less deleterious actions than upper-body fat and may offer protective functions. Some researchers have provided evidence that lower-body fat as well as peripheral SC adipose tissue have reverse correlation with cholesterol, triglycerides, blood pressure (Okura et al., 2004; Piche et al., 2008; Snijder et al., 2005), and the extent of arterial stiffness (Ferrerira et al., 2004; Snijder et al., 2004). Using selective venous catheterization to directly compare the metabolic properties of lower-body (gluteal) and upper-body (abdominal) SC adipose tissue in humans, Tan et al. (2004) found that lower-body fat has 67% lower blood flow and 87% lower lipolysis activity than abdominal adipose tissue.

2.3 Endocrine organ

As mentioned, besides being important for the storage and subsequent release of fatty acids, research over the past decade has shown that adipose tissue is also a source of hormones (Belanger et al., 2002; Fonseca-Alaniz et al., 2007; Henry and Clarke, 2008; Wang et al., 2007b). Numerous bioactive peptides and proteins are detected in adipose tissue, and act at the levels as autocrine/paracrine or intracrine or endocrine to affect adipose tissue remodeling, adipogenesis, lipid uptake and transport, angiogenesis, immune response and inflammation, neuron development, as well as initiating potent feedback processes in the regulation of appetite, food intake, glucose disposal, and energy expenditure. Taken together, evidence has led to the recognition that adipose tissue is an "endocrine organ" (Smith, 1996). The next few paragraphs will briefly discuss the most-studied adipokines.

Leptin is mostly secreted from adipocytes, and its levels in circulation directly correlates with adipose tissue mass (Maffei et al., 1995). Leptin was discovered as the product of the

ob gene in mice (Zhang et al., 1994) and acts on the hypothalamus to induce satiety and inhibit expression of NPY as well as the agouti peptide (AgRP), which are related to the actions of increasing nutritional intake and decreasing calorie utilization (Henry and Clarke, 2008; Schwartz et al., 2000). In animals with an obese gene defect, some abnormal signs and symptoms were found, including increases in corticosterone concentration, a thermogenesis deficit, a growth delay, exacerbated appetite, obesity, insulin resistance, and diabetes mellitus. Previous studies (Campfield et al., 1995; Halaas et al., 1995; Henry et al., 1999; Heymsfield et al., 1999; Schwartz et al., 2000; Tang-Christensen et al., 1999) demonstrated that reducing of food intake and promoting energy expenditure are the major metabolic activities of leptin in humans, non-human primates, rodents, and sheep. Leptin also has been known to produce effects on various physiological systems and metabolic processes, such as reproduction, angiogenesis, immunoresponse, blood pressure, and osteogenesis (Fruhbeck et al., 2001).

Adiponectin is an adipocyte-derived plasma protein and is best known for its role in the regulation of insulin sensitivity (Beltowski, 2003). Adiponectin has some favorable effects and is mostly secreted by mature adipocytes (Ahima, 2006). Qi et al (2004) have reported that intracerebroventricular administration of adiponectin decreases body weight in mice. Decreases in adiponectin levels are observed in obese individuals, particularly intraabdominal obesity (Reinehr et al., 2004), suggesting a negative correlation between degree of obesity and plasma adiponectin concentration contributes to the development of insulin resistance and hyperinsulinemia (Drolet et al., 2008; Yamauchi et al., 2001). Several other effects have been attributed to adiponectin, for example adiponectin attenuated activation of NFkB transcription factor (Ajuwon and Spurlock, 2005) and suppression of TNF-α (Thakur et al., 2006). A reciprocal association between adiponectin and C-reactive protein in patients with atherosclerosis has been found, indicating adiponectin may serve as an antiatherogenic plasma protein as well as exerting a protective action against vascular inflammation (Fonseca-Alaniz et al., 2007; Ouchi et al., 2003).

Resistin was first detected from experiments inducing insulin resistance in mice (Steppan et al., 2001), and is predominantly expressed by adipocytes in rodents. Previous studies

(Fonseca-Alaniz et al., 2007) suggest that resistin exhibits a proinflammatory action. However, in human beings, no significant correlation between resistin and obesity was reported (Fonseca-Alaniz et al., 2007). Adipsin is primarily expressed in adipoctyes of mice and both adipocytes and monocytes-macrophages in human subjects (Gabrielsson et al., 2003; Steppan et al., 2001). Visfatin is a newly identified adipokine which is produced and secreted primarily by visceral WAT (Fukuhara et al., 2005), and binds to and activates the insulin receptor, generating insulin-mimetic effects in both vitro and vivo. Several recent studies (Carlson, 2005; Smith et al., 2006b; Wang et al., 2007c) have revealed that circulating visfatin indirectly increases HDL-cholesterol and is involved in lipid metabolism.

As early as in 1987, Siiteri (1987) observed that several sex steroids were secreted by adipose tissue. Expression of various steroidogenic enzymes involved in steroid hormone conversion and local adipose tissue steroid metabolic processes were reported in adipose tissue (Belanger et al., 2002), such as 11β-hydroxy steroid dehydrogenase (11β-HSD) type 1 (Seckl and Walker, 2001), 17β-HSD (Deslypere et al., 1985; Folkerd and James, 1982), 5α-reductase (Longcope and Fineberg, 1985), 17α-hydroxylase (Puche et al., 2002), type 3 3α-HSD (Tchernof et al., 1997), 20α-HSD (Aldo-keto reductase 1 C 1, AKR1C1) (Blanchette et al., 2005). Adipose tissue has been shown to locally synthesize glucocorticoids through the action of 11β-HSD type 1 (Henry and Clarke, 2008). Kershaw et al. (Kershaw et al., 2005) have found that adipocyte-specific corticosterone inactivation leads to inhibition of diet-induced obesity in a rodent model. Adipose tissue can also be considered as a main site to secret oestrogens in postmenopausal women and men (Mattsson and Olsson, 2007), and adipose tissue aromatase expression is higher in gluteal regions than in the visceral fat depot. This complex network of steroid-converting enzymes in adipose tissue may dramatically impact the action of a steroid entering adipose tissue. In the present thesis, one study focused on AKR1C1, which inactivates progesterone.

2.4 Effects of sex hormones on adipose tissue

The striking sex difference in body fat distribution between men and women suggests that sex steroid hormones may play an important role in the modulation of adipose tissue distribution and accumulation as well as metabolism (Belanger et al., 2002; Mayes and

Watson, 2004). The following sections briefly describe studies on the impact of sex steroids on adipose cells. Males have five- to ten-fold higher plasma androgen concentration than females (Bolander, 1989). Sex steroid hormones have effects on adipose tissue mostly through the binding with high affinity to their specific receptors and subsequently initiate the transcriptional regulation of key proteins in adipose tissues. A number of studies supported this hypothesis and provided data that estrogen receptors (ER), progesterone receptors (PR), and the androgen receptors (AR) are detected in adipose tissue from rats, sheep, and humans (Mayes et al., 1996; McCann et al., 2001; Pedersen et al., 1996; Wade and Gray, 1978; Watson et al., 1993). However, nongenomic mechanisms may also be involved in the regulation by sex steroid hormones (Pietras et al., 2001). Nongenomic hormonal actions are mediated by plasma membrane receptors (Mayes and Watson, 2004). Anwar and co-workers (Anwar et al., 2001) have reported that both ER-alpha and ER-beta were detected in OM and SC adipocytes. Nongenomic actions are acute and normally maintained for less than 10 min, while transcriptional regulation at the genomic level generally lasts from several hours to days (Falkenstein et al., 2000; Kelly and levin, 2001; Schmidt et al., 2000).

Androgens have been reported to stimulate lipolysis in cultured male rat preadipocytes (Xu et al., 1990). Administration of testosterone in men can stimulate the release of fatty acids from adipocytes, and at the same time reduce TG storage, suggesting that testosterone also suppresses the action of LPL which is a major regulator of lipid storage in fat tissue (Marin et al., 1990; Marin et al., 1995). In both humans and rodent, androgen receptor and androgen binding were detected in adipocytes (De Pergola et al., 1990; Dieudonne et al., 1998; Pedersen et al., 1996). In addition to the actions of promoting lipid mobilization and inhibiting lipid uptake in adipose cells, androgens also exhibit effects to suppress the differentiation of adipose precursor cells into mature adipocytes (Gupta et al., 2008; Singh et al., 2006). Androgens appear to preferentially influence visceral adipose tissue metabolism and accumulation, whereas some studies suggest that their action is sex-specific (Garaulet et al., 2000). Treatment with testosterone has been shown to promote energy utilization in adipose tissue in men, while a stimulation of fat storage is observed after testosterone administration in women (De Pergola, 2001). However, the effects of the

non-aromatizable androgen 5α-dihydrotestosterone (DHT) remain unclear at the present time. One of the studies of this thesis focuses on the impact of DHT on the adipose tissue transcriptome in both sexes of mice.

Dehydroepiandrosterone-sulphate (DHEA-S) has been shown to be the most abundant steroid hormone in circulation (Nawata et al., 2004), and can be converted to different sex hormones (e.g. testosterone, adrostenedione, estradiol, etc.) (Dhatariya and Nair, 2003). Lea-Currie and co-workers (1998) have found that adipogenesis of 3T3-L1 adipose cells was arrested by DHEA-S. Many other experiments (Lea-Currie et al., 1997; Perez de heredia et al., 2007) reported that DHEA-S treatment may decrease fat content and development of adipocytes in rodent model. In morbidly obese patients, DHEA-S increases adiponectin gene expression in adipose tissue (hernandez-Morante et al., 2006). In addition, increased glycerol release from isolated adipocytes (rat model) was observed with oral treatment of DHEA (Tagliaferro et al., 1995). Serum DHEA-S was negatively correlated with visceral fat accumulation as assessed by CT in obese men, while serum DHEA-S concentrations were inversely associated with waist-to-hip ratio in obese female patients (hernandez-Morante et al., 2008). Lipolytic activity in vitro was also examined and it was demonstrated that DHEA-S treatment significantly promoted lipolysis in subcutaneous adipose tissue from women, and the similar effects were observed in visceral fat from men, indicating some sex- and depot-specific differences in the action of DHEA-S on adipose tissue metabolism. Taken together these data suggest that DHEA and DHEA-S may influence adipogenesis and lipolysis of adipose tissue in humans and rodent models in a manner that is similar to that of androgens.

Like androgens, estrogens have been reported to influence female adipose tissue metabolism and body fat distribution (Belanger et al., 2002; Tchernof et al., 1998). As early as 1978, Wade and Gray (1978) provided evidence that ER existed in adipose tissue of rats with a lower concentration but similar actions than in reproductive organs. Although earlier studies had extreme difficulties detecting ER in adipose tissue from different depots in normal women (Bronnegard et al., 1994; Rebuffe-Scrive et al., 1990), more recent molecular techniques made it possible to find low levels of ER in human adipose tissue

(Mayes and Watson, 2004). ER protein and ER mRNA were detected in human abdominal SC adipose tissue by using Scatchard analysis, Western blot analysis, and Northern blot analysis (Mizutani et al., 1994). Estrogens appear to inhibit lipid accumulation by suppressing the activity of LPL (Bjorntorp, 1996). Previous studies (Hamosh and Hamosh, 1975; Kim and Kalkhoff, 1975; Pedersen et al., 1992) reported that estrogens replacement decreased adipose tissue LPL activity which had been increased by ovariectomy in female rats.

In ovariectomized-adrenalectomized rats, progestin binding sites were found in adipose tissue (Gray and Wade, 1979). Other researchers (O'Brien et al., 1998) have also observed PR mRNA expression in abdominal SC fat samples from premenopausal women using northern blot analysis, and both PR protein isoforms (human PR-A and human PR-B) were also detected using Western blot analysis in those adipose tissues. The administration of progesterone alone may induce LPL activity in rat adipose tissue (Kim and Kalkhoff, 1975; Steingrimsdottir et al., 1980). In humans, local treatment of progesterone has been shown to increase adipose tissue LPL activity in the femoral region (Rebuffe-Scrive et al., 1983), indicating progestins may play a role in the regulation of adipose tissue LPL. Progesterone has also been shown to stimulate fat storage by promoting LPL activity, lipid synthesis, and steroid-mediated differentiation of adipocyte precursors (Lacasa et al., 2001; Mendes et al., 1985; Monjo et al., 2003; Rondinone et al., 1992; Shirling et al., 1981; Wiper-Bergeron et al., 2003).

As mentioned, Blanchette et al (2005) had demonstrated relatively high activity and expression of AKR1C1 (20α-HSD) in human adipose tissue. This enzyme had not been taken into account previously in studies on the impact of progesterone in adipose tissue. In the present thesis, one study focused on the impact of progesterone in preadipocyte cultures and considered local inactivation of the hormone by AKR1C1.

3. Genomic study of adipose tissue

Although increasing sedentary lifestyle and abundant energy-dense foods promoted the development of a global epidemic in obesity, not all individuals are influenced by those environmental stimulators to become overweight and obese, suggesting that molecular mechanism and multiple genetic factors may also be involved in the development of obesity. Disorders of adipose tissue lipid storage and functions will induce obesity and metabolic complications. Many investigators believe that abnormal expression of some genes in adipose tissue contributes to the development of obesity (Arner, 2000). Studies on these abnormal changes in adipose tissue gene expression may reveal unknown mechanisms involved in the development of obesity-related disease, and provide advantages to constitute novel and effective strategies for management of obesity. Over the last decades, most of researchers used mainly candidate gene and multiple genome-wide linkage analyses to identify genes and genetic variants in diseases and traits studies (Velculescu et al., 1995). However, available results indicated that gene discovery efforts for obesity have had only a limited impact.

3.1 Application of modern techniques

Constant evolution of technology, in particularly genomic high-throughput techniques, genome-wide association and the latest gene-finding strategies have accelerated the progress of gene discoveries and new possibilities arose for large-scale transcriptome analysis and comparison, such as cDNA or in situ synthesized oligonucleotide microarrays (Schena et al., 1995) and serial analysis of gene expression (SAGE) (Velculescu et al., 1995). Application of these modern molecular techniques makes it possible to simultaneously quantify and compare the levels of thousands of different messengers present in different types of cells as well as screen for patterns of sequence abnormalities in the DNA of these samples. Thus, the benefits of these approaches provide the opportunity to identify differences in the adipose tissue gene expression profile in relation to fat depot, adiposity or hormonal status.

The transcriptome is the set of transcripts including all messenger RNA molecules produced in one or a population of cells and can vary with external environmental factors. Examination on the transcriptome may reflect the genes that are being actively expressed at any given time under different conditions. Usually, high-throughput techniques are used in the study of transcriptomics evaluations on the expression level of mRNAs in a given cell population.

Microarrays have been used to assess changes in gene expression of cells, or to identify differentially-expressed genes between cell types or in various populations. This approach allows analyzing the expression patterns of tens of thousands of genes at the same time, and may provide extremely detailed examination on a disease or physiological disturbance without having to focus on some precognitive genes to study (Middleton et al., 2004). Microarray techniques can also provide benefits to categorize groups of genes according to their similar functions and identify a set of genes involved in relevant pathways as well as disease susceptibility genes. In recent years, DNA microarrays have been extensively used to search new candidate genes related to obesity or weight change in adipose tissue and adipocytes from humans and animals (Dahlman and Arner, 2007).

In addition to microarrays, other techniques also allow parallel quantification of a vast number of proteins and metabolites, and thereafter unravel interesting genes and processes associated with obesity (Kussmann and Affolter, 2006; Walsh et al., 2006). For example, SAGE may provide a large scale and quantitative analysis of the transcriptome while giving the opportunity of identifying new transcripts (Velculescu et al., 1995). During the last decades, studies in rodents or humans have used SAGE and microarrays to profile gene expression of adipocytes in different depots (Boeuf et al., 2001; Boeuf et al., 2002; Bolduc et al., 2004a; Bolduc et al., 2004b; Bullen, Jr. et al., 2004; Lopez et al., 2003; Vohl et al., 2004).

3.2 Discovery of adipose tissue genes

Adipose tissue transcriptome is highly complex. We postulated that transcriptomic studies would provide relevant information about the basis for determination of adipocyte number

and differentiation, differences in body fat distribution, or their association with metabolic disorders. Several studies have reported differences in gene expression (Atzmon et al., 2002; Linder et al., 2004; Vohl et al., 2004; von Eyben et al., 2004) and capacity of proliferation (Adams et al., 1997; Djian et al., 1983; Hauner and Entenmann, 1991; Kirkland et al., 1990; Tchkonia et al., 2002; Tchkonia et al., 2005) in adipose tissue taken from different depots in rodents and humans, suggesting that genetic programming could affect specific adipose depot development. A database showing the identity of altered adipose tissue transcriptome resulting from different treatments with hormones or in disease states (such as obesity and diabetes) would considerably contribute to general and specific knowledge on this tissue (Bolduc et al., 2004a; Vohl et al., 2004).

Modern approaches make it possible to discover adipose tissue genes contributing to obesity. The cell death-inducing DFFA-like effector a (CIDEA) gene was identified from adipose tissue (Dahlman et al., 2005b; Dahlman et al., 2005a) and considered as an obesity candidate gene. Using genomic-wide association analysis, some researchers have identified FTO gene (Frayling et al., 2007). A series of studies (Hinney et al., 2007; Peeters et al., 2008; Scuteri et al., 2007) subsequently confirmed FTO as an obesity-susceptibility gene in Caucasian populations. Adipose tissue located within the abdominal cavity has been suggested to be functionally and metabolically distinct from that of the SC compartment (Linder et al., 2004; Vohl et al., 2004) and a number of differentially-expressed genes encoding important functional properties may underlie abdominal obesity-related disorders (Vohl et al., 2004). Investigation about characterization of differences in gene expression between human SC and visceral adipose tissue also suggests genetic/developmental heterogeneity (Gesta et al., 2006).

4. Objectives and hypotheses

The link between obesity and chronic conditions such as type 2 diabetes and cardiovascular disease involve interactions of environmental factors and genetic predisposition. Several tissues and cell types are implicated and the regulatory pathways underlying obesity-related disease are extremely complex. At present, available data suggest that gene expression and cellular regulation of adipose tissue could play an important role in development of obesity-related complications. Identification and characterization of adipose tissue transcriptome is likely to reveal further insight into the endocrine function of adipose tissue and the relationship between energy homeostasis and other physiological systems. With the development of genomic tools, it has become possible to simultaneously study a very high number of genes possibly involved in obesity-associated diseases.

The **overall objective** of my Ph.D. studies was to study the transcriptome of adipose tissue in various physiological conditions. Genomic methods such as microarrays have been used. We seeked to gain insight into the molecular events that are critical for the development of obesity and other related diseases.

4.1 Gene expression variability in obese men

We first studied gene expression variability in SC and OM adipose tissue of obese men. Regional fat distribution accounts for an important part of the association between obesity and related metabolic complications. A higher risk of obesity-associated metabolic diseases is specifically associated with increased adipose tissue in the abdominal region (Chan et al., 2004; Wajchenberg, 2000). Analysis of variability in gene expression can be used to examine specific genes which could be related to adipose tissue function. Although adipocytes and adipose tissue have been shown to express more genes than initially thought (Fruhbeck and Gomez-Ambrosi, 2003; Yang et al., 2002), so far, only data on individual variability in gene expression of animals are available (Boeuf et al., 2001; Boeuf et al., 2002). No study has examined human adipose tissue gene expression variability using DNA arrays.

The first aim of my project is to investigate the interindividual variability of gene expression in abdominal SC and OM adipose tissue samples from obese men, using previously established microarrays (Vohl et al., 2004), and attempt to identify highly variable transcripts or pathways in these fat compartments based on the coefficient of variation (CV) in gene expression. The stduy design is shown in Figure 3. These data would provide novel information for identifying highly variable transcripts and cellular pathways that may explain the different metabolic and endocrine-paracrine function of these tissue, and possibly their different roles in the development of obesity-related disease. SC and OM adipose tissue samples were obtained surgically from 10 nondiabetic, normolipidemic massively obese men undergoing obesity surgery. Affymetrix human U133A microarrays (10 arrays for SC fat samples and 10 arrays for OM fat samples) were used to measure expression levels for 22,283 probesets (Vohl et al., 2004). Real time RT-PCR was used for confirmation with a subset of genes.

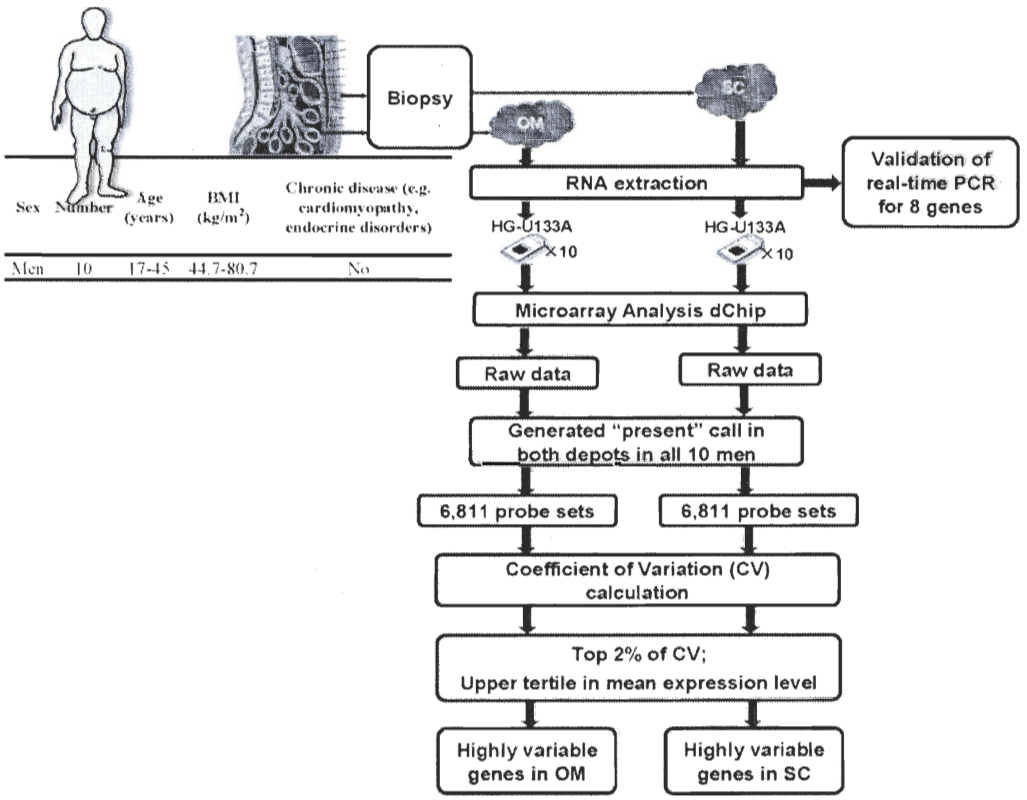
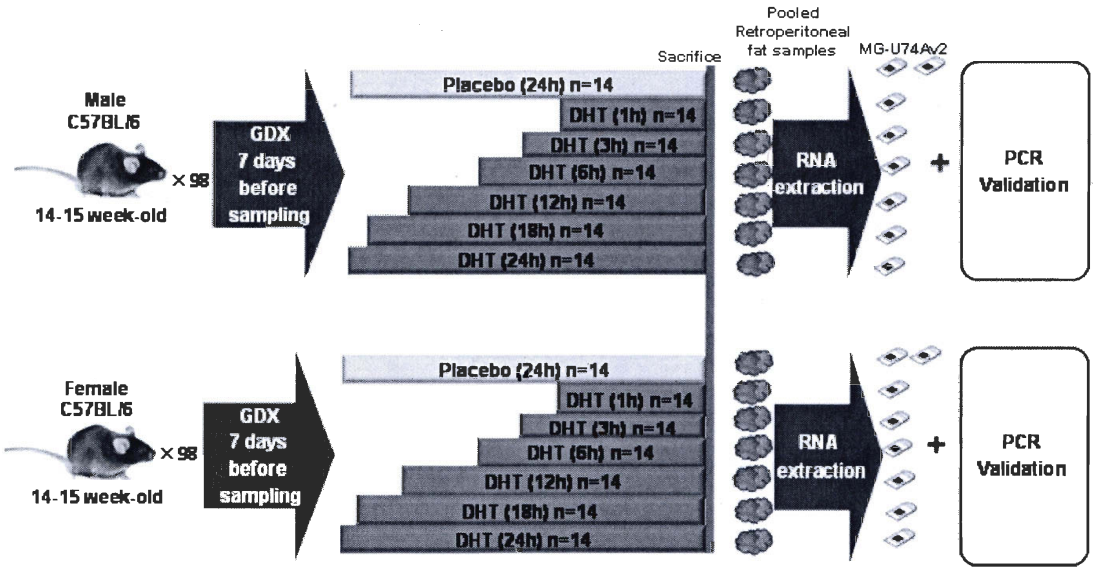


Figure 3. Study design for section 4.1.

4.2 Response to DHT in adipose tissue of mice

Second, we investigated DHT response of the adipose tissue transcriptome in mice. In humans, sex differences are observed in body fat distribution. These differences suggest a close association between sex steroid hormones and regional fat distribution (Belanger et al., 2002). Elbers et al. (1997) showed that female-to-male transsexuals treated with high-doses of androgens develop abdominal fat. The effects of testosterone on radioactive triglyceride accumulation in adipose tissue was studied in rats, and previous studies (Li and Bjorntorp, 1995) reported that castration significantly increased triglyceride accumulation in retroperitoneal and mesenteric fat. Another line of evidence supporting the involvement of androgens in the modulation of body fat distribution is that androgen receptor knock-out mice develop late onset obesity which is especially visceral (Fan et al., 2005; Sato et al., 2003). All these results indirectly suggest that androgens could be key steroids in the modulation of fat distribution in both human and rodent models. The impact of androgens on gene transcription in male and female adipose tissue has been examined using serial analysis of gene expression (Bolduc et al., 2004b). However, until recently, most of the investigations on gene expression were based on individual genes.

The second aim of my project is to study the response of adipose tissue transcriptome to DHT in mice. In this study, using DNA microarray, we investigated the response of male and female mice retroperitoneal adipose tissue to the non-aromatizable DHT, the most potent androgen. The study design is shown in Figure 4. We hypothesize that androgen-responsive genes in both male and female adipose tissue provide information about the signalling pathways linking fat distribution and androgens. Adipose tissue samples were obtained in gonadectomized (GDX) animals treated with placebo (control group), subcutaneous injection of 0.1mg DHT on 1, 3, 6, 12, 18 and 24h prior to killing in experimental groups. cRNA fragments were hybridized to MG-U74 v2 Genechip set. Androgen-responsive genes were identified using specific defined criteria. Real-time PCR was used to validate significant expression responses in a subset of 46 probsets in male mice and 98 probesets in female mice respectively.



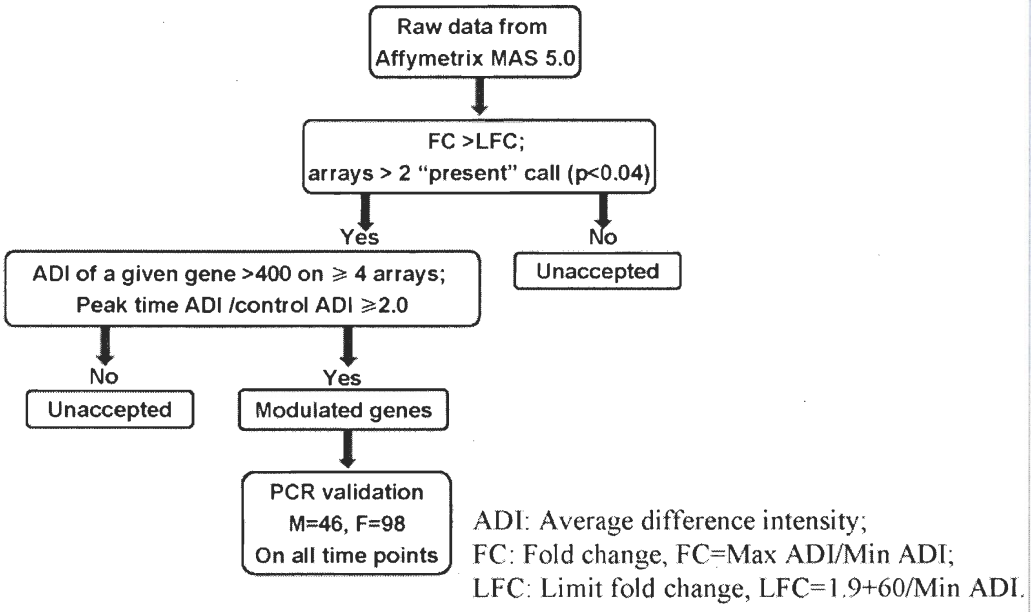


Figure 4. Study design for section 4.2.

4.3 Progesterone metabolism in adipose tissue

Third, we examined progesterone metabolism in human adipose cells. Progesterone has been suggested to be involved in the regulation of adipose tissue accumulation and body fat distribution. It can modulate adipose tissue metabolism by stimulating fat accretion through the regulation of LPL acitivity, lipogenesis and steroid-mediated differentiation of preadipocytes (Lacasa et al., 2001; Wiper-Bergeron et al., 2003). Specific sites of progesterone synthesis include the adrenal cortex, the corpus luteum in the ovary, the testes and placenta. Significant amounts of progesterone can be detected in the plasma of both men and women and we previously have shown high activity of the progesterone-metabolizing enzyme 20α-HSD in abdominal adipose tissue from both sexes.

The third aim of the project is to investigate pathways of progesterone metabolism in human adipose cells. We focused on the comparison of progesterone metabolites formation in preadipocytes and lipid-storing adipocytes. This study included samples from twenty-four women aged 26 to 62 years (BMI 42.11 ± 16.62 kg/m^2, range 21.4 to 74.31 kg/m^2) undergoing abdominal gynecological (n=9) or bariatric (n=15) surgery. Isolated mature adipocytes, primary preadipocytes, and inducing-differentiated adipoctyes were incubated with radiolabelled progesterone for 24h. The metabolites were identified using thin layer chromatography. The study design is shown in Figure 5.

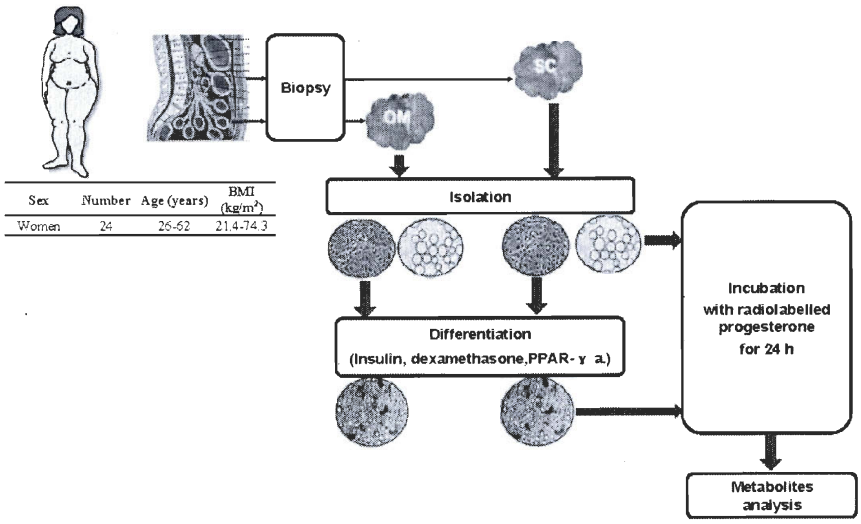


Figure 5. Study design for section 4.3.

4.4 Adipose tissue metabolism and gene expression in monkeys

Then, we assessed the impact of hormonal treatments on the monkey adipose tissue transcriptome. Ovariectomy has been shown to increase food intake and modulate abdominal adipose tissue metabolism in humans and rodent models.

In the present study, we investigate the impact of ovariectomy on abdominal adipose tissue metabolism and gene expression in female cynomolgus monkeys (Macaca Fascicularis) from the Molecular Endocrinology and Oncology Research Center monkey colony. Fourteen female cynomolgus monkeys were divided into two groups of 7 animals (age 4 to 10 years). Monkeys bear a unique identification numbers (and/or letters) tattooed on their chest and/or limb. The first group (INT, intact) remained intact while the second group was ovariectomized (OVX) to eliminate steroids from gonadal origin. All animals were strictly fed identical regimens. Throughout the study period, the animals were strictly fed 8 cookies per day divided in two separate meals. Animal were fed 1 fruit and 1 vegetable twice a week (same fruit and same vegetable for all animals on a given day). Abdominal SC and mesenteric fat samples were collected at necropsy seven days after the beginning of the menses in the INT group and 14 days after ovariectomy in the OVX group. Adipose cells were isolated with collagenase digestion. LPL activity and lipolysis were measured using standard procedures. Affymetrix HG-U133A microarrays were used to measure gene expression profiles of pooled mesenteric tissue samples obtained from two groups. Total RNA was isolated by Trizol and in vitro transcribed to produce a biotinylated cRNA target. Expression levels were analyzed using the Limma package and GeneSprint software. The background subtraction and normalization of probe set intensities was performed using the method of Robust Multiarray Analysis (RMA). To identify differentially expressed genes, gene expression intensities were compared using a moderated t-test and by using empirical Bayers methods followed by a false discovery rate (FDR) filtering. The study design is shown in Figure 6.

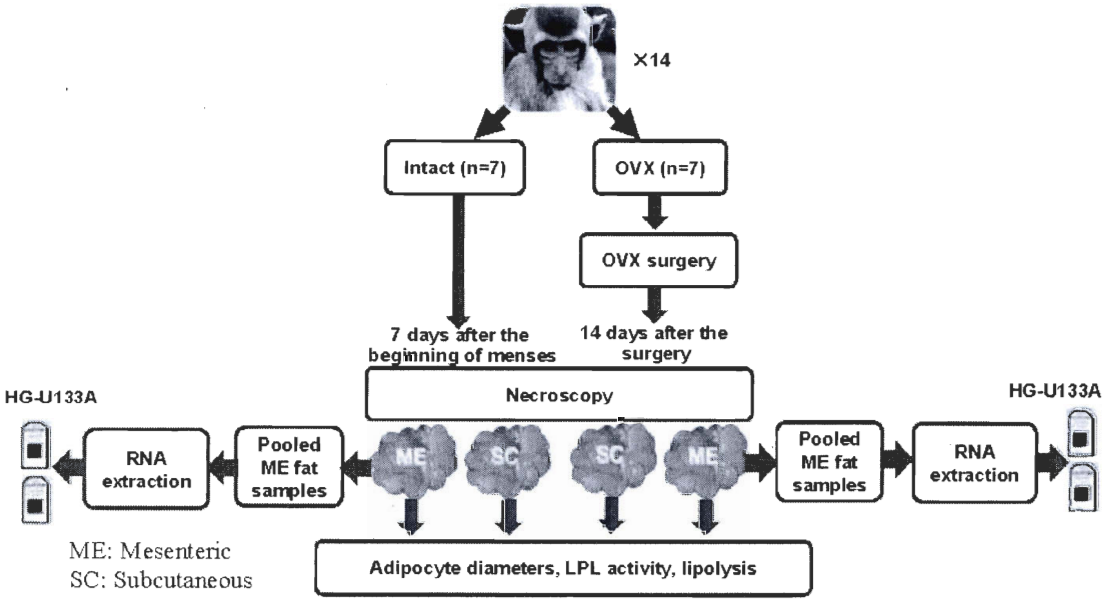


Figure 6. Study design for section 4.4.

Part A: Gene expression variability in subcutaneous and omental adipose tissue of obese men

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Gene expression variability in subcutaneous and omental adipose tissue of obese men

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Nonstandard abbreviations: CV, coefficient of variation; Om, omental adipose tissue; Sc, subcutaneous adipose tissue

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ABSTRACT

We investigated interindividual variability in gene expression in abdominal subcutaneous (SC) and omental (OM) adipose tissue of 10 massively obese men. Affymetrix human U133A microarrays were used to measure gene expression levels. A total of 6811 probesets generated significant signal in both depots in all samples. Interindividual variability in gene expression was rather low, with more than 90% of transcripts showing a coefficient of variation (CV) lower than 23.6% and 21.7% in OM and SC adipose tissues, respectively. The distributions of CV were similar between the two fat depots. A set of highly variable genes was identified for both tissues on the basis of a high CV and elevated gene expression level. Among the set of highly regulated genes, 18 transcripts were involved in lipid metabolism and 28 transcripts were involved in cell death for SC and OM samples, respectively. In conclusion, gene expression interindividual variability was rather low and globally similar between fat compartments, and the adipose tissue transcriptome appeared as relatively stable, although specific pathways were found to be highly variable in SC and OM depots.

Keywords: Adipose tissue; Omental; Subcutaneous; Microarrays; Obese men

INTRODUCTION

A higher risk of obesity-related metabolic diseases has been associated with increased adipose tissue mass in the abdominal region (5,24). Using imaging methods, studies have shown that abdominal, and especially visceral or intra-abdominal obesity, in both men and women, is closely associated with a dyslipidemic state which includes hypertriglyceridemia, low high density lipoprotein (HDL)-cholesterol levels, elevated apolipoprotein B, a greater proportion of small, dense low-density lipoprotein (LDL) particles and increased LDL-cholesterol to HDL-cholesterol ratio (6). This condition is also associated with hyperinsulinemia and insulin resistance (7,24).

Adipose tissue located within the abdominal cavity has been suggested to be functionally and metabolically distinct from that of the subcutaneous compartment (23,15) and a number of differentially-expressed genes encoding important functional properties may underlie abdominal obesity-related disorders (23). Many studies have now used microarray profiling of adipose tissue to investigate gene expression in obesity (3,2,4,16). Analysis of variability in gene expression has been used to examine specific genes which could be related to adipose tissue function. However, so far, only animal data are available (3,2), and no large-scale genomic study has been performed to examine the variability of gene expression in human adipose tissue. In this study, we investigated the interindividual variability in gene expression in abdominal subcutaneous (SC) and omental (OM) adipose tissue samples from 10 nondiabetic, normolipidemic obese men, using previously established microarrays (23).

SUBJECTS AND METHODS

Patient selection. The study group included 10 massively obese men undergoing biliopancreatic diversion at the Laval Hospital (Quebec City). This surgical procedure involves bypassing the small intestine and diverting the bile and pancreatic juice to the distal ileum, which produces maldigestion and selective malabsorption essentially for fat and starch (17). Following clinical examination, none of the patients had identified chronic diseases such as cardiomyopathy and endocrine disorders. Body weight was stable at the time of study and no subject had been on a diet or involved in a weight reduction program in the last 6 months. All patients provided informed-written consent prior to their inclusion in the study. Adipose tissue samples were obtained at the beginning of the surgery from the abdominal subcutaneous wall (close to the umbilicus) and from the greater omentum. Body weight, height, waist and hip circumferences were measured according to standardized procedures.

RNA extraction, reverse transcription and probe preparation. Adipose tissue samples were homogenized in Trizol reagent and centrifuged to separate the lipid fraction. Total RNA was prepared from the cleared homogenate according to the manufacturer's protocol (Invitrogen, Carlsbad, CA). RNA was repurified using RNEasy mini columns (Qiagen, Hilden, Germany). RNA integrity was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Probes for microarray experiments were prepared using 10 micrograms of total RNA and hybridized overnight to Affymetrix HG-U133A Gene Chips (Affymetrix, Santa Clara, CA). Non-specifically bound probe was removed by washing using the Agilent GeneChip Fluidics Station 400. Detection of specifically bound probes was performed by incubating the arrays with a biotinylated anti-streptavidin antibody (Vector Laboratories, Burlingame, CA) prior to staining with SAPE (streptavidin phycoerthryin: Molecular Probes, Eugene, OR). Detailed protocols for probe synthesis and hybridization reactions have been previously described (20). Real-time RT-PCR was used for confirmation with a subset of genes (23).

Array data extraction and analysis. The arrays were scanned using an Agilent GeneArray Scanner and raw data was extracted from scanned images and scaled to 1000 units mean intensity using Microarray Analysis D-Chip software (PM-MM model). A significant signal was considered when the DChip software indicated a "present" call based on the modified algorithm

of Microarray Suite analysis software 4 (Affymetrix). Interindividual variance in mean expression level and coefficient of variation (CV) calculations were performed for each transcript from the normalized signal obtained in both fat depots of all 10 subjects of the study (one array per fat sample, for a total of 20 arrays). Non-parametric spearman rank correlation coefficients were computed to quantify associations between variance and mean expression levels or CV and mean expression levels in SC and OM fat samples either combined or separately. Log-10 transformation for all variables was used to normalize values. SC and OM variances or CVs were compared among fat depots by paired t-test. The selection of the most variable genes in SC and OM fat samples was based on the following 3 criteria: 1) probesets which generated significant signal ("present" call) in both depots in all 10 subjects (n=6811); 2) probesets that were in the top 2 percentile of CV for each depot (n=136 for SC and OM); and 3) probesets that generated a mean expression level that was in the upper tertile (n=68 and 69 for SC and OM respectively). In addition, we examined variability in the probesets which generated significant signal ("present" call) in at least one and up to nine individuals.

Biological Pathway Analyses. Cellular pathways related to these transcripts were identified using the Kegg database (http://www.genome.ad.jp/kegg/) and genecards (http://www.genecards.org/). The Ingenuity Pathway Analysis System (Ingenuity® Systems, www.ingenuity.com) was also used to visualize gene expression data in the context of biological pathways. Two input files were uploaded in the Ingenuity Pathway Analysis system considering 1) probesets highly variable in SC tissues (n=68), 2) probesets highly variable in OM tissues (n=69). Analyses were performed on both files individually, and a comparison analysis was also performed.

RESULTS

Men of the study were 17.0 to 45.0 years old and were in the morbid obesity range with BMI values ranging from 44.7 to 80.7 kg/m^2. They were characterized by a normal lipid profile, and were slightly hypertensive (9). Of the 22,283 probesets present on the array, significant signal ("present" call) was obtained for 6811 probesets in both fat compartments of all 10 subjects. A total of 9076 and 8590 probesets generated significant signal ("present" call) in at least one and up to nine individuals in OM and SC samples respectively.

Figure 1 shows the correlations between gene expression variance or CV (%variance) and mean gene expression levels for all 6811 positive signals, regardless of the fat depot (Fig. 1A), in the OM (Fig.1B) or SC (Fig.1C) fat compartments. As expected, highly-expressed genes had higher absolute variance in their expression levels as reflected by a positive correlation between mean transcript expression levels and absolute gene expression variance. However, mean gene expression levels were negatively correlated with CV, indicating slightly higher variability at low expression levels. The distribution of CV in gene expression in OM and SC is shown in Fig 2. The left panels show the 6811 probesets which generated significant signal in both compartments in all 10 fat samples. More than 90% of clones showed a CV lower than 23.6% and 21.7% in OM and SC adipose tissues, respectively. The right panels show CV distributions of genes that generated significant signal ("present" call) in at least one and up to nine individuals in the OM and SC fat samples. More than 90% of clones showed a CV lower than 22.0% and 20.3% in OM and SC samples respectively. No difference in CV was observed between fat depots in both subsets of transcripts.

Among the 6811 probesets that generated significant signal ("present" call) in both depots in all 10 subjects, we selected probesets that were in the top 2 percentile of CV in each depot, and then identified the ones (69 and 68 probesets in OM and SC samples respectively) which were in the upper tertile of mean gene expression level (see appended tables). Sixty-three genes were obtained in both fat compartments. Selected pathways with highly variable transcripts in SC and OM adipose tissue are shown in Table 1. Some pathways were highly variable in both fat depots, including pathways of hematopoietic cell lineage, the Fc epsilon RI signaling pathway, genes involved in glycerophospholipid metabolism, leukocyte transendothelial migration, and the

GnRH signaling pathway (PLA2G2A and TFRC). Conversely, several pathways were highly variable only in OM or SC adipose tissue samples. Transcripts related to the Jak-STAT, Wnt, adipocytokine, apoptosis and MAPK signaling pathways were more variable among OM samples. We also found that pyruvate kinase (PKM2), a transcript related to insulin signaling, glycolysis/gluconeogenesis and type 2 diabetes, was more variable among SC samples. The Ingenuity Pathway Analysis system revealed that 18 transcripts in the SC dataset were involved in lipid metabolism, which was clearly the top function associated with this dataset, whereas 28 transcripts were associated with cell death in OM fat (Table 2).

DISCUSSION

Regional fat distribution accounts for an important part of the association between obesity and related metabolic complications. In the present study, we used SC and OM adipose tissue samples from 10 obese men for microarray hybridizations, and measured expression levels for ~22,200 probesets. We studied the interindividual variability of gene expression in both depots, and attempted to identify highly variable transcripts or pathways in these fat compartments. Interindividual variability in gene expression in both depots in all subjects was rather low. In addition, no difference in the distribution of CVs was observed among fat depots. This provides evidence that gene expression within abdominal OM and SC adipose tissue samples is relatively homogenous; and indirectly suggests that primary characteristics of adipose tissue from both the SC and OM compartments are relatively similar. Several studies have now used microarrays to investigate gene expression profiling of adipose tissue in rodents (3,2,4,16), and humans (23,15,12). However, no study had examined human adipose tissue gene expression variability. Individual analyses of adipose tissue gene expression within a homogeneous study group or population might help to identify possible new functional links between different genes. This is the largest microarray study of human SC and OM fat performed to date and the first to provide information on the interindividual variability of gene expression in human adipose tissue.

SC and OM fat have been demonstrated as being very different in terms of lipolysis, cytokine secretion and linking to disease risks such as insulin resistance and dyslipidemia (6,19,22). This wide heterogeneity among individuals could potentially be reflected by different patterns of gene expression in each fat depot. We measured some variability in gene expression in the present analysis. However, the adipose tissue transcriptome appeared as relatively stable, since interindividual variability was rather low, with more than 90% of clones showing a CV lower than 23.6% and 21.7% in OM and SC adipose tissues in the 6811 probsets which generated significant signal in both fat depots in all 10 subjects, respectively. Variability in the probesets that were silenced in at least one and up to nine individuals out of ten in both depots showed similar variability, and no difference in distributions of CVs was observed between fat depots. Interestingly, Boeuf et al. (2) obtained strikingly similar data when analyzing the individual variability of gene expression in subcutaneous white and brown adipose tissue of hamsters. They found that individual variability of gene expression in both types of fats was also low, with more

than 80% of clones showing a CV lower than 30%. These results led the authors to conclude that gene expression in adipose tissue was rather robust and stable for animals, under identical environmental conditions. In the present study, gene expression variability was very consistent with that observed by Boeuf et al (2). We suggest that even in human subjects not under controlled physiological, metabolic and environmental situations, adipose tissue gene expression is relatively homogeneous. Our results also indicate that the larger portion of genes in SC and OM adipose tissue have stable expression and suggest that only a few pivotal genes might be responsible for the demonstrated regional differences in adipose tissue physiology and related complications.

Among the set of highly variable transcripts, we found that genes in SC samples were mostly involved in lipid metabolism. We also found that a transcript related to insulin signaling, PKM2 was more variable among SC than OM samples. Insulin increases glucose uptake in muscle and fat, and promotes the storage of substrates in fat, liver and muscle by stimulating lipogenesis, glycogen and protein synthesis, and by inhibiting lipolysis, glycogenolysis and protein breakdown. PKM2 is a glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, generating ATP (13). Kim et al. (14) examined mice with tissue-specific overexpression of LPL and their findings indicated a direct and causative relationship between the accumulation of intracellular fatty acid-derived metabolites and insulin resistance mediated via alterations in the insulin signaling pathway. This phenomenon has been suggested to occur as a mean to prevent further fat accumulation in a given tissue, the reduction in insulin action seen in insulin-resistant states affecting metabolic fuel partitioning (8). High variability of SC adipose tissue genes of fat storage and insulin signaling way reflect high variability in the capacity to store fat in this depot in the presence of energy excess.

Cytokines regulate several aspects of adipose tissue metabolism (11,18). Some possibly mediate their responses through activation of the JAK-STAT pathway. We found a transcript related to JAK-STAT pathway that was more variable among OM samples. Another interesting finding of this study is that transcripts related to the MAPK, Wnt and adipocytokine signaling pathways, and to apoptosis were also more variable among Om samples. These transcripts were PIM1, NFKBIA, JUN, PLA2G2A, HSPA1A, HSPA1B, GADD45B, MAP2K3. Transcripts related to

these cellular processes are involved in cell cycle, apoptosis, growth, proliferation, fate determination, development, immunity, and ubiquitin mediated proteolysis. The proto-oncogene PIM1 has been shown to prevent the normal process of apoptosis, acting as a cell survival factor. GADD45B is involved in cell cycle arrest, apoptosis, signal transduction and cell survival. The human HSPA multigene family encodes several highly conserved proteins which are expressed in response to heat shock and a variety of other stress stimuli including oxidative free radicals and toxic metal ions (21). At the same time, we found that among highly variable genes in OM adipose tissue samples, 28 transcripts showing high variability were involved in cell death. These results suggest high interindividual variability in programmed OM fat cell death.

Obesity has been recently suggested as a proinflammatory state (10), and white adipose tissue is no longer considered an inert tissue mainly devoted to energy storage but is emerging as an active participant in regulating physiologic and pathologic processes, including immunity and inflammation. Many of these cellular pathways were highly variable in both fat depots. For example, pathways of hematopoietic cell lineage, the Fc epsilon RI signaling pathway, glycerophospholipid metabolism and leukocyte transendothelial migration included highly variable genes in both fat compartments. Two main genes were responsible for this finding (PLA2G2A, TFRC). PLA2G2A plays an important role in a variety of cellular processes, including the production of precursors for inflammatory reactions. Furthermore, it is a key enzyme in eicosanoid synthesis and is therefore an interesting candidate gene in the context of inflammation (1). TFRC encodes the transferrin receptor, which plays an important role in controlling cell growth through iron uptake. Both genes are involved in inflammation, proliferation, growth and oncogenesis. Our results may reflect high variability in inflammatory responses in both fat compartments in obesity.

In summary, our data demonstrated that interindividual variability of gene expression in abdominal SC and OM adipose tissue samples from obese men was rather low. Future studies are required to investigate relations between different phenotypes (such as obesity, insulin, blood lipids) and expression of these transcripts.

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REFERENCES

1. Bertsch T.; Aufenanger J. Phospholipase A2 in inflammatory bowel disease. Gut. 41:859-860; 1997.

2. Boeuf S.; Keijer J.; Franssen-Van Hal N.L.; Klaus S. Individual variation of adipose gene expression and identification of covariated genes by cDNA microarrays. Physiol Genomics. 11:31-36; 2002.

3. Boeuf S.; Klingenspor M.; Van Hal N.L.; Schneider T.; Keijer J.; Klaus S. Differential gene expression in white and brown preadipocytes.Differential gene expression in white and brown preadipocytes. Physiol Genomics. 7:15-25; 2001.

4. Bullen J.W. Jr; Ziotopoulou M.; Ungsunan L.; Misra J.; Alevizos I.; Kokkotou E.; Maratos-Flier E.; Stephanopoulos G.; Mantzoros C.S. Short-term resistance to diet-induced obesity in A/J mice is not associated with regulation of hypothalamic neuropeptides. Am J Physiol Endocrinol Metab. 287:E662-E670; 2004.

5. Chan D.C.; Watts G.F.; Sussekov A.V.; Barrett P.H.; Yang Z.; Hua J.; Song S. Adipose tissue compartments and insulin resistance in overweight-obese Caucasian men. Diabetes Res Clin Pract. 63:77-85; 2004.

6. Despres J.P.; Moorjani S.; Ferland M.; Tremblay A.; Lupien P.J.; Nadeau A.; Pinault S.; Theriault G.; Bouchard C. Adipose tissue distribution and plasma lipoprotein levels in obese women. Importance of intra-abdominal fat. Arteriosclerosis. 9:203-210; 1989.

7. Despres J.P.; Nadeau A.; Tremblay A.; Ferland M.; Moorjani S.; Lupien P.J.; Theriault G.; Pinault S.; Bouchard C. Role of deep abdominal fat in the association between regional adipose tissue distribution and glucose tolerance in obese women. Diabetes. 38:304-309; 1989.

8. Eckel R.H. Insulin resistance: an adaptation for weight maintenance. Lancet. 340:1452-1453; 1992.

9. Expert Panel on Detection, E.a.T.o.H.B.C.i.A.Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA. 285:2486-2497; 2001.

10. Fantuzzi G. Adipose tissue, adipokines, and inflammation. J Allergy Clin Immunol. 115:911-999; 2005.

11. Fried S.K.; Bunkin D.A.; Greenberg A.S. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid.Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. J Clin Endocrinol Metab. 83:847-850; 1998.

12. Gomez-Ambrosi J.; Catalan V.; Diez-Caballero A.; Martnez-Cruz L.A.; Gil M.J.; Garcia-Foncillas J.; Cienfuegos J.A.; Salvador J.; Mato J.M.; Fruhbeck G. Gene expression profile of omental adipose tissue in human obesity. FASEB J. 18:215-217; 2004.

13. Ikeda Y.;Noguchi T. Allosteric regulation of pyruvate kinase M-2 isozyme involves a cysteine residue in the intersubunit contact. J Biol Chem. 273:12227-12233; 1998.

14. Kim J.K.; Fillmore J.J.; Chen Y.; Yu C.; Moore I.K.; Pypaert M.; Lutz E.P.; Kako Y.; Velez-Carrasco W.; Goldberg I.J.; Breslow J.L.; Shulman G.I. Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance.Tissue-specific overexpression of lipoprotein lipase causes tissue-specific insulin resistance. Proc Natl Acad Sci U S A. 98:7522-7527; 2001.

15. Linder K.; Arner P.; Flores-Morales A.; Tollet-Egnell P.; Norstedt G. Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women. J Lipid Res. 45:148-154; 2004.

16. Lopez I.P.; Marti A.; Milagro F.I.; Zulet Md Mde L.; Moreno-Aliaga M.J.; Martinez J.A.; De Miguel C. DNA microarray analysis of genes differentially expressed in diet-induced (cafeteria) obese rats. Obes Res. 11:188-194; 2003.

17. Marceau P.; Biron S.; Hould F.S.; Marceau S.; Simard S.; Thung S.N.; Kral J.G. Liver pathology and the metabolic syndrome X in severe obesity. J Clin Endocrinol Metab. 84:1513-1517; 1999.

18. Mohamed-Ali V.; Pinkney J.H.; Coppack S.W. Adipose tissue as an endocrine and paracrine organ. Int J Obes Relat Metab Disord. 83:1145-1158; 1998.

19. Motoshima H.; Wu X.; Sinha M.K.; Hardy V.E.; Rosato E.L.; Barbot D.J.; Rosato F.E.; Goldstein B.J. Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: effects of insulin and rosiglitazone. J Clin Endocrinol Metab. 87:5662-5667; 2002.

20. Novak J.P.; Sladek R.; Hudson T.J. Characterization of variability in large-scale gene expression data: implications for study design. Genomics. 79:104-113; 2002.

21. Pelham H.R. Speculations on the functions of the major heat shock and glucose-regulated proteins. Cell. 46:959-961; 1986.

22. Tchernof A.; Belanger C.; Morisset A.S.; Richard C.; Mailloux J.; Laberge P.; Dupont P. Regional differences in adipose tissue metabolism in women: minor effect of obesity and body fat distribution. Diabetes. 55:1353-1360; 2006.

23. Vohl M.C.; Sladek R.; Robitaille J.; Gurd S.; Marceau P.; Richard D.; Hudson T.J.; Tchernof A. A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men. Obes Res. 12:1217-1222; 2004.

24. Wajchenberg B.L. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev. 21:697-738; 2000.

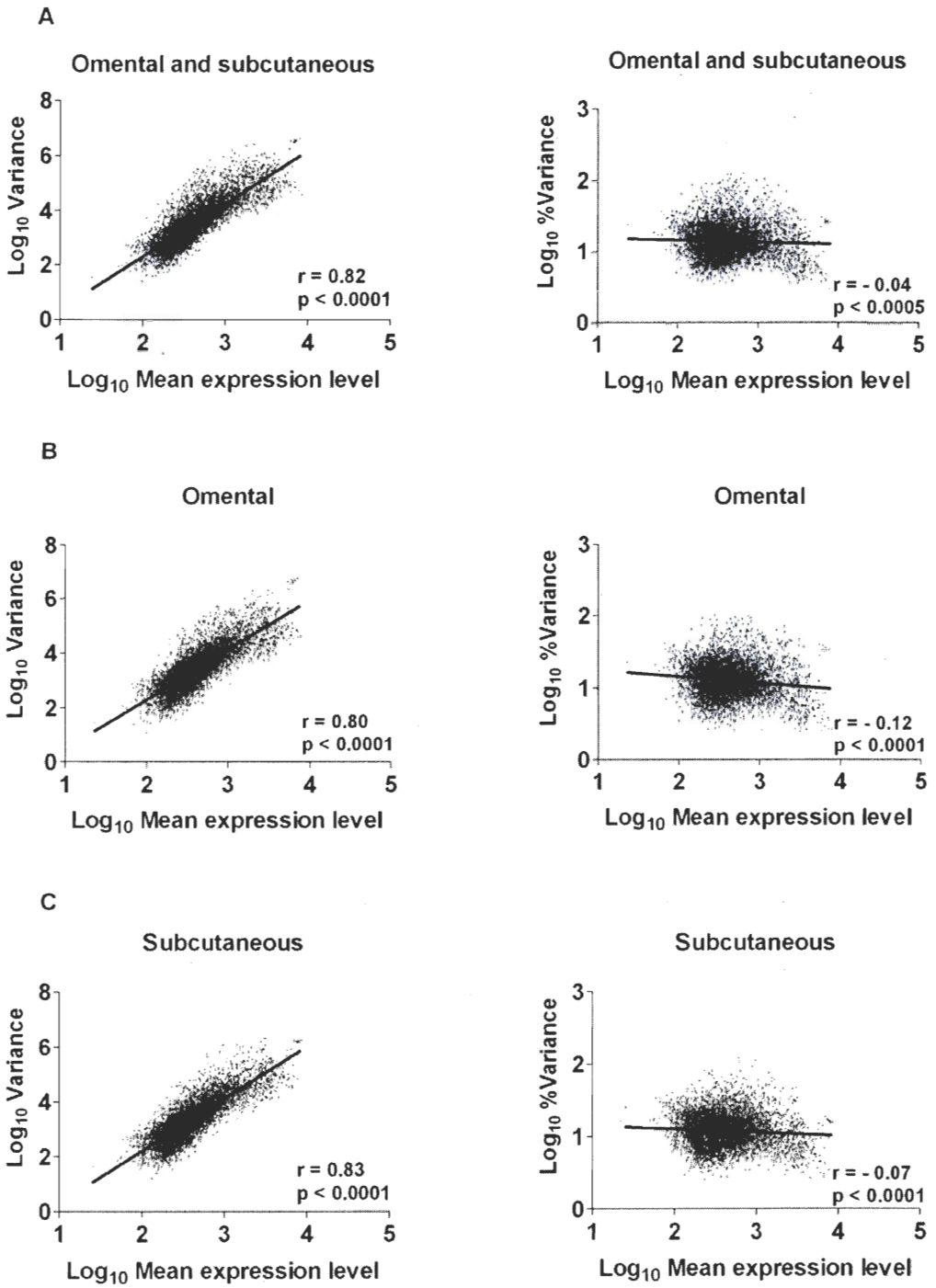

Figure 1. Correlations between gene expression variance or coefficient of variation (%variance) and mean gene expression levels for all 6811 positive signals regardless of fat depot (A), or in the OM or SC fat compartments (B and C).

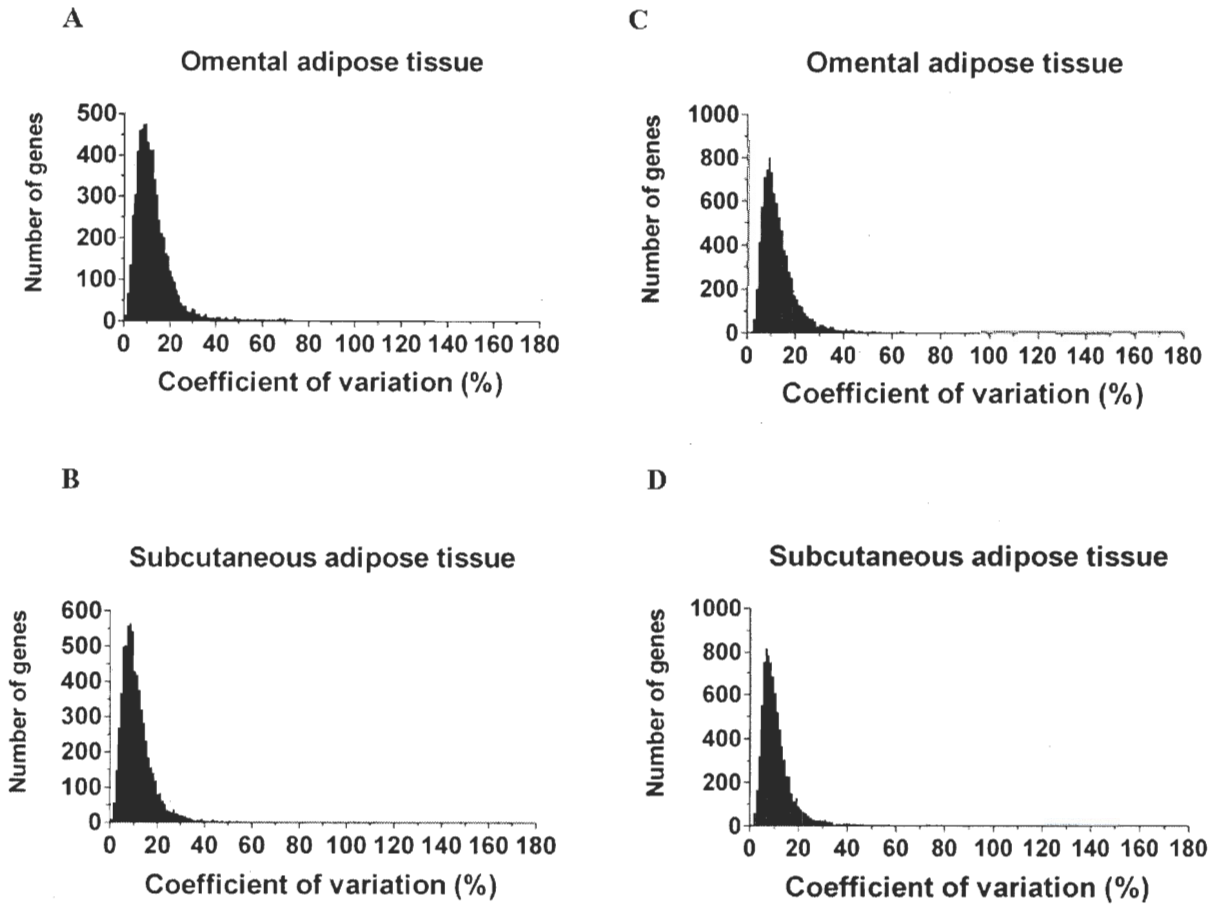

Figure 2. Distribution of coefficients of variation in OM or SC adipose tissue of 6811 probesets which generated significant signal in both fat depots in all 10 subjects (left panel, A and B). In OM and SC adipose tissue samples, 9076 and 8590 probesets which only presented significant signal in at least one and up to nine individuals (right panel, C and D). Interindividual variability was similar in both sets of transcripts. No difference in CV distribution was observed between fat depots.

Table 1: Selected pathways with highly variable transcripts in OM and SC adipose tissue, based on the coefficient of variation in gene expression level.

Omental adipose tissue			Subcutaneous adipose tissue		
Pathway	**Number of Genes**	**Symbols**	**Pathway**	**Number of Genes**	**Symbols**
Aminosugars metabolism	2	UAP1, GFPT2	Aminosugars metabolism	1	HEXB
Antigen processing and presentation	2	HSPA1A, HSPA1B	Antigen processing and presentation	4	IFI30, CTSB**, CTSS, CTSL
Arachidonic acid metabolism	1	PLA2G2A	Arachidonic acid metabolism	1	PLA2G2A
Cell adhesion molecules (CAMs)	1	ICAM1*	Cell adhesion molecules (CAMs)	2	ITGB2, ALCAM
Cell Communication	1	LMNA*	Cell Communication	3	SPP1, COL1A2, THBS1
Cell cycle	1	GADD45B	Cell cycle	1	CDC16
Fc epsilon RI signaling pathway	2	PLA2G2A, MAP2K3	Fc epsilon RI signaling pathway	2	PLA2G2A, FCER1G
Focal adhesion	1	JUN	Focal adhesion	3	SPP1, COL1A2, THBS1
Glycan structures - biosynthesis 2	1	UGCG	Glycan structures - degradation	2	GUSB,HEXB
Glycerophospholipid metabolism	1	PLA2G2A	Glycerophospholipid metabolism	1	PLA2G2A
GnRH signaling pathway	2	PLA2G2A, MAP2K3	GnRH signaling pathway	1	PLA2G2A
Hematopoietic cell lineage	1	TFRC*	Hematopoietic cell lineage	2	TFRC,CD9
Leukocyte transendothelial migration	1	ICAM1*	Leukocyte transendothelial migration	3	ITGB2, MMP9, TFRC
Linoleic acid metabolism	1	PLA2G2A	Linoleic acid metabolism	1	PLA2G2A
Long-term depression	1	PLA2G2A	Long-term depression	1	PLA2G2A
MAPK signaling pathway	6	GADD45B, HSPA1A, HSPA1B, JUN, PLA2G2A, MAP2K3	MAPK signaling pathway	1	PLA2G2A
mTOR signaling pathway	1	HIF1A	mTOR signaling pathway	1	TSC1
Natural killer cell mediated cytotoxicity	2	ICAM1*, FCGR3B	Natural killer cell mediated cytotoxicity	3	ITGB2, FCER1G, TYROBP
Ribosome	1	RPS26	Ribosome	1	RPS26
Toll-like receptor signaling pathway	4	NFKBIA, MAP2K3, TBK1, JUN	Toll-like receptor signaling pathway	1	TBK1
VEGF signaling pathway	1	PLA2G2A	VEGF signaling pathway	1	PLA2G2A
Wnt signaling pathway	1	JUN	Alkaloid biosynthesis II	1	LIPA
Adipocytokine signaling pathway	1	NFKBIA	Alzheimer's disease	1	APOE*
Apoptosis	1	NFKBIA	ATP synthesis	1	ATP6V0B
B cell receptor signaling pathway	2	NFKBIA, JUN	Bile acid biosynthesis	1	LIPA
Cytokine-cytokine receptor interaction	1	CCL8	Carbon fixation	1	PKM2
Epithelial cell signaling in Helicobacter pylori infection	1	NFKBIA	Cholera - Infection	1	ATP6V0B
Glutamate metabolism	1	GFPT2	ECM-receptor interaction	3	SPP1, COL1A2, THBS1
Glycosphingolipid metabolism	1	UGCG	Globoside metabolism	1	HEXB
Jak-STAT signaling pathway	1	PIM1	Glutathione metabolism	1	GSTO1
Methionine metabolism	1	MAT2A	Glycerolipid metabolism	1	LIPA
Nicotinate and nicotinamide metabolism	2	NNMT, PBEF1	Glycolysis / Gluconeogenesis	1	PKM2
Selenoamino acid metabolism	1	MAT2A	Glycosaminoglycan degradation	2	GUSB, HEXB
T cell receptor signaling pathway	2	NFKBIA, JUN	Insulin signaling pathway	2	PKM2, TSC1
			Metabolism of xenobiotics by cytochrome P450	1	GSTO1
			Neurodegenerative Disorders	1	APOE*
			N-Glycan degradation	1	HEXB
			Oxidative phosphorylation	1	ATP6V0B
			Pentose and glucuronate interconversions	1	GUSB
			Porphyrin and chlorophyll metabolism	1	GUSB
			Pyruvate metabolism	1	PKM2*
			Regulation of actin cytoskeleton	2	ITGB2, ARPC1B
			SNARE interactions in vesicular transport	1	VAMP8
			Starch and sucrose metabolism	1	GUSB
			TGF-beta signaling pathway	1	THBS1
			Thiamine metabolism	1	THTPA
			Type II diabetes mellitus	1	PKM2
			Ubiquitin mediated proteolysis	1	CDC16

** Two probesets generated similar results for these genes.*

Table 2. Gene associated with the top function in the Om and Sc datasets containing highly variable transcripts.

Category	Process	Genes
Omental adipose tissue		
Cell Death		
	cell death	ATF3, CYR61, DNAJB1, EMP1, GADD45B, HIF1A, HSPA1A, HSPA1B, ICAM1, IER3, JUN, JUNB, KLF4, MAP2K3, MCL1, MT1X, MT2A, NFIL3, NFKBIA, PBEF1, PIM1, PRG1, S100A8, SGK, TBK1, TFRC, TNFAIP3, UGCG
	apoptosis	ATF3, CYR61, GADD45B, HIF1A, HSPA1A, HSPA1B, ICAM1, IER3, JUN, KLF4, MAP2K3, MCL1, MT2A, NFIL3, NFKBIA, PBEF1, PIM1, PRG1, S100A8, SGK, TBK1, TFRC, TNFAIP3, UGCG
	killing	HSPA1A, HSPA1B, S100A8
	cell viability	LMNA, MCL1, MT2A, NFKBIA, TNFAIP3, UGCG
	cytotoxicity	FCGR3B, MT2A, TNFAIP3
	survival	CYR61, HIF1A, HSPA1B, JUN, MCL1, NFIL3, NFKBIA, PIM1, UGCG
	colony survival	JUN
	inhibition	HSPA1B, IER3, MCL1
	activation-induced cell death	ICAM1
Subcutaneous adipose tissue		
Lipid Metabolism		
	storage	ADFP, GM2A, LIPA, SCD
	quantity	APOC1, APOE, CTSS, FCER1G, LIPA, NR1H3, PLA2G2A, SCD, UCP2, IL1RN
	synthesis	APOE, CD9, FCER1G, NR1H3, PLA2G2A, SCD
	release	CTSB, FCER1G, IL1RN, PLA2G2A
	modification	APOE, PLA2G2A, SCD, UCP2, ITGB2

efflux	APOE, NR1H3, SAA1
hydrolysis	GM2A, HEXB, PLA2G2A
accumulation	APOE, NR1H3, IL1RN, UCP2, APOC1
production	APOE, IL1RN, ITGB2, PLA2G2A, NR1H3
activation	NR1H3
esterification	APOE, SCD
oxidation	APOE, SCD, UCP2
peroxidation	APOE, PLA2G2A
co-capping	ITGB2
uptake	APOC1, APOE
metabolism	APOC1, APOD, IL1RN, PLA2G2A, SCD
exchange	APOC1
liberation	PLA2G2A
degradation	GM2A, PLA2G2A
desaturation	SCD
secretion	APOE, SCD
steroidogenesis	APOE
transport	ADFP

Appended Table 1: List of the 69 Om adipose tissue transcripts in upper tertile of mean expression level and top 2 percentile of the coefficient of variation.

Probeset	Symbol	Description	Cytogen.Band	Accession	%CV
217739_s_at	PBEF1	Pre-B-cell colony-enhancing factor	7q22.2	NM_005746	100.5
202241_at	TRIB1	Phosphoprotein regulated by mitogenic pathways	8q24.13	NM_025195	95.0
204472_at	GEM	GTP binding protein overexpressed in skeletal muscle	8q13-q21	NM_005261	91.3
202643_s_at	TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	6q23	AI738896	86.6
202644_s_at	TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	6q23	NM_006290	83.3
202637_s_at	ICAM1	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	19p13.3-p13.2	AI608725	78.8
202638_s_at	ICAM1	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor	19p13.3-p13.2	NM_000201	46.6
212724_at	RND3	Ras homolog gene family, member E	2q23.3	BG054844	77.6
36711_at	MAFF	V-maf musculoaponeurotic fibrosarcoma oncogene homolog F (avian)	22q13.1	AL021977	76.3
204007_at	FCGR3B	Fc fragment of IgG, low affinity IIIb, receptor for (CD16)	1q23	J04162	71.4
202917_s_at	S100A8	S100 calcium binding protein A8 (calgranulin A)	1q21	NM_002964	70.9
203574_at	NFIL3	Nuclear factor, interleukin 3 regulated	9q22	NM_005384	66.0
221541_at	CRISPLD2	Hypothetical protein DKFZp434B044	16q24.1	AL136861	64.2
221477_s_at	MGC5618	Hypothetical protein MGC5618		BF575213	63.3
202388_at	RGS2	Regulator of G-protein signalling 2, 24kD	1q31	NM_002923	63.1
217546_at	MT1M	Metallothionein 1M	16q13	R06655	62.2
200798_x_at	MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)	1q21	NM_021960	61.3
200797_s_at	MCL1	Myeloid cell leukemia sequence 1 (BCL2-related)	1q21	AI275690	39.1
207574_s_at	GADD45B	Growth arrest and DNA-damage-inducible, beta	19p13.3	NM_015675	61.1
208152_s_at	DDX21	DEAD (Asp-Glu-Ala-Asp) box polypeptide 21	10q21	NM_004728	60.7
204881_s_at	UGCG	UDP-glucose ceramide glucosyltransferase	9q31	NM_003358	60.1
201325_s_at	EMP1	Epithelial membrane protein 1	12p12.3	NM_001423	60.1
201502_s_at	NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	14q13	AI078167	59.5
202672_s_at	ATF3	Activating transcription factor 3	1q32.3	NM_001674	59.1
209193_at	PIM1	Pim-1 oncogene	6p21.2	M24779	59.0
201858_s_at	PRG1	Proteoglycan 1, secretory granule	10q22.1	J03223	56.0
203411_s_at	LMNA	Lamin A/C	1q21.2-q21.3	NM_005572	55.9
212086_x_at	LMNA	Lamin A/C	1q21.2-q21.3	AK026584	51.2

201631_s_at	IER3	Immediate early response 3	6p21.3	NM_003897	54.4
202391_at	BASP1	Brain abundant, membrane attached signal protein 1	5p15.1-p14	NM_006317	53.7
208836_at	ATP1B3	ATPase, Na+/K+ transporting, beta 3 polypeptide	3q23	U51478	53.3
207332_s_at	TFRC	Transferrin receptor (p90, CD71)	3q29	NM_003234	53.1
208691_at	TFRC	Transferrin receptor (p90, CD71)	3q29	BC001188	52.5
200800_s_at	HSPA1A	Heat shock 70kD protein 1A	6p21.3	NM_005345	51.9
208470_s_at	HPR	Haptoglobin-related protein	16q22.1	NM_020995	51.7
208151_x_at	DDX17	DEAD (Asp-Glu-Ala-Asp) box polypeptide 17	22q13.1	NM_030881	51.2
209340_at	UAP1	UDP-N-acteylglucosamine pyrophosphorylase 1	1q23.3	S73498	51.1
202581_at	HSPA1B	Heat shock 70kD protein 1B	6p21.3	NM_005346	50.8
200768_s_at	MAT2A	Methionine adenosyltransferase II, alpha	2p11.2	BC001686	49.6
214038_at	CCL8	Chemokine (C-C motif) ligand 8	17q11.2	AI984980	48.6
201466_s_at	JUN	V-jun sarcoma virus 17 oncogene homolog (avian)	1p32-p31	NM_002228	48.0
201739_at	SGK	Serum/glucocorticoid regulated kinase	6q23	NM_005627	47.5
200666_s_at	DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	19p13.2	NM_006145	47.5
213629_x_at	MT1F	Metallothionein 1F (functional)	16q13	BF246115	47.0
217165_x_at	MT1F	Metallothionein 1F (functional)	16q13	M10943	41.2
200831_s_at	SCD	Stearoyl-CoA desaturase (delta-9-desaturase)	10q23-q24	AA678241	46.7
212185_x_at	MT2A	Metallothionein 2A	16q13	NM_005953	46.5
200704_at	LITAF	Lipopolysaccharide-induced TNF factor	16p13.13	AB034747	45.7
211456_x_at	LOC645745	Metallothionein 1H-like protein	1q43	AF333388	45.5
208581_x_at	MT1X	Metallothionein 1X	16q13	NM_005952	44.9
202238_s_at	NNMT	Nicotinamide N-methyltransferase	11q23.1	NM_006169	44.6
204419_x_at	HBG2	Hemoglobin, gamma G	11p15.5	NM_000184	44.6
221841_s_at	KLF4	Kruppel-like factor 4 (gut)	9q31	BF514079	44.0
202081_at	IER2	Immediate early response 2	19p13.13	NM_004907	43.9
203649_s_at	PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)	1p35	NM_000300	43.6
220046_s_at	CCNL1	Cyclin L1	3q25.32	NM_020307	43.2
205100_at	GFPT2	Glutamine-fructose-6-phosphate transaminase 2	5q34-q35	NM_005110	43.1
201289_at	CYR61	Cysteine-rich, angiogenic inducer, 61	1p31-p22	NM_001554	42.8
215499_at	LOC651423	Similar to mitogen-activated protein kinase kinase 3 isoform A	17q11.2	AA780381	42.3
201473_at	JUNB	Jun B proto-oncogene	19p13.2	NM_002229	42.0
205516_x_at	CIZ1	CDNK1A interacting zinc finger protein	9q34.1	NM_012127	42.0
216336_x_at	IBRDC3	Consensus includes gb: AL031602 /DEF=Human DNA	1p35.1	AL031602	41.7

		sequence from clone RP5-1174N9 on chromosome 1p34.1-35.3. Contains the gene for a novel protein with IBR domain, a (pseudo?) gene for a novel protein similar to MT1E (metallothionein 1E (functional)), ESTs, S			
221651_x_at	IGKC	Immunoglobulin kappa constant	2p12	BC005332	41.3
204745_x_at	MT1G	Metallothionein 1G	16q13	NM_005950	41.3
200881_s_at	DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1	9p13-p12	NM_001539	41.2
217753_s_at	RPS26	Ribosomal protein S26	12q13	NM_001029	40.7
218520_at	TBK1	TANK-binding kinase 1	12q14.1	NM_013254	40.4
200989_at	HIF1A	Hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	14q21-q24	NM_001530	39.7
212859_x_at	MT1E	Metallothionein 1E (functional)	16q13	BF217861	39.2

Appended Table 2: List of the 68 Sc adipose tissue transcripts in the upper tertile of mean expression level and top 2 percentile of the coefficient of variation.

Probeset	Symbol	Description	Cytogen.Band	Accession	%CV
212657_s_at	IL1RN	Interleukin 1 receptor antagonis	2q14.2	U65590	121.4
209875_s_at	SPP1	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	4q21-q25	M83248	111.9
209395_at	CHI3L1	Chitinase 3-like 1 (cartilage glycoprotein-39)	1q32.1	M80927	107.9
203936_s_at	MMP9	Matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase)	20q11.2-q13.1	NM_004994	92.0
208691_at	TFRC	Transferrin receptor (p90, CD71)	3q29	BC001188	79.9
207332_s_at	TFRC	Transferrin receptor (p90, CD71)	3q29	NM_003234	66.3
201952_at	ALCAM	Activated leucocyte cell adhesion molecule	3q13.1	AA156721	78.8
201422_at	IFI30	Interferon, gamma-inducible protein 30	19p13.1	NM_006332	75.5
201850_at	CAPG	Capping protein (actin filament), gelsolin-like	2p11.2	NM_001747	71.1
201847_at	LIPA	Lipase A, lysosomal acid, cholesterol esterase (Wolman disease)	10q23.2-q23.3	NM_000235	64.2
201720_s_at	LAPTM5	Lysosomal-associated multispanning membrane protein 5	1p34	AI589086	62.6
201721_s_at	LAPTM5	Lysosomal-associated multispanning membrane protein 5	1p34	NM_006762	60.7
203523_at	LSP1	Lymphocyte-specific protein 1	11p15.5	NM_002339	62.6
213274_s_at	CTSB	Cathepsin B	8p22	AA020826	61.9
200838_at	CTSB	Cathepsin B	8p22	NM_001908	57.5
200839_s_at	CTSB	Cathepsin B	8p22	NM_001908	44.9
213275_x_at	CTSB	Cathepsin B	8p22	W47179	38.9
219454_at	EGFL6	EGF-like-domain, multiple 6	Xp22	NM_015507	60.8
202803_s_at	ITGB2	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	21q22.3	NM_000211	59.2
202902_s_at	CTSS	Cathepsin S	1q21	NM_004079	58.2
203337_x_at	ITGB1BP1	Integrin beta 1 binding protein 1	2p25.2	NM_004763	55.4
212737_at	GM2A	GM2 ganglioside activator	5q31.3-q33.1	AL513583	52.5
209122_at	ADFP	Adipose differentiation-related protein	9p22.1	BC005127	51.3
208607_s_at	SAA2	Serum amyloid A2	11p15.1-p14	NM_030754	51.2
205516_x_at	CIZ1	CDKN1A interacting zinc finger protein 1	9q34.1	NM_012127	49.7
202546_at	VAMP8	Vesicle-associated membrane protein 8 (endobrevin)	2p12-p11.2	NM_003761	49.6
200766_at	CTSD	Cathepsin D (lysosomal aspartyl protease)	11p15.5	NM_001909	48.4

202409_at	IGF2	Insulin-like growth factor 2 (somatomedin A)	11p15.5	X07868	48.1
204122_at	TYROBP	TYRO protein tyrosine kinase binding protein	19q13.1	NM_003332	46.8
218520_at	TBK1	TANK-binding kinase 1	12q14.1	NM_013254	46.6
214456_x_at	SAA1	Serum amyloid A1	11p15.1	M23699	46.6
204232_at	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	1q23	NM_004106	46.3
221651_x_at	IGKC	Immunoglobulin kappa constant	2p12	BC005332	46.2
201141_at	GPNMB	Glycoprotein (transmembrane) nmb	7p15	NM_002510	46.0
201201_at	CSTB	Cystatin B (stefin B)	21q22.3	NM_000100	45.9
221269_s_at	SH3BGRL3	SH3 domain binding glutamic acid-rich protein like 3	1p35-p34.3	NM_031286	45.3
203649_s_at	PLA2G2A	Phospholipase A2, group IIA (platelets, synovial fluid)	1p35	NM_000300	42.9
218540_at	THTPA	Thiamine triphosphatase	14q11.2	NM_024328	42.1
209659_s_at	CDC16	CDC16 cell division cycle 16 homolog (S. cerevisiae)	13q34	AF164598	41.9
200831_s_at	SCD	Stearoyl-CoA desaturase (delta-9-desaturase)	10q23-q24	AA678241	41.6
213553_x_at	APOC1	Apolipoprotein C-I	19q13.2	W79394	41.4
213101_s_at	ACTR3	ARP3 actin-related protein 3 homolog (yeast)	2q14.1	Z78330	40.0
202605_at	GUSB	Glucuronidase, beta	7q21.11	NM_000181	39.7
201050_at	PLD3	Phospholipase D family, member 3	19q13.2	NM_012268	39.6
202399_s_at	AP3S2	Adaptor-related protein complex 3, sigma 2 subunit	15q26.1	NM_005829	39.5
202404_s_at	COL1A2	Collagen, type I, alpha 2	7q22.1	NM_000089	39.5
201108_s_at	THBS1	Thrombospondin 1	15q15	BF055462	38.9
201470_at	GSTO1	Glutathione-S-transferase omega 1	10q25.1	NM_004832	38.8
201251_at	PKM2	Pyruvate kinase, muscle	15q22	NM_002654	38.5
200078_s_at	ATP6V0B	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit b	1p32.3	BC005876	37.9
213515_x_at	HBG1	Hemoglobin, gamma A	11p15.5	AI133353	37.7
217118_s_at	C22orf9	Chromosome 22 open reading frame 9	22q13.31	AK025608	37.6
203382_s_at	APOE	Apolipoprotein E	19q13.2	NM_000041	37.6
201944_at	HEXB	Hexosaminidase B (beta polypeptide)	5q13	NM_000521	37.2
209390_at	TSC1	Tuberous sclerosis 1	9q34	AF013168	37.0
203381_s_at	APOE	Apolipoprotein E	19q13.2	N33009	36.8
201005_at	CD9	CD9 molecule	12p13.3	NM_001769	36.7
218109_s_at	MFSD1	Major facilitator superfamily domain containing 1	3q25.33	NM_022736	36.5
203920_at	NR1H3	Nuclear receptor subfamily 1, group H, member 3	11p11.2	NM_005693	36.4
207168_s_at	H2AFY	H2A histone family, member Y	5q31.3-q32	NM_004893	36.4
208998_at	UCP2	Uncoupling protein 2 (mitochondrial, proton carrier)	11q13.4	U94592	35.7

207977_s_at	DPT	Dermatopontin	1q12-q23	NM_001937	35.7
203416_at	CD53	CD53 moecule	1p13	NM_000560	35.7
201954_at	ARPC1B	Actin related protein 2/3 complex, subunit 1B, 41 kDa	7q22.1	NM_005720	35.6
201525_at	APOD	Apolipoprotein D	3q26.2-qter	NM_001647	35.4
209183_s_at	C10orf10	Chromosome 10 open reading frame 10	10q11.21	AL136653	35.0
202087_s_at	CTSL	Cathepsin L	9q21-q22	NM_001912	34.9
217753_s_at	RPS26	Ribosomal protein S26	12q13	NM_001029	34.7

Part B: Response of the adipose tissue transcriptome to dihydrotestosterone in mice

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Response of the adipose tissue transcriptome to dihydrotestosterone in mice

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ABSTRACT

Androgens have been postulated to be important modulators of adipose tissue metabolism and fat cell function. In the present study, we investigated the response of male and female mice retroperitoneal adipose tissue to the non-aromatizable androgen dihydrotestosterone (DHT). Adipose tissue samples were obtained in gonadectomized (GDX) animals treated with vehicle (control group), or injected with 0.1mg DHT 1, 3, 6, 12, 18 and 24h prior to necropsy. Fourteen animals were pooled at each time point.(total 196 animals). Transcripts which were significantly modulated were considered as androgen-responsive genes. Quantitative real-time RT-PCR was used to confirm results from the microarry analysis in a subset of 46 probesets in male mice and 98 probesets in female mice. Considering peak time versus control, 74.0% and 63.3% of the modulated genes were confirmed by PCR in males and females, respectively. Four genes were significantly stimulated in a similar manner by DHT in both sexes, namely metallothionein 1 (Mt1), growth arrest and DNA-damage-inducible 45 gamma (Gadd45g), cyclin-dependent kinase inhibitor 1A (Cdkn1a), and fk506-binding protein 5 (Fkbp5). All these genes appear to be involved in the regulation of adipocyte differentiation/proliferation and adipogenesis. In conclusion, this study which evaluated the acute transcriptome response of adipose tissue to DHT in male and female mice suggests that DHT consistently modulates genes involved in the regulation of adipogenesis in retroperitoneal adipose tissue of both male and female animals.

Keywords: Retroperitoneal; Microarray; Adipogenesis

INTRODUCTION

Several studies suggest that sex hormones modulate adipose tissue deposition at the level of preadipocyte differentiation and/or proliferation as well as adipocyte lipogenesis and/or lipolysis (2; 36; 40). Cross-sectional (35) and longitudinal (18; 22) studies found that circulating androgen levels are negatively associated with abdominal obesity and visceral fat accumulation in men. A number of studies also reported negative associations between circulating androgens and abdominal adiposity in women (3; 10; 47). On the other hand, female-to-male transsexuals treated with high-doses of androgens preferentially accumulate intra-abdominal fat and progressively lose gluteal-femoral fat (15). In women with the polycystic ovary syndrome, a positive association between abdominal obesity and plasma androgen levels is found (13). Triglycerides in retroperitoneal and mesenteric adipose tissue increase after castration in rats, but decrease when animals are treated with testosterone (25). Additionally, androgen receptor knock-out mice develop late onset obesity through reduced adipose tissue lipolysis in animals of both sexes (16; 42). All these findings suggest that androgens may be key steroids in the modulation of fat accumulation and distribution in both humans and rodent models.

The impact of androgens on gene transcription in adipose tissue from male mice has been previously examined by serial analysis of gene expression in one study (7). Although very informative, that approach did not consider temporal expression regulation in response to the hormone. In the present study, using DNA microarrays, we examined the acute response of the adipose tissue transcriptome to dihydrotestosterone (DHT) in gonadectomized male and female mice. We tested the general hypothesis that transcriptional profiling of androgen-responsive genes in both male and female mice would bring new insights on possible signaling pathways linking fat distribution and sex hormones.

METHODS

Mice and RNA sample preparation. Male and female, 14-15 week-old, C57BL6 mice were obtained from Charles River Canada Inc (St-Constant, Québec, Canada). Fourteen animals were pooled for one replica at each time point (1, 3, 6, 12, 18, and 24 h) and fourteen mice were pooled to generate two replica of the control group in each sex. A total of 196 animals was used in our study. Mice were housed (1 per cage) in plastic cages under 12 hours light/dark cycles; the animals had access to Lab Rodent Diet No. 5002 and given tap water *ad libitum*. Bilateral gonadectomy (castration for male mice and ovariectomy for female mice) were performed under isoflurane-induced anesthesia 7 days prior to sacrifice and organ collection for the seven gonadectomized (GDX) groups (n=14 mice per group). DHT (0.1mg) was injected subcutaneously 1, 3, 6, 12, 18 and 24 h prior to sacrifice in DHT-treated groups. One group was retained as control and received a vehicle solution (0.4% (w/v) Methocel A15LV Premium; 5% ethanol). Retroperitoneal adipose tissue samples from all mice of the same group were collected, rapidly trimmed, snap-frozen in liquid nitrogen and stored at -80°C prior to mRNA extraction. These fat samples were pooled to eliminate interindividual variation and to extract a sufficient amount of mRNA. For blood DHT measurements, gonadectomized C57BL6 male and female mice were injected with 0.1 mg DHT 1, 3, 6, 12, 18 or 24h prior to blood sampling (n=12 mice/timepoint/sex). Control mice (n=24 mice/sex) were injected with the vehicle alone (5% ethanol-0.4% methylcellulose) 24h prior to necropsy. Mice under isoflurane anesthesia were exsanguinated by cardiac venipuncture. Blood samples were processed for serum preparation and kept frozen at -80C until use for the determination of serum DHT concentrations by gas chromatography mass spectrometry (GC-MS) as previously described (23) with the exception of the serum volume used (0.15 to 0.3mL in mice instead of 0.75mL in humans). Serum samples from two mice of the same group were pooled for each determination of DHT levels (n=6 for DHT groups; n=12 for the control group). The experiment was conducted in an animal facility approved by the Canadian Council on Animal Care (CCAC) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The study was performed in accordance with the CCAC Guide for Care and Use of Experimental Animals.

Target preparation. Total mRNA was isolated using Trizol (Invitrogen, Burlington, Ontario) following the manufacturer's protocol. Total mRNA (20μg) was converted to cDNA by incubation with 400 U SuperScript II reverse transcriptase (Invitrogen) using a T7-oligo-$(dt)_{24}$ primer [5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGC GG-$(dt)_{24}$-3'] 1x first strand buffer (50

mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT) and 0.5 mM dNTPs at 42°C for 1h. Second-strand synthesis was performed using 40 U DNA polymerase I (Invitrogen), 10 U *E. Coli* DNA ligase (Invitrogen), 2 U RNAse H (Invitrogen), 1x reaction buffer [18.8 mM Tris-HCl pH 8.3, 90.6 mM KCl, 4.6 mM $MgCl_2$, 3.8 mM DTT, 0.15 mM NAD, 10 mM $(NH_4)_2SO_4$] and 0.2 mM dNTPs at 16°C for 2h. cDNAs were blunt-ended with 10 U of T4 DNA polymerase (Invitrogen) incubated 5 min at 16°C. cDNA was extracted with phenol-chloroform using phase lock gels (Brinkman, Mississauga, Ontario), ethanol precipitated and resuspended in 10 µL of DEPC-treated H_2O. cDNA was *in vitro* transcribed using a T7 BioArray High Yield RNA Transcript Labeling Kit (Enzo Diagnostics, Farmingdale, NY) to produce biotinylated cRNA. The mixture (20 µL final volume) was incubated at 37°C for 5h, with gentle mixing every 30 min. Labelled cRNA was purified with an RNeasy Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. Purified cRNA was fragmented to 30-200 mer using a fragmentation buffer (100 mM potassium acetate-30 mM magnesium acetate-40 mM Tris-acetate pH 8.1), for 20 min at 94°C. The quality of total RNA, cDNA synthesis, cRNA amplification and cRNA fragmentation was monitored by micro-capillary electrophoresis (Bioanalizer 2100, Agilent Technologies, Palo Alto, CA).

Microarray hybridization and scanning. Microarray analysis was performed using Murine GeneChip U74Av2 Genechips (Affymetrix, Santa Clara, CA) which allow the analysis of the expression level of more than 12,400 mouse genes and ESTs on each array in the present study. Fragmented cRNA (15 µg) was incubated with 1x hybridization buffer (0.1 mg/ml herring sperm DNA, 0.5 mg/ml acetylated BSA, 5 nM control oligonucleotide B2) and 1x eukaryotic hybridization control solution (1.5 pM *BioB*, 5 pM *BioC*, 25 pm *BioD* and 100 pM *cre*), for 16h at 45°C with constant rotation (60 rpm). Microarrays were processed using an Affimetrix GeneChip Fluidic Station 400 (protocol EukGE-WS2Av4). Staining was performed with streptavidin-conjugated phycoerythrin (SAPE) followed by amplification with a biotinylated anti-streptavidin antibody and by a second round of SAPE solution. Genechips were scanned using an Agilent GeneArray Scanner (Agilent Technologies). Signal intensities for β-actin and GAPDH genes were used as internal quality controls. The ratio of fluorescent intensities for the 5' and 3' ends of these housekeeping genes was < 2. Scanned images were analyzed with Microarray Suite 5.0 (Affimetrix). The fourteen animals at each time point were pooled and the cRNA probe corresponding to each time point was hybridized once on separate microarrays. The fourteen animals of the control group were pooled and the cRNA probe from the control group was hybridized on two microarrays (duplicate). A total of

sixteen microarrays were used in microarray experiments. The microarray data have been deposited in the GEO repository (series number GSE9631).

Real-time RT-PCR. First strand cDNA synthesis was performed using 5 μg of RNA extracted from each time pool in a reaction containing 200 units of Superscript II Rnase H-reverse transcriptase (Invitrogen), 300 ng of oligo dT18, 500 μM dNTP, 10 mM DTT and 34 units of human RNase inhibitor (Amersham Pharmacia) in a final volume of 50 μL. The resulting products were then treated with 1 μg of Rnase A for 30 min at 37°C and purified thereafter with Qiaquick PCR purification kits (Qiagen). For quantitative PCR analyses, a Light-Cycler PCR (Roche Diagnostics) was used to confirm the expression level of cDNAs measured in the microarray method. The FastStart DNA Master SYBR green kit (Roche Diagnostics) was used in a final reaction volume of 20 μL containing 3 mM $MgCl_2$ and cDNA corresponding to 20 ng total RNA. The PCR was carried out according to the following conditions: 95°C/10 minutes, 50 cycles of (95°C / 10 seconds, 55-65°C / 5 seconds, 72°C / 11 seconds) with a temperature transition of 3°C / second. PCR results were calculated and normalized using the second-derivative and double-correction method as described previously (29), and with use of the housekeeping gene ATP synthase O subunit (Atp5o). This gene was found to display remarkably stable expression levels from embryonic life through adulthood in various tissues (48). Messenger RNA levels were expressed as the number of copies per microgram of total RNA using a standard curve of crossing points (Cp) vs. logarithm of quantity. The standard curve was established using known cDNA amounts of 10, 10^2, 10^3, 10^4, 10^5, and 10^6 copies of Atp5o. The Light Cycler 3.5 program was provided by the manufacturer (Roche). Expression of the housekeeping gene was constant at all time points and not significant influenced by DHT treatment in the present experiment.

Data processing and statistical analysis. Raw data of microarrays were collected and analyzed using the Affymetrix Microarray Suite 5.0 (MAS) software. To control for variability of the measurement, which is greater at low expression level (50), we established a relationship between fold changes and expression levels as described previsouly (31). In order to select differentially expressed genes, we used the following criteria: 1) the fold change (FC) had to be higher than the limit fold change (LFC) over the time course; and 2) at least two "present calls" (p-value < 0.04) had to be obtained across all arrays. The limit fold change (LFC) was defined as LFC= a + b/X, where X is the minimum Average Difference Intensity (ADI, i.e. absolute expression level) for a given gene. The parameters a and b were estimated based on the distribution of ratios calculated from replicated chips (1.9 and 60 respectively in the present experiment).

The fold change (FC) was calculated with the following equation: FC = Max ADI / Min ADI over the time course and control. Average ADI of two technique replica of control were used in data analysis. To identify genes to be validated by quantitative real-time RT-PCR, genes were further filtered following additional criteria: 1) ADI of a given gene greater than 400 on more than 60% of arrays; 2) absolute FC on peak time (peak time ADI / control ADI) $\geq$ 2.0. Annotations of interesting probesets were collected from different available databases using the NetAffx online tool (http://www.affymetrix.com/analysis/index.affx), and their identity was verified using the BLAST tool (http://www.ncbi.nlm.nih.gov). The Ingenuity Pathway Analysis System (Ingenuity® Systems, www.ingenuity.com) was also used to visualize gene expression data in the context of biological pathways.

Validation analyses were performed using peak time vs. control comparisons to examine the direction of fold changes with the microarray and RT-PCR methods. Since data for all time points were also compared to those of real-time RT-PCR for the genes selected, Spearman rank non-parametric correlation analysis was also used to compare time courses obtained with microarrays and real time RT-PCR. Time points from the microarray analysis were correlated to time points from the real-time RT-PCR analysis and DHT responses that generated correlation coefficients with a $p \leq 0.1$ were considered similar. This permissive cutoff was chosen to avoid underestimation of time course reproducibility.

RESULTS

In both male and female mice, serum DHT reached the maximal level 1h after treatment and decreased thereafter to reach baseline at 24h (Figure 1). The overall objective of the data mining approach was the identification of candidate genes within adipose tissue that were sensitive to DHT treatment. Arrays containing approximately 30,000 probesets for murine genes and EST clusters were available. Globally, based on the criteria mentioned in the methods, we found that 94 probesets were significantly modulated by DHT on chip arrays from male mice retroperitoneal adipose tissue, representing 93 genes (Appended Table 1). A total of 116 probesets were significantly modulated by DHT on chip arrays from female mice retroperitoneal adipose tissue, representing 112 genes (Appended Table 2). These genes exhibited gene expression changes that were higher than or equal to 2.0 fold comparing peak time point to control.

The distributions of peaking times for significantly modulated probesets were examined (Figure 2). In male mice, 38 (40%) probesets presented peak times at 1h. A total of 49 (42.2%) and 20 (17.2%) probesets in female mice presented peak times at 18h and 24h, respectively. In male mice, 33 (35.1%) up-regulated probesets and 61 (64.9%) down-regulated probesets were identified. In female mice, 51 (44%) probesets were up-regulated and 65 (56%) probesets were down-regulated.

Among the microarray data, a total of 21 genes were found to be significantly modulated in adipose tissue samples from both male and female mice. Sixteen of them changed in a similar direction in response to DHT. Five genes showed opposite regulation trends in males and females, including LOC677213, Tnnc2, Ddx6 Mgea5 and Myh1. Four of the latter genes were down-regulated in male mice and up-regulated in female mice. Only the Mgea5 gene was up-regulated in male mice and down-regulated in female mice (data not shown).

To confirm gene expression responses using an independent method, quantitative real-time RT-PCR validation was conducted. We validated subsets of 46 and 98 transcripts in male and female mice, respectively (Appended Tables 1 and 2). Using peak time vs. control comparisons, 74.0% and 63.3% of modulated genes were confirmed by PCR in male and female mice, respectively. We also used a more stringent approach to precisely compare time courses between the two methods. Using $p \leq 0.1$ as a cut-off for significance, Spearman non-parametric correlation analysis was performed and 17 genes (37.0%) showed reproducible time courses in PCR validation in male mice (Table 1). In female mice, 26 genes

(26.5%) showed a spearman rank correlation coefficient with a P value lower than 0.10, indicating identical time courses in PCR validation (Table 2). Among up-regulated genes with positive PCR validations in male mice, 5 were related to cell cycle, 4 to DNA replication, recombination and repair, 10 to cell death, 7 to cellular development, and 8 to cellular growth and proliferation. Among down-regulated genes, 5 were related to carbohydrate metabolism, 3 to cellular compromise, 2 to protein degradation, 4 to cell death, and 3 to cell-to-cell signaling and interaction. In female mice, among up-regulated genes with positive PCR validations, 9 were related to cellular development, 8 to cellular growth and proliferation, 8 to cell death, 7 to cellular assembly and organization, and 4 to cellular compromise. Among down-regulated genes, 7 were related to lipid metabolism, 9 to molecular transport, 3 to nucleic acid metabolism, 12 to small molecule biochemistry, and 6 to cell cycle.

Table 3 shows that four genes generated similar responses to the DHT treatment in both sexes, namely FK506 binding protein 5 (Fkbp5), cyclin-dependent kinase inhibitor 1A (p21 or Cdkn1a), growth arrest and DNA-damage-inducible 45 gamma (Gadd45g) and metallothionein 1 (Mt1). The DHT response of all these genes was validated using quantitative real-time RT-PCR. They all showed highly significant Spearman rank correlation coefficients with p values of 0.0001, 0.014, 0.003 and 0.023 in fat samples from male mice and 0.0001, 0.01, 0.07 and 0.0001 in fat samples from female mice. These genes were all up-regulated by DHT in both sexes (Table 3 and Figure 3).

DISCUSSION

This work represents the first microarray investigation on the effects of DHT treatment in retroperitoneal adipose tissue from a rodent model. Serum DHT measures obtained over the course of the experiment suggest that our study should be considered as an acute response experiment. A significant modulation of the adipose tissue transcriptome was observed in response to DHT in both male and female mice. A number of genes involved in distinct cellular functions were modulated in a sex-specific manner. A total of 21 genes were found to be simultaneously modulated in fat samples from both male and female mice. Sixteen of them showed similar regulatory patterns and 5 genes showed opposite regulatory patterns.

Bolduc et al. (7) previously examined the effects of DHT on adipose tissue in male mice using serial analysis of gene expression (SAGE) at 2 time points (3 and 24 h). They found that various pathways were regulated by DHT in retroperitoneal adipose tissue. Of note, DHT treatment indicated a stimulation of genes involved in fatty acid and triacylglycerol production as well as lypolysis in fat samples. They concluded that almost all aspects of cell function were affected by DHT through the modulation of gene expression. Much like the latter study, several cell functions were affected by DHT in the present analysis. For example, in male mice, we found that some genes related to cell cycle, DNA replication and recombination as well as repair, molecular transport, and cell death were up-regulated. Other genes involved in cellular function and maintenance were down-regulated. In female mice, some genes related to connective tissue development and function or tissue morphology were up-regulated, and some genes involved in cell cycle and cell death were down-regulated.

Only four genes showed identical regulatory patterns in both male and female mice. These genes were Fkbp5, Cdkn1a, Gadd45g and Mt1. All these genes, when up-regulated, appear to be related to a negative regulation of adipocyte differentiation and adipogenesis. Fkbp5 is considered as a glucocorticoid receptor heat shock protein 90 (Hsp90)-associated co-chaperone and plays a central role in steroid hormone signaling (33). Fkbp51 over-expression induces glucocorticoid resistance in squirrel monkeys by reducing hormone-binding affinity (38). Our finding indirectly suggests that up-regulated expression of Fkbp5 in response to DHT treatment may mediate inhibitory effects of DHT on glucocorticoid-induced fat cell differentiation and lipogenesis. Androgens stimulate lipolysis in adipose tissue and induce an antiadipogenic effect, at least in primary cultured preadipocytes, in adipocyte precursor cells and in 3T3-L1 preadipocytes (11; 43; 44).

The Gadd45 family of genes is involved in cell cycle and programmed cell death. Specifically, proteins of the Gadd45 family play a role in the inhibition of cellular growth (17; 49) and promote apoptotic cell death (19; 21; 28; 45). They are powerful suppressors of cell growth (20). Testosterone and DHT are thought to inhibit adipocyte differentiation through an AR-mediated nuclear translocation of β-catenin and activation of downstream Wnt signalling (43). Studies in a different model have shown that Gadd45a plays a role in the negative regulation of cell development through the suppression of β-catenin (20). Elevated expression of the Gadd45g gene in the present study may relate to reduced adipogenesis in response to DHT through this pathway.

Regarding MT1, obesity is induced in older MT-null transgenic mice (5), which are characterized by increases in body weight and white adipose tissue that are similar to that of obese diabetic (*db/db*) mice. Under cold exposure and induction by catecholamines, the MT1 gene is abundantly detected in brown adipose tissue (BAT) of rats (4; 6). Secretion or expression of MT protein is also observed in white adipose tissue (WAT) from mice (46), dog (14) and humans (12). All these observations indicate that expression of MT genes is linked to the regulation of energy balance. Our data on increased MT1 in response to DHT relate androgens to adipose tissue metabolism and energy homeostasis.

As an adipogenic transcription factor, PPARγ induces growth arrest and is involved in adipocyte differentiation through cyclin-dependent kinase inhibitors p18 and p21 (30). p27/p21 double knockout mice become obese and show adipocyte hyperplasia and related metabolic consequences (32). Significantly up-regulated Cdkn1a expression was detected in the present study, which suggests that DHT treatment may decrease adipocyte number and lead to reductions in retroperitoneal adiposity in male and female mice. Taken together, we hereby provide evidence indicating that DHT signalling involves cross-talks between adipocyte proliferation, apoptosis and differentiation pathways as well as energy homeostasis in retroperitoneal fat. These phenomena appear to be independent of the sex of the animal.

We identified five genes that were simultaneously and significantly modulated in fat samples from both male and female mice but showed opposite regulatory patterns. LOC677213, Tnnc2, Ddx6 and Myh1 were down-regulated in male mice and up-regulated in female mice. These genes are involved in the differentiation of skeletal muscle cells (9; 34) or immunity (1). Mgea5 was up-regulated in male mice and

down-regulated in female mice. The meningioma-expressed antigen 5 (hyaluronidase) gene (Mgea5) encodes a β-O-linked N-acetylglucosaminidase (O-GlcNAcase), and is involved in Type 2 diabetes as a biological and positional candidate gene (8).

Several other genes which were significantly modulated in the microarray analysis and positively validated by quantitative real-time RT-PCR in both male and female retroperitoneal fat samples were myogenic in nature. These genes included myosin light polypeptide kinase (Mylk), myosin heavy polypeptide 4 (Myh4), enolase 3 (Eno3), phosphofructokinase (Pfkm) and myosin heavy polypeptide 1 (Myh1). The procedure for fat sampling was carefully reviewed to ascertain that the sampling included only retroperitoneal adipose tissue, and that the tissues were carefully trimmed. Similar findings of myogenic genes in fat were also reported in other studies (24; 41; 51). Adipose tissue apparently shows mesenchymal plasticity for myogenic differentiation (24), and adipocyte precursors and skeletal myoblasts are capable of differentiating along similar developmental lineages (39). The use of whole tissue samples prevents us from reaching conclusions on which cell type is responsible for the androgenic response observed and should be acknowledged. Nevertheless, Lin et al. (26) found that fat pad weight and total lipid content of myostatin-null mice were significantly lowered compared to wild-type mice and suggested that increased muscle development in myostatin knockout mice resulted in reduced adipogenesis and leptin secretion. Rebbapragada also found that BMP7-induced adipogenesis is restrained by myostatin in both mesenchymal precursor cells and preadipocytes (37). These previous reports and the present findings support the notion that a strong relationship between myogenic and adipose genes may exist in adipose tissue of both male and female mice.

In this study, we used 6 time points to examine trends of gene modulation in mice from both sexes. Significant modulations were observed with microarray data using criteria described in the methods section. With respect to validation using RT-PCR, most previous studies used only two time points on a limited number of genes. In the present study, a very large number of genes were validated using RT-PCR on all 6 time points and in the vehicle group. Using a rather stringent validation procedure, our analysis indicates that microarray results are moderately reproducible when compared to quantitative real-time RT-PCR over several time points. Indeed, only 37.0% and 26.5% of transcripts in male and female mice respectively showed identical time courses. Using only peak time vs. control comparisons to validate the direction of fold changes in gene expression, valid findings increased to 74.0% and 63.3% of modulated

genes in male and female mice, respectively. Because we used the pooled samples, our analysis may have contributed to yield lower than expected reproducibility rates. The use of several arrays would have allowed us to use repeated measures ANOVA to identify significantly modulated genes. This may have led to higher reproducibility rates when comparing with RT-PCR results. We suggest, however, that the use of multiple time points and a rather stringent filtering procedure decreased the likelihood of identified false positives. Our results nevertheless suggest that careful validation on a different platform remains an absolute necessity (27).

In conclusion, our study indicates that microarray technology can be used to identify androgen-responsive genes in retroperitoneal adipose tissue from male and female mice. We found that DHT consistently stimulates genes that are associated with the regulation of adipogenesis in adipose tissue. This study also provides evidence to support the hypothesis that a myogenic response may be triggered by DHT in fat.

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REFERENCES

1. **Akao Y and Matsuda Y**. Identification and chromosome mapping of the mouse homologue of the human gene (DDX6) that encodes a putative RNA helicase of the DEAD box protein family. *Cytogenet Cell Genet* 75: 38-44, 1996.

2. **Anderson LA, McTernan PG, Barnett AH and Kumar S**. The effects of androgens and estrogens on preadipocyte proliferation in human adipose tissue: influence of gender and site. *J Clin Endocrinol Metab* 86: 5045-5051, 2001.

3. **Armellini F, Zamboni M, Castelli S, Robbi R, Mino A, Todesco T, Bergamo-Andreis IA and Bosello O**. Interrelationships between intraabdominal fat and total serum testosterone levels in obese women. *Metabolism* 43: 390-395, 1994.

4. **Beattie JH, Black DJ, Wood AM and Trayhurn P**. Cold-induced expression of the metallothionein-1 gene in brown adipose tissue of rats. *Am J Physiol* 270: R971-R977, 1996.

5. **Beattie JH, Wood AM, Newman AM, Bremner I, Choo KH, Michalska AE, Duncan JS and Trayhurn P**. Obesity and hyperleptinemia in metallothionein (-I and -II) null mice. *Proc Natl Acad Sci U S A* 95: 358-363, 1998.

6. **Beattie JH, Wood AM, Trayhurn P, Jasani B, Vincent A, McCormack G and West AK**. Metallothionein is expressed in adipocytes of brown fat and is induced by catecholamines and zinc. *Am J Physiol Regul Integr Comp Physiol* 278: R1082-R1089, 2000.

7. **Bolduc C, Larose M, Yoshioka M, Ye P, Belleau P, Labrie C, Morissette J, Raymond V, Labrie F and St-Amand J**. Effects of dihydrotestosterone on adipose tissue measured by serial analysis of gene expression. *J Mol Endocrinol* 33: 429-444, 2004.

8. **Cameron EZ, Martinez-Marignac VL, Chan A, Valladares A, Simmonds LV, Wacher N, Kumate J, McKeigue P, Shriver MD, Kittles R, Cruz M and Parra EJ**. MGEA5-14 polymorphism and type 2 diabetes in Mexico City. *Am J Hum Biol* 19: 593-596, 2007.

9. **Davis RL, Weintraub H and Lassar AB**. Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* 51: 987-1000, 1987.

10. **De Pergola G, Triggiani V, Giorgino F, Cospite MR, Garruti G, Cignarelli M, Guastamacchia E and Giorgino R**. The free testosterone to dehydroepiandrosterone sulphate molar ratio as a marker of visceral fat accumulation in premenopausal obese women. *Int J Obes Relat Metab Disord* 18: 659-664, 1994.

11. **De Pergola G, Xu XF, Xang SM, Giorgino R and Bjorntorp P**. Up-regulation of androgen receptor binding in male rat fat pad adipose precursor cells exposed to testosterone:study in a whole cell assay system. *J Steroid Biochem Mol Biol* 37: 553-558, 1990.

12. **Do MS, Nam SY, Hong SE, Kim KW, Duncan JS, Beattie JH and Trayhurn P**. Metallothionein gene expression in human adipose tissue from lean and obese subjects. *Horm Metab Res* 34: 348-351, 2002.

13. **Dunaif A**. Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev* 18: 774-800, 1997.

14. **Eisele I, Wood IS, German AJ, Hunter L and Trayhum P**. Adipokine gene expression in dog adipose tissues and dog white adipocytes differentiated in primary culture. *Horm Metab Res* 37: 474-481, 2005.

15. **Elbers JM, Asscheman H, Seidell JC, Megens JA and Gooren LJ**. Long-term testosterone administration increases visceral fat in female to male transsexuals. *J Clin Endocrinol Metab* 82: 2044-2047, 1997.

16. **Fan W, Yanase T., Nomura M., Okabe T., Goto K., Sato T., Kawano H., Kato S. and Nawata H.** Androgen receptor null male mice develop late-onset obesity caused by decreased energy expenditure and lipolytic activity but show normal insulin sensitivity with high adiponectin secretion. *Diabetes* 54: 1000-1008, 2005.

17. **Fornace AJ Jr, Alamo I Jr and Hollander MC**. DNA damage-inducible transcripts in mammalian cells. *Proc Natl Acad Sci U S A* 85: 8800-8804, 1988.

18. **Gapstur SM, Gann PH, Kopp P, Colangelo L, Longcope C and Liu K**. Serum androgen concentrations in young men: a longitudinal analysis of associations with age, obesity, and race. The CARDIA male hormone study. *Cancer Epidemiol Biomarkers Prev* 11: 1041-1047, 2002.

19. **Harkin DP, Bean JM, Miklos D, Song YH, Truong VB, Englert C, Christians FC, Ellisen LW, Msheswaran S, Oliner JD and Haber DA**. Induction of GADD45 and JNK/SAPK-dependent apoptosis following inducible expression of BRCA1. *Cell* 97: 575-585, 1999.

20. **Hildesheim J, Belova GI, Tyner SD, Zhou X, Vardanian L and Fornace AJ Jr**. Gadd45a regulates matrix metalloproteinases by suppressing DeltaNp63alpha and beta-catenin via p38 MAP kinase and APC complex activation. *Oncogene* 23: 1829-1837, 2004.

21. **Hildesheim J, Bulavin DV, Anver MR, Alvord WG, Hollander MC, Vardanian L and Fornace AJ Jr**. Gadd45a protects against UV irradiation-induced skin tumors, and promotes apoptosis and stress signaling via MAPK and p53. *Cancer Res* 62: 7305-7315, 2002.

22. **Khaw KT and Barrett-Connor E**. Lower endogenous androgens predict central adiposity in men. *Ann Epidemiol* 2: 675-682, 1992.

23. **Labrie F, Belanger A, Belanger P, Berube R, Martel C, Cusan L, Gomez J, Candas B, Castiel I, Chaussade V, Deloche C and Leclaire J**. Androgen glucuronides, instead of testosterone, as the new markers of androgenic activity in women. *J Steroid Biochem Mol Biol* 99: 182-188, 2006.

24. **Lee GH and Kemp DM**. Human adipose-derived stem cells display myogenic potential and perturbed function in hypoxic conditions. *Biochem Biophys Res Commun* 341: 882-888, 2006.

25. **Li M. and Bjorntorp P**. Effects of testosterone on triglyceride uptake and mobilization in different adipose tissues in male rats in vivo. *Obes Res* 3: 113-119, 1995.

26. **Lin J, Armold HB, Della-Fera MA, Azain MJ, Hartzell DL and Baile CA**. Myostatin knockout in mice increases myogenesis and decreases adipogenesis. *Biochem Biophys Res Commun* 291: 701-706, 2002.

27. **Liu YZ, Dvornyk V, Lu Y, Shen H, Lappe JM, Recker RR and Deng HW**. A novel pathophysiological mechanism for osteoporosis suggested by an in vivo gene expression study of circulating monocytes. *J Biol Chem* 280: 29011-29016, 2005.

28. **Lu B, Yu H, Chow C, Li B, Zheng W, Davis RJ and Flavell RA**. GADD45gamma mediates the activation of the p38 and JNK MAP kinase pathways and cytokine production in effector TH1 cells. *Immunity* 14: 583-590, 2001.

29. **Luu-The V, Paquet N, Calvo E and Cumps J**. Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction. *Biotechniques* 38: 287-293, 2005.

30. **Morrison RF and Farmer SR**. Role of PPARgamma in regulating a cascade expression of cyclin-dependent kinase inhibitors, p18(INK4c) and p21(Waf1/Cip1), during adipogenesis. *J Biol Chem* 274: 17088-17097, 1999.

31. **Mutch DM, Berger A, Mansourian R, Tytz A and Roberts MA**. The limit fold change model: a practical approach for selecting differentially expressed genes from microarray data. *BMC Bioinformatics* 3: 17, 2002.

32. **Naaz A, Holsberger DR, Iwamoto GA, Nelson A, Kiyokawa H and Cooke PS**. Loss of cyclin-dependent kinase inhibitors produces adipocyte hyperplasia and obesity. *FASEB J* 18: 1925-1927, 2004.

33. **Nuber UA, Kriaucionis S, Roloff TC, Guy J, Selfridge J, Steinhoff C, Schulz R, Lipkowitz B, Ropers HH, Holmes MC and Bird A**. Up-regulation of glucocorticoid-regulated genes in a mouse model of Rett syndrome. *Hum Mol Genet* 14: 2247-2256, 2005.

34. **Parmacek MS and Leiden JM**. Structure, function, and regulation of troponin C. *Circulation* 84: 991-1003, 1991.

35. **Pasquali R, Casimirri F, Cantobelli S, Melchionda N, Morselli Labate AM, Fabbri R, Capelli M and Bortoluzzi L**. Effect of obesity and body fat distribution on sex hormones and insulin in men. *Metabolism* 40: 101-104, 1991.

36. **Price TM, O'Brien SN, Welter BH, George R, Anandjiwala J and Kilgore M**. Estrogen regulation of adipose tissue lipoprotein lipase--possible mechanism of body fat distribution. *Am J Obstet Gynecol* 178: 101-107, 1998.

37. **Rebbapragada A, Benchabane H, Wrana JL, Celeste AJ and Attisano L**. Myostatin signals through a transforming growth factor beta-like signaling pathway to block adipogenesis. *Mol Biol Cell* 23: 7230-7242, 2003.

38. **Reynolds PD, Ruan Y, Smith DF and Scammell JG**. Glucocorticoid resistance in the squirrel monkey is associated with overexpression of the immunophilin FKBP51. *J Clin Endocrinol Metab* 84: 663-669, 1999.

39. **Rink C, Roy S, Khanna S, Rink T, Bagchi D and Sen CK**. Transcriptome of the subcutaneous adipose tissue in response to oral supplementation of type 2 Leprdb obese diabetic mice with niacin-bound chromium. *Physiol Genomics* 27: 370-379, 2006.

40. **Roncari DA and Van RL**. Promotion of human adipocyte precursor replication by 17beta-estradiol in culture. *J Clin Invest* 62: 503-508, 1978.

41. **Sasao N, Hirayama E and Kim J**. Characterization of heterokaryons between skeletal myoblasts and preadipocytes: myogenic potential of 3T3-L1 preadipocytes. *Eur J Cell Biol* 82: 97-103, 2003.

42. **Sato T., Matsumoto T., Yamada T., Watanabe T., Kawano H. and Kato S.** Late onset of obesity in male androgen receptor-deficient (AR KO) mice. *Biophys Res Commun* 300: 167-171, 2003.

43. **Singh R, Artaza JN, Taylor WE, Braga M, Yuan X, Gonzalez-Cadavid NF and Bhasin S**. Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: nuclear translocation of androgen receptor complex with beta-catenin and T-cell factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors. *Endocrinology* 147: 141-154, 2006.

44. **Singh R, Artaza JN, Taylor WE, Gonzalez-Cadavid NF and Bhasin S**. Androgens stimulate myogenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent cells through an androgen receptor-mediated pathway. *Endocrinology* 144: 5081-5088, 2003.

45. **Takekawa M and Saito H**. A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. *Cell* 95: 521-530, 1998.

46. **Trayhurn P, Duncan JS, Wood AM and Beattie JH**. Metallothionein gene expression and secretion in white adipose tissue. *Am J Physiol Regul Integr Comp Physiol* 279: R2329-R2335, 2000.

47. **Turcato E, Zamboni M, De Pergola G, Armellini F, Zivelonghi A, Bergamo-Andreis IA, Giorgino R and Bosello O**. Interrelationships between weight loss, body fat distribution and sex hormones in pre- and postmenopausal obese women. *J Intern Med* 241: 363-372, 1997.

48. **Warrington JA, Nair A, Mahadevappa M and Tsyganskaya M**. Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes. *Physiol Genomics* 2: 143-147, 2000.

49. **Zhan Q, Antinore MJ, Wang XW, Carrier F, Smith ML, Harris CC and Fornace AJ Jr**. Association with Cdc2 and inhibition of Cdc2/Cyclin B1 kinase activity by the p53-regulated protein Gadd45. *Oncogene* 18: 2892-2900, 1999.

50. **Zhang Y, Bosse Y, Marceau P, Biron S, Lebel S, Richard D, Vohl MC and Tchernof A**. Gene expression variability in subcutaneous and omental adipose tissue of obese men. *Gene Expr* 14: 35-46, 2007.

51. **Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P and Hedrick MH**. Human Adipose Tissue Is a Source of Multipotent Stem Cells. *Mol Biol Cell* 13: 4279-4295, 2002.

Table 1. In male mice, seventeen genes were considered as responsive to DHT treatment. Time course responses of these genes were reproducible in the microarray and quantitative real-time RT-PCR experiments.

Probeset	Gene symbol	Description	Accession	p-value
98308_at	Myh1	myosin, heavy polypeptide 1, skeletal muscle, adult	AJ002522	0.000
102736_at	Ccl2	chemokine (C-C motif) ligand 2	M19681	0.000
93482_at	Mylk	myosin, light polypeptide kinase	AI117835	0.000
94297_at	Fkbp5	FK506 binding protein 5	U16959	0.000
101979_at	Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	AF055638	0.003
98092_at	Plac8	placenta-specific 8	AA790307	0.003
96344_at	Eno3	enolase 3, beta muscle	X61600	0.007
160894_at	Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	X61800	0.014
94429_at	Eef1a2	eukaryotic translation elongation factor 1 alpha 2	L26479	0.014
94881_AT	Cdkn1a	cyclin-dependent kinase inhibitor 1A	AW048937	0.014
93573_at	Mt1	metallothionein 1	V00835	0.023
102906_at	Tgtp	T-cell specific GTPase	L38444	0.036
92599_at	Pgam2	phosphoglycerate mutase 2	AF029843	0.036
160754_at	Pygm	muscle glycogen phosphorylase	AI850363	0.052
100323_at	Amd2	S-adenosylmethionine decarboxylase 2	Z23077	0.071
94438_at	Pfkm	phosphofructokinase, muscle	AI852672	0.094
99667_at	Cox6a2	cytochrome c oxidase, subunit VI a, polypeptide 2	U08439	0.094

Table 2. In female mice, twenty-six genes were considered as responsive to DHT treatment. Time course responses of these genes were reproducible in the microarray and quantitative real-time RT-PCR experiments.

Probeset	Gene Symbol	Description	Accession	p-value
94297_at	Fkbp5	FK506 binding protein 5	U16959	0.00
93573_at	Mt1	metallothionein 1	V00835	0.00
93783_at	Ela1	elastase 1, pancreatic	M27347	0.00
98488_at	Myh4	myosin, heavy polypeptide 4, skeletal muscle	AJ223361	0.00
94418_at	Elovl6	ELOVL family member 6, elongation of long chain fatty acids (yeast)	AI839004	0.00
96588_at	Ptger3	prostaglandin E receptor 3 (subtype EP3)	D10204	0.00
97402_at	Inmt	indolethylamine N-methyltransferase	M88694	0.00
97524_f_at	Amy2	amylase 2, pancreatic	X02578	0.01
99571_at	Acaa1b	acetyl-Coenzyme A acyltransferase 1B	AW012588	0.01
94881_AT	Cdkn1a	cyclin-dependent kinase inhibitor 1A	AW048937	0.01
100022_at	Cish	cytokine inducible SH2-containing protein	D89613	0.02
101922_at	Kdelr2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	AW123408	0.04
92885_at	Tnnt3	troponin T3, skeletal, fast	L48989	0.04
100605_at	Tpm2	tropomyosin 2, beta	M81086	0.04
99939_at	Cel	carboxyl ester lipase	U37386	0.04
98308_at	Myh1	myosin, heavy polypeptide 1, skeletal muscle, adult	AJ002522	0.05
93028_at	H19	H19 fetal liver mRNA	X58196	0.05
94326_r_at	Mrps18a	mitochondrial ribosomal protein S18A	AV339603	0.05
103606_r_at	Rgs19	regulator of G-protein signaling 19	AW121438	0.05
101979_at	Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	AF055638	0.07
92592_at	Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)	M25558	0.07
97867_at	Hsd11b1	hydroxysteroid 11-beta dehydrogenase 1	X83202	0.07
101578_f_at	Actb	actin, beta, cytoplasmic	M12481	0.09
160711_at	Decr1	2,4-dienoyl CoA reductase 1, mitochondrial	AI844846	0.09
100566_at	Igfbp5	insulin-like growth factor binding protein 5	L12447	0.09
98441_at	Fmr1	fragile X mental retardation syndrome 1 homolog	L23971	0.09

Table 3: In both male and female mice, four genes were modulated in an identical manner. Time course responses of these genes were reproducible in the microarray and quantitative real-time RT-PCR experiments.

Gene Symbol	Description	Accession	Male		Female	
			Peaking time (h)	Fold change	Peaking time (h)	Fold change
Cdkn1a	cyclin-dependent kinase inhibitor 1A	AW048937	6	2.80	6	2.69
Fkbp5	FK506 binding protein 5	U16959	12	3.86	12	6.19
Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	AF055638	6	4.55	6	3.53
Mt1	metallothionein 1	V00835	12	2.70	6	3.44

The fold change (FC) is peak time absolute FC = Peak Time ADI / Average Control ADI.
All these data are from microarray analysis.

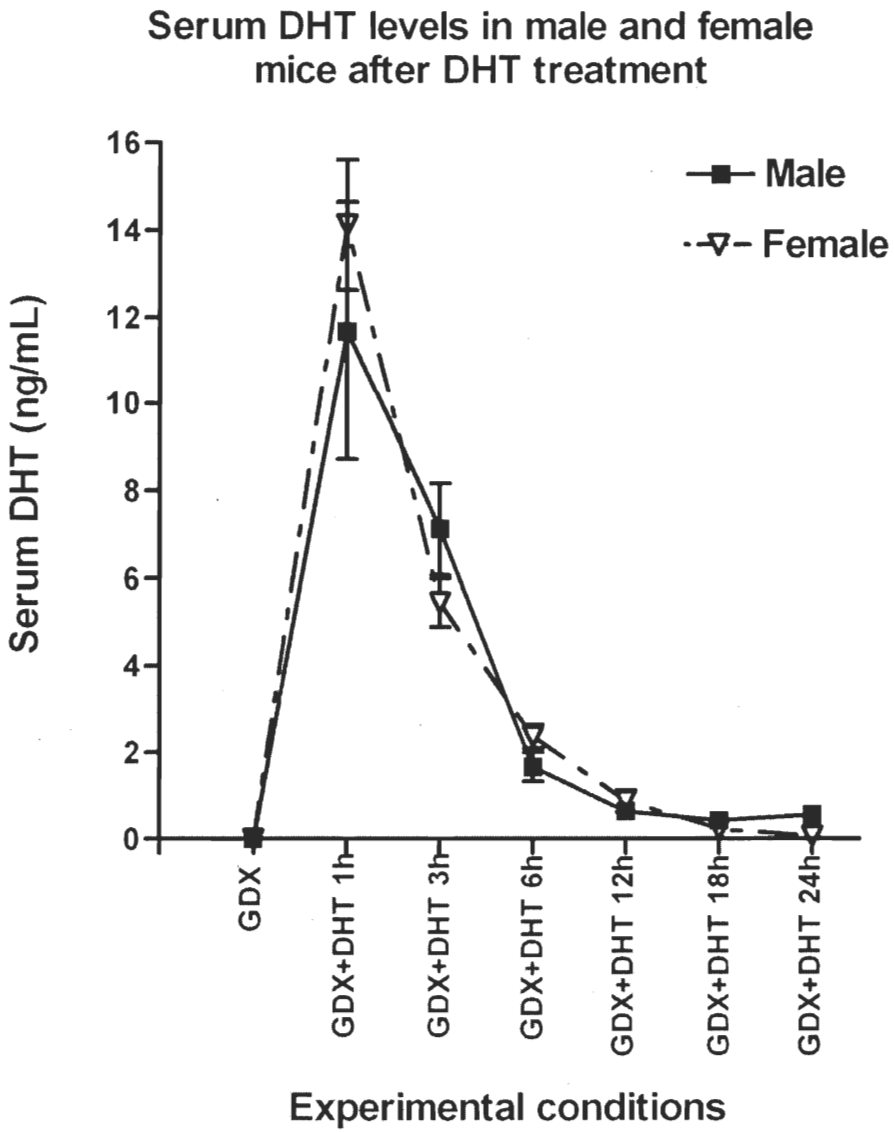

Figure 1. Serum DHT was measured at each time point after subcutaneous injection with 0.1mg DHT in each male and female mouse as well as each of control GDX animals. Data are presented as Mean ± SEM.

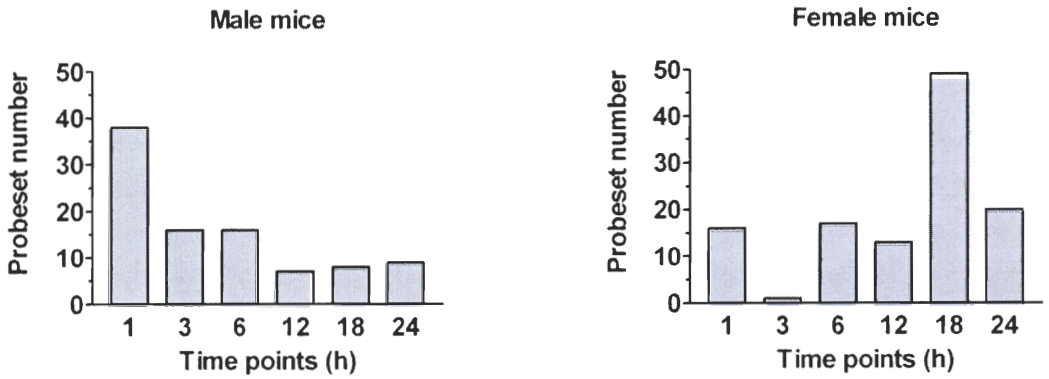

Figure 2: Distribution of peaking times for probesets which were significantly modulated in male and female retroperitoneal adipose tissue samples.

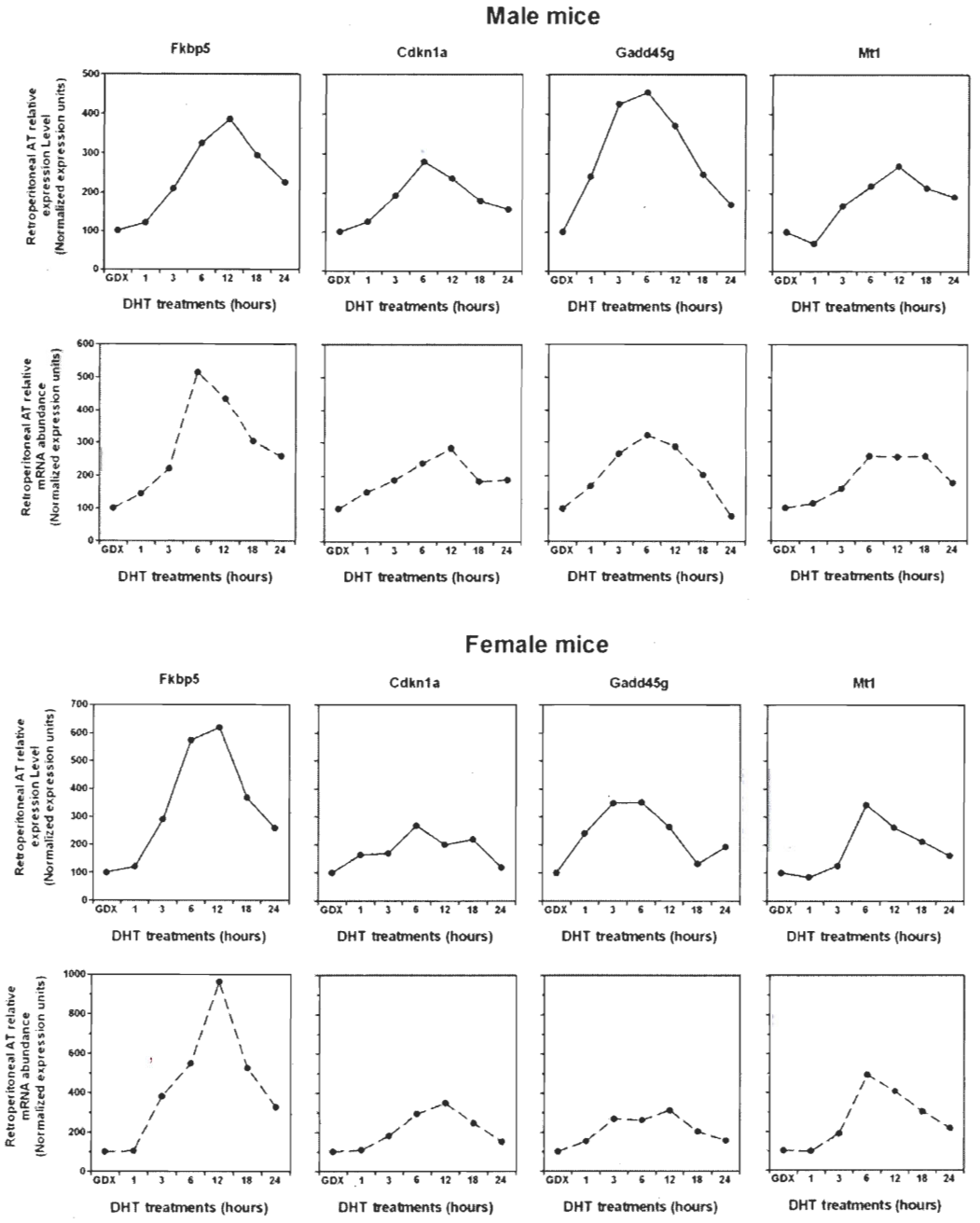

Figure 3. Effect of DHT after 1, 3, 6, 12, 18 or 24 h of treatment or vehicle (GDX) on mRNA expression levels of 4 androgen-responsive genes in retroperitoneal adipose tissue (AT) from both male and female mice. Microarray results (—) were validated by quantitative real-time RT-PCR (---). Data are shown in relative expression units for the results from the microarray analysis and in relative mRNA abundance for the results of RT-PCR validation.

Appended Table 1: 94 significantly modulated probesets in response to DHT representing 93 genes in retroperitoneal adipose tissue from male mice, 46 of these genes were validated by quantitative real-time RT-PCR. (* No quantitative RT-PCR validation performed.)

Probeset ID	Symbol	Description	Accession	Microarray data		Real-time PCR data	
				Peak Fold	Peak Time	Peak	Peak Tim
*160171_f_at	Acot9	acyl-CoA thioesterase 9	AI854821	-2.46	1	-	-
101578_f_at	Actb	actin, beta, cytoplasmic	M12481	-12.77	1	1.55	6
95705_s_at	Actb	actin, beta, cytoplasmic	J04181	-5.72	1	- 2.25	24
93512_f_at	Adk	adenosine kinase	AW121801	2.05	12	1.40	24
*99038_at	Adss	adenylosuccinate synthetase, non muscle	L24554	-2.59	3	-	-
96069_at	Akr7a5	aldo-keto reductase family 7, member A5 (aflatoxin aldehyde reductase)	AI840094	-2.29	12	- 1.75	24
100323_at	Amd2	S-adenosylmethionine decarboxylase 2	Z23077	2.27	12	2.31	6
*101621_at	Apbh	androgen-binding protein eta	AF008595	-2.80	1	-	-
*96650_at	Auh	AU RNA binding protein/enoyl-coenzyme A hydratase	AI837724	-2.06	1	-	-
102736_at	Ccl2	chemokine (C-C motif) ligand 2	M19681	2.49	24	2.62	24
*97930_f_at	Cd151	CD151 antigen	AF033620	2.04	3	-	-
95661_at	Cd9	CD9 antigen	L08115	-2.91	3	2.65	1
94881_at	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	AW048937	2.80	6	2.83	12
160894_at	Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	X61800	2.08	6	3.15	6
161569_f_at	Ckm	creatine kinase, muscle	AV086797	-2.62	6	- 6.22	18
99667_at	Cox6a2	cytochrome c oxidase, subunit VI a, polypeptide 2	U08439	-4.54	6	- 5.81	24
100069_at	Cyp2f2	cytochrome P450, family 2, subfamily f, polypeptide 2	M77497	-2.68	1	- 9.24	1
160611_at	Cyp4v3	cytochrome P450, family 4, subfamily v, polypeptide 3	AA212964	2.31	6	2.35	18
93964_s_at	Ddx6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	AF038995	-2.14	24	- 1.91	24
*102797_at	Dhrs3	dehydrogenase/reductase (SDR family) member 3	X95281	-2.78	1	-	-

*97880_at	Dlst	dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex)	AW061024	2.01	1	-	-
95426_at	Echs1	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	AW048512	-2.63	1	- 1.79	12
94429_at	Eef1a2	eukaryotic translation elongation factor 1 alpha 2	L26479	-2.39	6	- 2.86	18
96344_at	Eno3	enolase 3, beta muscle	X61600	-2.03	6	- 2.71	6
*92809_r_at	Fkbp4	FK506 binding protein 4	X17069	-2.17	1	-	-
94297_at	Fkbp5	FK506 binding protein 5	U16959	3.86	12	5.14	6
*160516_at	Gaa	glucosidase, alpha, acid	U49351	-2.27	1	-	-
101979_at	Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	AF055638	4.55	6	3.21	6
97934_at	Galnt1	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1	U73820	2.05	18	1.26	18
*102402_at	Gbas	glioblastoma amplified sequence	AJ001261	-3.16	1	-	-
*103737_at	Gfpt2	glutamine fructose-6-phosphate transaminase 2	AB016780	-2.84	3	-	-
99141_at	Gm2a	GM2 ganglioside activator protein	U09816	-2.78	1	1.34	1
*97458_at	Gnb1	guanine nucleotide binding protein, beta 1	AI845935	-3.36	1	-	-
*100574_f_at	Gpi1	glucose phosphate isomerase 1	L09104	-2.10	1	-	-
*101954_at	H2afz	H2A histone family, member Z	U70494	-2.31	3	-	-
98284_f_at	H2-T18	histocompatibility 2, T region locus 18	X03052	-2.74	1	4.28	3
*104194_at	Heph	hephaestin	AF082567	2.10	1	-	-
*103833_at	Hipk2	homeodomain interacting protein kinase 2	AF077659	-2.28	3	-	-
*102790_at	Jtb	jumping translocation breakpoint	AB016490	-2.13	3	-	-
101922_at	Kdelr2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	AW123408	-2.74	1	1.57	1
*96876_at	Laptm4a	lysosomal-associated protein transmembrane 4A	U34259	-2.28	1	-	-
*160778_at	LOC677213	similar to U2AF homology motif (UHM) kinase 1	AI846236	-3.74	1	-	-
97420_at	Lrg1	leucine-rich alpha-2-glycoprotein 1	AW230891	2.29	24	2.20	24
*98402_at	Macf1	microtubule-actin crosslinking factor 1	AI843799	-3.16	1	-	-
*92607_at	Mest	mesoderm specific transcript	AF017994	2.32	3	-	-
*95594_at	Mfn1	mitofusin 1	AI847486	-2.14	18	-	-
94499_at	Mgea5	meningioma expressed antigen 5 (hyaluronidase)	AI835427	2.63	1	- 1.48	18

94224_s_at	Mnda	myeloid cell nuclear differentiation antigen (Interferon-inducible protein p204)	M74123	2.41	6	2.14	3
93573_at	Mt1	metallothionein 1	V00835	2.70	12	2.59	18
101561_at	Mt2	metallothionein 2	K02236	3.97	6	- 3.19	6
98308_at	Myh1	myosin, heavy polypeptide 1, skeletal muscle, adult	AJ002522	-8.83	6	- 4.46	6
102108_f_at	Myh9	myosin, heavy polypeptide 9, non-muscle	AI505453	-2.38	1	- 1.78	24
93482_at	Mylk	myosin, light polypeptide kinase	AI117835	2.29	12	2.31	12
160991_at	Nkiras1	NFKB inhibitor interacting Ras-like protein 1	AI846893	2.16	18	1.86	6
*98507_at	Nr1d1	nuclear receptor subfamily 1, group D, member 1	AI834950	-2.01	1	-	-
103052_r_at	Nr2f2	nuclear receptor subfamily 2, group F, member 2	X76653	-2.36	24	- 1.41	3
*94362_at	Nras	neuroblastoma ras oncogene	AI843682	2.01	1	-	-
94438_at	Pfkm	phosphofructokinase, muscle	AI852672	-2.52	6	- 1.90	12
92599_at	Pgam2	phosphoglycerate mutase 2	AF029843	-2.68	18	2.34	1
*162032_f_at	Pkm2	pyruvate kinase, muscle	AV368209	2.58	1	-	-
98092_at	Plac8	placenta-specific 8	AA790307	2.01	24	2.46	24
*96464_at	Plxnb2	plexin B2	N28179	-2.15	18	-	-
*103054_at	Polr2a	polymerase (RNA) II (DNA directed) polypeptide A	U37500	2.11	3	-	-
*98846_f_at	Psg17	pregnancy specific glycoprotein 17	M83344	-2.27	1	-	-
96698_at	Psmd5	proteasome (prosome, macropain) 26S subunit, non-ATPase, 5	AI835520	-2.02	6	1.65	6
160754_at	Pygm	muscle glycogen phosphorylase	AI850363	-2.64	18	- 2.03	18
*101113_at	Rhoa	ras homolog gene family, member A	AI846668	-2.70	1	-	-
*101965_at	Rnf13	ring finger protein 13	AF037205	-2.47	3	-	-
97506_at	Rnf2	ring finger protein 2	AI043016	-2.64	24	1.30	18
*94068_at	Rps19	ribosomal protein S19	AW048899	-2.70	1	-	-
*98086_r_at	Rps28	ribosomal protein S28	U11248	2.46	3	-	-
*92578_at	Scye1	small inducible cytokine subfamily E, member 1	U10118	-2.25	3	-	-
*95104_at	Sdc2	syndecan 2	U00674	-2.36	3	-	-

98590_at	Sdc4	syndecan 4	D89571	-2.65	1	- 1.41	24
*94017_s_at	Sfrs2	splicing factor, arginine/serine-rich 2 (SC-35)	X98511	-2.24	1	-	-
*103863_at	Sft2d1	SFT2 domain containing 1	AW049769	-2.53	1	-	-
103070_at	Sirpa	signal-regulatory protein alpha	AB018194	2.14	24	1.80	24
*161149_r_at	Slc23a3	solute carrier family 23 (nucleobase transporters), member 3	AV222871	-2.09	1	-	-
*93486_at	Slc27a1	solute carrier family 27 (fatty acid transporter), member 1	U15976	-2.16	24	-	-
*96832_at	Slc39a1	solute carrier family 39 (zinc transporter), member 1	AA606878	-3.92	1	-	-
100538_at	Sod1	superoxide dismutase 1, soluble	M35725	-7.52	1	- 1.26	24
*94292_at	Strap	serine/threonine kinase receptor associated protein	AF096285	2.36	1	-	-
*160117_at	Tef	thyrotroph embryonic factor	AI850638	-2.25	3	-	-
102906_at	Tgtp	T-cell specific GTPase	L38444	2.17	6	3.31	6
103869_at	Thbs3	thrombospondin 3	U16175	-2.48	24	1.66	6
*97392_at	Tmem129	transmembrane protein 129	AW050086	2.44	18	-	-
*94829_at	Tmem70	transmembrane protein 70	AW121838	2.75	6	-	-
*160267_at	Tmem93	transmembrane protein 93	AI845987	-2.50	1	-	-
162325_f_at	Tnnc2	troponin C2, fast	AV083137	-2.05	1	- 2.51	6
92885_at	Tnnt3	troponin T3, skeletal, fast	L48989	-2.70	6	1.72	18
*102916_s_at	Tnxb	tenascin XB	AB010266	-2.00	18	-	-
*160376_at	Trp53inp2	transformation related protein 53 inducible nuclear protein 2	AW125508	2.00	12	-	-
*160678_at	Tspan12	tetraspanin 12	AA871166	2.14	1	-	-
*102308_at	Tulp3	tubby-like protein 3	AF045582	2.06	3	-	-

Appended Table 2: 116 significantly modulated probesets in response to DHT representing 112 genes in retroperitoneal adipose tissue from female mice, 98 of these genes were validated by quantitative real-time RT-PCR. (* No quantitative RT-PCR validation performed.)

Probeset ID	Symbol	Description	Accession	Microarray data		Real-time PCR data	
				Peak Fold	Peak time	Peak Fold	Peak time
*161085_r_at	A4galt	alpha 1,4-galactosyltransferase	AA682038	2.9	18	-	-
92913_at	Abcd2	ATP-binding cassette, sub-family D (ALD), member 2	Z48670	2.1	24	1.22	1
99571_at	Acaa1b	acetyl-Coenzyme A acyltransferase 1B	AW012588	-3.2	18	-2.29	18
160207_at	Acly	ATP citrate lyase	AW121639	-2.1	12	-2.05	12
101578_f_at	Actb	actin, beta, cytoplasmic	M12481	-12.6	1	1.38	18
95705_s_at	Actb	actin, beta, cytoplasmic	J04181	-6.4	1	-2.28	24
101947_at	Akap8l	A kinase (PRKA) anchor protein 8-like	AB028921	-3.6	18	-2.01	1
97524_f_at	Amy2	amylase 2, pancreatic	X02578	-27.7	1	51.06	12
102815_at	Anxa11	annexin A11	U65986	-2.3	18	-1.39	18
*103006_at	Atf5	activating transcription factor 5	AB012276	-2.2	1	-	-
160581_at	Atg16l1	autophagy-related 16-like 1 (yeast)	AI848078	2.1	24	-1.27	6
162223_f_at	Atp2a1	ATPase, Ca++ transporting, cardiac muscle, fast twitch 1	AV241808	-2.7	18	8.27	18
102302_at	Bckdhb	branched chain ketoacid dehydrogenase E1, beta polypeptide	L16992	-2.1	18	1.10	24
101475_at	Bmi1	B lymphoma Mo-MLV insertion region 1	M64068	-2.1	18	-1.18	1
99584_at	Cd82	CD82 antigen	D14883	-3.0	24	2.33	12
95037_at	Cdk9	cyclin-dependent kinase 9 (CDC2-related kinase)	AW046639	-2.0	24	-1.75	6
94881_at	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	AW048937	2.7	6	3.48	12
160894_at	Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	X61800	2.3	6	2.84	3
99939_at	Cel	carboxyl ester lipase	U37386	13.8	12	-244.97	1
100022_at	Cish	cytokine inducible SH2-containing protein	D89613	-2.4	12	-2.34	12
103709_at	Col1a1	procollagen, type I, alpha 1	AA763466	-3.8	6	1.65	18
100308_at	Col8a1	procollagen, type VIII, alpha 1	X66976	2.4	18	1.69	18
103492_at	Cpxm1	carboxypeptidase X 1 (M14 family)	AF077738	-2.2	18	-1.52	6

103646_at	Crat	carnitine acetyltransferase	X85983	-2.1	18	1.47	3
162469_r_at	Cyc1	cytochrome c-1	AV069997	2.2	18	-1.95	3
95529_at	Dbnl	drebrin-like	U58884	-2.2	24	-1.86	6
92969_at	Ddr2	discoidin domain receptor family, member 2	X76505	2.3	18	1.49	3
93964_s_at	Ddx6	DEAD (Asp-Glu-Ala-Asp) box polypeptide 6	AF038995	2.3	1	1.22	18
160711_at	Decr1	2,4-dienoyl CoA reductase 1, mitochondrial	AI844846	-2.1	18	-1.42	6
*102797_at	Dhrs3	dehydrogenase/reductase (SDR family) member 3	X95281	-3.2	1	-	-
161474_r_at	Dpep3	dipeptidase 3	AV043191	2.3	18	-2.15	3
95426_at	Echs1	enoyl Coenzyme A hydratase, short chain, 1, mitochondrial	AW048512	-2.4	18	-1.14	6
93783_at	Ela1	elastase 1, pancreatic	M27347	11.3	12	-5.86	1
100755_at	Ela2	elastase 2, neutrophil	U04962	2.4	18	-3.63	1
94418_at	Elovl6	ELOVL family member 6, elongation of long chain fatty acids (yeast)	AI839004	-2.6	12	-1.69	12
98526_r_at	Erdr1	Erythroid differentiation regulator 1	AI854606	3.0	18	-1.21	6
93974_at	Errfi1	ERBB receptor feedback inhibitor 1	AW212475	2.6	1	2.98	12
93975_at	Errfi1	ERBB receptor feedback inhibitor 1	AI853531	2.1	12	2.23	18
94297_at	Fkbp5	FK506 binding protein 5	U16959	6.2	12	9.61	12
98441_at	Fmr1	fragile X mental retardation syndrome 1 homolog	L23971	-2.1	18	-1.54	6
93459_s_at	Fzd4	frizzled homolog 4 (Drosophila)	AW122897	2.4	18	-1.28	3
101979_at	Gadd45g	growth arrest and DNA-damage-inducible 45 gamma	AF055638	3.5	6	3.12	12
94192_at	Gdap10	ganglioside-induced differentiation-associated-protein 10	Y17860	3.4	1	-1.62	24
161826_r_at	Glul	glutamate-ammonia ligase (glutamine synthetase)	AV381947	-2.0	24	-1.64	24
99141_at	Gm2a	GM2 ganglioside activator protein	U09816	-3.0	1	1.12	3
100514_at	Gna13	guanine nucleotide binding protein, alpha 13	M63660	2.3	24	1.27	18
*94854_g_at	Gnb1	guanine nucleotide binding protein, beta 1	U29055	-3.1	1	-	-

97458_at	Gnb1	guanine nucleotide binding protein, beta 1	AI845935	-4.0	1	1.22	18
101867_at	Gpam	glycerol-3-phosphate acyltransferase, mitochondrial	U11680	-2.5	18	-2.03	18
92592_at	Gpd1	glycerol-3-phosphate dehydrogenase 1 (soluble)	M25558	-2.1	12	-4.55	6
93028_at	H19	H19 fetal liver mRNA	X58196	2.0	6	1.97	6
*93023_f_at	Hist1h3g	histone cluster 1, H3g	M32459	2.9	18	-	-
97867_at	Hsd11b1	hydroxysteroid 11-beta dehydrogenase 1	X83202	2.1	24	3.27	24
161446_r_at	Htra2	HtrA serine peptidase 2	AV353694	-2.3	24	-1.48	1
93321_at	Ifi203	interferon activated gene 203	AF022371	-3.7	18	1.29	3
160092_at	Ifrd1	interferon-related developmental regulator 1	V00756	-2.8	18	-1.13	12
*162392_r_at	Ift88	intraflagellar transport 88 homolog (Chlamydomonas)	AV266216	-2.5	24	-	-
*94952_at	Igf2bp2	insulin-like growth factor 2 mRNA binding protein 2	AI152780	-2.1	6	-	-
100566_at	Igfbp5	insulin-like growth factor binding protein 5	L12447	-2.1	12	-1.39	6
97402_at	Inmt	indolethylamine N-methyltransferase	M88694	5.0	18	6.03	18
97859_at	Inpp5a	inositol polyphosphate-5-phosphatase A	AA762325	-2.0	18	-1.37	6
104652_at	Kcnk2	potassium channel, subfamily K, member 2	AI849601	-2.4	18	-2.89	6
161796_r_at	Kcnq1	potassium voltage-gated channel, subfamily Q, member 1	AV367240	-2.2	24	1.29	12
101922_at	Kdelr2	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 2	AW123408	-2.7	1	1.88	18
*160778_at	LOC677213	similar to U2AF homology motif (UHM) kinase 1	AI846236	5.3	18	-	-
94499_at	Mgea5	meningioma expressed antigen 5 (hyaluronidase)	AI835427	-2.3	18	1.37	12
96258_at	Mgst3	microsomal glutathione S-transferase 3	AI843448	-2.1	24	-1.39	3
161767_r_at	Mrps18a	mitochondrial ribosomal protein S18A	AV339603	2.4	18	-1.78	24
94326_r_at	Mrps18a	mitochondrial ribosomal protein S18A	AW060921	2.1	18	-1.78	24
93573_at	Mt1	metallothionein 1	V00835	3.4	6	4.91	6
101561_at	Mt2	metallothionein 2	K02236	6.5	6	5.33	3
160262_at	Mtch2	mitochondrial carrier homolog 2 (C. elegans)	AI839901	-2.8	18	-1.14	3

99613_at	Mut	methylmalonyl-Coenzyme A mutase	X51941	-2.3	12	-1.89	1
98308_at	Myh1	myosin, heavy polypeptide 1, skeletal muscle, adult	AJ002522	2.5	6	1.91	6
98488_at	Myh4	myosin, heavy polypeptide 4, skeletal muscle	AJ223361	2.1	6	1.82	6
102108_f_at	Myh9	myosin, heavy polypeptide 9, non-muscle	AI505453	-2.1	6	-1.85	6
*93050_at	Myl2	myosin, light polypeptide 2, regulatory, cardiac, slow	M91602	2.4	6	-	-
*94537_at	Mylc2b	myosin light chain, regulatory B	AI838859	-2.2	18	-	-
98507_at	Nr1d1	nuclear receptor subfamily 1, group D, member 1	AI834950	-2.1	6	-4.49	6
103288_at	Nrip1	nuclear receptor interacting protein 1	AF053062	2.0	18	-1.33	1
97581_at	Odz4	odd Oz/ten-m homolog 4 (Drosophila)	AF059485	-2.1	24	-2.01	18
94804_at	Pbx1	pre B-cell leukemia transcription factor 1	L27453	-2.3	24	-1.69	6
*102049_at	Pdk4	pyruvate dehydrogenase kinase, isoenzyme 4	AJ001418	2.3	3	-	-
96588_at	Ptger3	prostaglandin E receptor 3 (subtype EP3)	D10204	-2.9	12	-1.85	12
*94929_at	Ptpn1	protein tyrosine phosphatase, non-receptor type 1	M97590	2.1	1	-	-
99032_at	Rasd1	RAS, dexamethasone-induced 1	AF009246	-2.2	18	-1.54	6
*160213_at	Reg1	regenerating islet-derived 1	D14010	7.8	12	-	-
103606_r_at	Rgs19	regulator of G-protein signaling 19	AW121438	-2.3	6	-1.44	6
162468_at	Rhoc	ras homolog gene family, member C	AV064502	2.0	18	-2.39	6
96859_at	Rnf10	ring finger protein 10	AB026621	-2.5	18	1.56	24
161327_f_at	Rpl10a	ribosomal protein L10A	AV104703	-2.4	18	-1.21	1
*100732_at	Rps8	ribosomal protein S8	X73829	-2.2	1	-	-
*161062_r_at	Rufy3	RUN and FYVE domain containing 3	AW049573	2.2	24	-	-
*161378_r_at	Runx2	runt related transcription factor 2	AV245229	-2.6	24	-	-
*92756_r_at	Sct	secretin	X73580	2.4	18	-	-
98590_at	Sdc4	syndecan 4	D89571	-3.0	1	2.25	18
161149_r_at	Slc23a3	solute carrier family 23 (nucleobase transporters), member 3	AV222871	3.3	18	-1.27	6
96832_at	Slc39a1	solute carrier family 39 (zinc transporter), member 1	AA606878	-3.9	1	-2.24	24
103371_at	Slc39a7	solute carrier family 39 (zinc transporter),	AF100956	-2.1	18	-1.20	6

		member 7					
92471_i_at	Slfn2	schlafen 2	AF099973	-2.3	18	1.79	24
100538_at	Sod1	superoxide dismutase 1, soluble	M35725	-4.8	6	-1.28	18
100032_at	Sp1	trans-acting transcription factor 1	X60136	2.5	18	1.25	18
100051_at	Stom	stomatin	U17297	-2.1	24	-1.37	6
160300_at	Tff1	trefoil factor 1	Z21858	2.1	18	-2.16	1
103958_g_at	Tfrc	transferrin receptor	X57349	-2.1	24	-1.45	1
93882_f_at	Tgoln1	trans-golgi network protein	D50032	2.2	24	1.45	24
162325_f_at	Tnnc2	troponin C2, fast	AV083137	2.2	24	1.86	24
92885_at	Tnnt3	troponin T3, skeletal, fast	L48989	-2.1	18	1.82	1
99532_at	Tob1	transducer of ErbB-2.1	D78382	-2.3	12	1.25	24
95694_at	Top1	topoisomerase (DNA) I	X70956	-2.0	18	-1.30	6
100605_at	Tpm2	tropomyosin 2, beta	M81086	2.0	6	2.17	6
94020_at	Twf2	twinfilin, actin-binding protein, homolog 2 (Drosophila)	Y17808	2.3	18	-1.36	1
161284_r_at	Txnrd3	Thioredoxin reductase 3	AV299386	2.3	18	-1.23	6
160110_at	Wwp2	WW domain containing E3 ubiquitin protein ligase 2	AW124007	2.2	18	-1.39	6
95627_at	Wwtr1	WW domain containing transcription regulator 1	AW046038	2.1	18	1.35	12
*92202_g_at	Zbtb16	zinc finger and BTB domain containing 16	AI553024	2.7	6	-	-

Part C: Progesterone metabolism in adipose cells

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Progesterone metabolism in adipose cells

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ABSTRACT

The aim of the present study was to investigate pathways of progesterone metabolism in human adipose cells. Adipose tissue samples from the omental (OM) and subcutaneous (SC) fat compartments were surgically obtained in women. In isolated mature adipocytes, progesterone was converted to 20α-hydroxyprogesterone as the main metabolite, most likely through the activity of aldo-keto reductases 1C1, 2 and 3 (20α-HSD, 3α-HSD type 3 and 17β-HSD type 5 respectively). In cultured preadipocytes, progesterone was converted to several metabolites identified using bidimensional thin layer chromatography, with or without the dual inhibitor of 5α-reductase type 1 and 2 (17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA)). Major metabolites identified in OM and SC preadipocytes which were incubated for 24 hours with ^{14}C-labelled progesterone were 20α-hydroxyprogesterone, 5α-pregnane-3α/β-ol-20-one, 5α- and 5β-pregnanedione, 5α- and 5β-pregnane-20α-ol-3-one, 5α-pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β-20α-diol. Induction of preadipocyte differentiation increased expression levels of AKR1C1 and modified the pattern of progesterone metabolism substantially, leaving 20α-hydroxyprogesterone as the main metabolite generated. On the other hand, progesterone itself showed no consistent effect on adipocyte differentiation. In conclusion, preadipocytes and lipid-storing, mature adipocytes efficiently generate progesterone metabolites in women, which is consistent with rather modest effects progesterone on abdominal fat cell differentiation.

Keywords: 20α-hydroxyprogesterone; Adipocyte; Omental; Differentiation; Aldo-keto reductases 1C

INTRODUCTION

Findings of several studies (Anderson et al., 2001; Price et al., 1998; Roncari and Van, 1978) suggest that sex steroids may be regarded as critical modulators of adipose tissue deposition through the regulation of preadipocyte proliferation and/or differentiation as well as lipogenesis and/or lipolysis of mature adipocytes. A series of *in vivo* and *in vitro* observations (Lacasa et al., 2001; Mendes et al., 1985; Monjo et al., 2003; Rondinone et al., 1992; Shirling et al., 1981; Wiper-Bergeron et al., 2003) has shown that progesterone may stimulate fat accretion by increasing lipoprotein lipase (LPL) activity, lipid synthesis and steroid-mediated differentiation of preadipocytes. These results, however, are not unanimous, as Hamosh et al. (Hamosh and Hamosh, 1975) reported no effect of progesterone on LPL activity in rat adipose tissue. Björntorp (Bjorntorp, 1997) suggested that progesterone could be involved in the presence of a female fat distribution pattern via an anti-glucocorticoid action in abdominal adipose tissue. This notion was partially supported by studies showing that progesterone inhibited glucocorticoid-induced fat cell differentiation, lipogenesis, or body fat accumulation (Pedersen et al., 2003; Xu et al., 1990).

Regarding progesterone metabolism, we have reported high adipose tissue levels of 20α-hydroxysteroid dehydrogenase (20α-HSD, AKR1C1), an enzyme involved in the inactivation of progesterone (Blanchette et al., 2005). Findings of our previous studies indicated that the 20α-reduction of progesterone was increased in omental (OM) adipose tissue of women characterized by abdominal obesity (Blanchette et al., 2005; Blouin et al., 2005). In addition to the 20α-hydroxy group, the progesterone molecule contains additional functional groups that may be modified by other steroid-converting enzymes also expressed in adipose tissue such as 5α-reductase and 3α-hydroxysteroid dehydrogenase type 3 (3α-HSD-3, AKR1C2) (Belanger et al., 2002).

As a member of the aldo-keto reductase (AKR) superfamily, AKR1C1 is mainly involved in the conversion of progesterone into its biologically inactive metabolite 20α-hydroxyprogesterone (Penning TM, 1997), the reverse reaction being minor within a cellular context (Rizner et al., 2006). This enzyme has been detected in rat ovary, testes, adrenals, placenta, liver, thymus, T-dependent areas of other lymphoid organs, kindney and lung tissue of mice (Imamura et al., 2007), as well as human uterus (Mori and Wiest, 1979; Penning et al., 2000; Wilcox and Wiest, 1966; Wiest and Wilcox, 1961; Weinstein, 1977; Zhang et al., 2000). Findings from the group of Penning suggest that AKR1C1 appears to possess the plasticity which allows it to interconvert potent androgens, estrogens, and progestins into their cognate

inactive metabolites, the most catalytically efficient conversion being that of progesterone to its inactive metabolite (20α-hydroxyprogesterone) (Penning et al., 2000). In mouse liver cytosol, both NADPH and NADH are involved in the activity of 20α-HSD as the cofactors, whereas NADPH is the only cofactor for the 20α-HSD effect in mouse kidney (Shimade et al., 2006). Additionally, 20α-HSD activity is found to be catalyzed by type 1 or type 2 17β-HSD in human placenta (Penning, 1997). In human renal cytosol (Quinkler et al., 2004), large-scale conversion of progesterone to 20α-dihydro-progesterone was found. Wiebe et al. (Wiebe, 2006) reported that progesterone was directly converted to the 4-pregnenes, 3α-hydroxy-4-pregnen-20-one (3α-dihydroprogesterone; 3αHP) and 20α-hydroxyprogesterone through 3α-HSD and 20α-HSD activities, and through the irreversible catalysis of 5α-reductase, and then these metabolites were converted to 5α-pregnane, 5α-pregnane-3,20-dione (5α-dihydroprogesterone; 5αP). These previous reports indicated that 20α-HSD activity is also found in other non-reproductive tissues and appears to generate differences in the effect of progesterone.

The discrepancies in studies that have examined the impact of progesterone on adipose tissue function and the newly described possibility of pre-receptor progesterone inactivation through 20α-HSD and other enzymes in adipose tissue prompted us to re-examine progesterone action and metabolism in cultured adipose cells from women. The present study focuses on the comparison of progesterone metabolite formation in preadipocytes and lipid-storing adipocytes.

SUBJECTS AND METHODS

Subjects and adipose tissue sampling. Samples were obtained from twenty-four women aged 26 to 62 years (BMI 42.11 ± 16.62 kg/m^2, range 21.4 to 74.31 kg/m^2) undergoing abdominal gynecological (n=9) or bariatric (n=15) surgery. According to their BMI value, 7 women had normal BMI (29.2%), 4 were obese (16.6%), and 13 were morbidly obese (54.2%). Medication and drug history included anti-diabetic therapy (n = 7); lipid-lowering (n = 4) or antihypertensive therapy (n = 5), diuretics (n = 2), anti-depressants (n = 4). All these treatments were used in obese (n = 4) and morbidly obese (n = 11) patients undergoing bariatric surgery. Approval of the medical ethics committees of Laval University, Laval University Medical Research Center and Laval Hospital was obtained. All subjects provided written informed consent before their inclusion in the study.

Adipose tissue samples were collected during the surgical procedure at the site of incision (subcutaneous adipose tissue, SC) and from the greater omentum (omental adipose tissue, OM). Samples were immediately carried to the laboratory in 0.9% saline preheated at 37°C. A portion of the biopsy was used for adipocyte isolation and primary cultures, and the remaining tissue was immediately frozen at -80°C for subsequent analyses.

Adipocyte isolation and primary preadipocyte cultures. Tissue samples were digested with collagenase type I in Krebs-Ringer-Henseleit (KRH) buffer for 45 minutes at 37 °C according to a modified version of the Rodbell method (Rodbell, 1964). Adipocyte suspensions were filtered through nylon mesh and washed 3 times with KRH buffer. Mature adipocyte suspensions were incubated with ^{14}C-labelled progesterone at 37°C with shaking for 24 h. Preadipocytes were isolated using a modified method previously described by Hauner (Hauner, 1990; Hauner et al., 2001). Briefly, the residual buffer of the adipocyte isolation was centrifuged and the pellet was washed in DMEM-F12 supplemented with 10% fetal bovine serum, 2.5 µg/ml amphotericin B, 1.00 U/ml penicillin and 50g/ml streptomycin. Cells were treated with erythrocyte lysis buffer (154 mM NH_4Cl, 10mM $KHCO_3$, and 0.1 mM EDTA, pH 7.5) and washed again with DMEM-F12. Preadipocytes were seeded in 12-well culture plates and cultured at 37 °C under a 5% CO_2 atmosphere. Medium was changed every 2-3 days. For steroid converting activities before and after fat cell differentiation, preadipocytes were incubated 24h with ^{14}C-labelled progesterone at baseline and 16 to 20 days after inducing differentiation using differentiation medium with insulin, dexamethasone and a PPARγ agonist (Zen-Bio, Research Triangle Park, NC). The concentration of radiolabeled progesterone was 0.44

μM per well which is close to the *K*m value of human 20α-HSD in intact transfected cells, 0.6 μM (Zhang et al., 2000).

Glycerol-3-phosphate dehydrogenase (G3PDH) activity measurements and lipid accumulation. G3PDH activity was used as a marker of adipocyte differentiation and measured according to Sottile *et al.* (Sottile and Seuwen, 2001) with some modifications. Differentiated cells from 3 separate wells of 96-well plates were washed with PBS. Cold homogenization solution (100 μL/well; 20 mM tris, 1 mM EDTA, 1 mM β-mercaptoethanol, pH 7.3) was added to harvest cells and samples were kept at -80°C until analysis. After thawing, samples were assayed for G3PDH activity in 96-well plates. To 100 μL of the sample, 90 μL of reaction mix (100 mM triethanolamine, 2.5 mM EDTA, 0.1 mM β-mercaptoethanol, 353 μM NADH, pH 7.7) was added and incubated for 10 minutes at 37°C. The assay was initiated by the addition of dihydroxyacetone phosphate (10 μL/well of a 8 mM stock solution) and a THERMOmax microplate reader (Molecular Devices, Sunnyvale, CA) was used to measure optical density at 340 nm at repeated intervals during 5 minutes. Purified G3PDH enzyme was used to generate a standard curve and calculate G3PDH activity in mU of purified enzyme. Proteins were quantified in duplicate by the BCA method in 2 separate wells and used to normalize for the amount of biological material. G3PDH activity was expressed as mU/μg protein.

Oil red O staining followed by spectrophotometric analysis was performed to measure lipid accumulation as previously described (Ramirez-Zacarias et al., 1992). Cells from 3 different wells of a 96-well plate were washed with PBS and fixed with formalin for 1h. An oil red O solution in isopropanol was added to the wells and incubated for 2h. After washing 3 times with dH_2O, oil red O retained by lipid droplets was eluted with isopropanol containing 4% Igepal CA-630. Optical density was measured at 490 nm.

Based on previous studies suggesting that progesterone may have insulin-like (Lacasa et al., 2001) or anti-glucocoticoid-like action (Bjorntorp, 1997; Pedersen et al., 2003; Xu et al., 1990), cultured SC or OM preadipocytes were differentiated by incubation with various concentrations of progesterone (10^{-5} to 10^{-8}M) or without progesterone in differentiation media containing the following insulin and dexamethasone concentrations: 1) 100nM insulin and 1μM dexamethasone; 2) 25nM insulin and 1μM dexamethasone; 3) 100nM insulin and 0.25μM dexamethasone; 4) 25nM insulin and 0.25μM dexamethasone. Ethanol alone was added in untreated controls. A PPARγ agonist was included in all four experimental conditions. After

differentiation, G3PDH enzymatic activity measurement and oil red O lipid staining were performed to assess the effect of progesterone on fat cell differentiation and lipid accumulation.

Enzymatic activities and metabolite formation. After incubation with ^{14}C-labelled progesterone for 24h, steroids were extracted twice with 1 volume ether as described previously (Blouin et al., 2003). The organic phases were pooled and evaporated to dryness. Steroids were solubilized in 50µl dichloromethane (reference standards were solubilized in 100% ethanol) and applied to Silica Gel 60 thin layer chromatogram (TLC) plates (Merk, Dàrmstadt, Germany). The separation was performed by migration in toluene-acetone (4:1). Unlabelled 20α-hydroxyprogesterone was used as a standard and was detected under ultraviolet light. Bidimensional thin layer chromatography was performed according to (Wiebe JP et al., 2000), and samples were applied to Silica Gel 60 TLC plates and separated by two successive migrations in chloroform-ether (10:3) and two migrations in hexane-ethyl acetate (5:2). The extracted sample was applied on the lower right hand corner of 20 × 20 cm silica gel 60 F254 aluminium sheet (Merck KGaA, Germany), and separated by two successive migrations in solvent 1 (chloroform:ether, 10:3, v/v) and then turned 90° to perform two successive migrations in solvent system 2 (hexane:ethyl acetate, 5:2, v/v). The migrations of cold steroid reference standards were also performed at same time to identify metabolites. Plates were analyzed using a Storm 860 PhosphoImager (Amersham Pharmacia Biotech Inc.) and the proportion of each metabolite was quantified using the ImageQuant software version 5.1 (Amersham Pharmacia Biotech Inc.).

Real-time PCR measurements of enzyme mRNA abundance. Total RNA was isolated from primary differentiated and non-differentiated cultures using Rneasy Lipid Tissue Mini Kit (Qiagen) following the manufacturer's recommendations. RNA quality was assessed with a bioanalyzer (Agilent Technologies) and RNA from 2 separate culture wells was pooled for real-time RT-PCR quantifications performed in duplicate. First strand cDNA synthesis was accomplished using 0.5-5 µg of the isolated RNA in a reaction containing 200 units of Superscript III Rnase H- reverse transcriptase (Invitrogen Life Technologies), 300 ng of oligo dT_{18}, 500 µM dNTP, 5 mM DTT and 40 units of Protector RNase inhibitor (Roche Diagnostics) in a final volume of 50 µL. Resulting cDNA was then treated with 1 µg of Rnase A for 30 min at 37°C and purified thereafter with Qiaquick PCR purification kits (Qiagen). For quantitative PCR analyses, a Light-Cycler PCR (Roche Diagnostics) was used to measure the mRNA abundance of AKR1C1 and

progesterone receptor (PR). The sets of primers were: 5'-CCTATA-GTG-CTC-TGG-GAT-CCC-AC-3', and 5'-AGG-ACC-ACA-ACC-CCA-CGC-TGT-3' (AKR1C1); and 5'-TGAGCT-TAA-TGG-TGT-TTG-GTC-TAG-GA-3', and 5'-TTC-TTT-CAT-CCG-CTG-TTC-ATT-TAG-TATT-3' (progesterone receptor). The FastStart DNA Master Plus SYBRGreen I kit (Roche Diagnostics) was used in a final reaction volume of 20 μL containing 3 mM $MgCl_2$, 20 ng of each primer and 20-200 ng of the cDNA template. The PCR was carried out according to the following conditions: 50 cycles of (95°C/10 sec, 59-66°C/5 sec, 72°C/11 sec and reading at 75°C/3 sec) and temperature transition was 3°C/sec for all reactions. PCR results were normalized according to subunit O of ATP synthase expression levels. This gene was found to display remarkably stable expression levels from embryonic life through adulthood in various tissues (Warrington et al., 2000). A universal standard curve was generated with ATPase from an amplification with perfect efficiency (i.e. efficiency coefficient E = 2.00) using cDNA amounts of 0, 10^2, 10^3, 10^4, 10^5, and 10^6 copies. The crossing points (Cp) to calculate the amount of copies in initial cDNA specimens were determined using the double derivative method (Luu-The et al., 2005). For each sample, the Cp value was divided by that of the housekeeping gene. In order to further minimize inter-assay variability, this Cp ratio was then multiplied by the average Cp generated for housekeeping gene amplifications of all samples examined in the present experiment. PCR data were expressed in normalized number of copies per μg total RNA.

Statistical analysis. Unpaired t-tests were used in comparisons of metabolite mean radioactivity intensities between cultured preadipocytes and adipocytes induced to differentiate, including: 1) 5α- pregnanedione, 2) unconverted progesterone, 3) 5α/β-pregnane-20α-ol-3-one and 5α-pregnane-3α/β-ol-20-one, 3) 20α-hydroxyprogesterone, 4) 5β-pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β, 20α-diol, 5) as well as total metabolites. Paired t tests were performed to compare the difference in the relative intensity of the above-mentioned metabolites as well as unconverted progesterone, between cultured preadipocytes and differentiated adipocytes. Analyses were performed using the JMP statistical software (SAS Institute, Cary, NC).

RESULTS

Figure 1A shows a thin-layer chromatography of steroid products in mature adipocytes and preadipocytes from SC and OM samples after incubation with radiolabelled progesterone for 24 hours. In both fat depots, we found that 20α-hydroxyprogesterone was the predominant conversion product of progesterone. These results are representative of experiments performed with mature adipocytes and isolated preadipocytes from several other patients. In time course experiments, 20α-reduction of progesterone was linear over 48h (not shown).

By using bidimensional thin layer chromatography, we performed a more detailed analysis of progesterone metabolites generated by intact primary SC and OM preadipocytes from women. Figure 2 shows a bidimensional thin layer chromatography of steroid products obtained after incubating SC and OM preadipocytes for 24 hours with radiolabelled progesterone. Metabolites generated included 20α-hydroxyprogesterone, 5α-pregnane-3α/β-ol-20-one, 5α- and 5β-pregnanedione, 5α- and 5β-pregnane-20α/β-ol-3-one, 5α-pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β-20α-diol. Progesterone was mainly converted to 20α-hydroxyprogesterone, whereas 5β-pregnane-3α/β-20α-diol was also generated after incubating with the dual inhibitor of 5α-reductase type 1 and 2 (17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA)) (Figure 2B). These results are representative of several other preadipocyte cultures. The postulated pathways of progesterone inactivation in preadipocytes are shown in Figure 3.

We found that the pattern of metabolites generated was altered during adipocyte differentiation (Figure 4). A total of 6 adipose tissue samples were examined (3 SC fat samples and 3 OM fat samples). At baseline, intact preadipocyte cultures generated a mixture of metabolites similar to that shown in Figure 2. However, in lipid-storing adipocytes after differentiation, 20α-hydroxyprogesterone formation was proportionally increased. Table 1 shows the quantification of relative intensities of several metabolites based on the radioactivity of the TLC. Proportions of 20α-hydroxyprogesterone, 5β-pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β, 20α-diol were significantly higher in differentiated adipocytes compared to preadipocytes. The proportion of total metabolite formation also increased in differentiated adipocytes. Unconverted progesterone, 5α/β-pregnane-20α-ol-3-one, 5α-pregnane-3α/β-ol-20-one, and 5α-pregnanedione were consistently lower in differentiated adipocytes compared to preadipocytes.

In a similar experiment, expression levels of AKR1C1 (20α-HSD) mRNA were obtained in preadipocytes (before differentiation) or post-differentiated adipose cells from SC (n=6) and OM (n=4) adipose tissue (Figure 5). We found that the expression level of AKR1C1 mRNA in differentiated adipocytes was significantly higher than in preadipocytes in the SC depot. We also found that the expression level of AKR1C1 mRNA was increased following adipocyte differentiation in OM compartments, although the trend failed to reach significance. PR expression was examined in whole SC and OM adipose tissue samples of one patient. We found that expression levels were very low. For this reason, PR expression was not investigated in isolated cells.

Using differentiation media with various concentrations of insulin and dexamethasone, cultured SC or OM preadipocytes were incubated with (10^{-5} to 10^{-8}M) or without progesterone. No consistent effect of progesterone was observed on biochemical measurements of fat cell differentiation including G3PDH enzymatic activity and oil red O measurements (data not shown).

DISCUSSION

Although the effects of progesterone on adipose tissue and adipocytes from humans (Belanger et al., 2002; Bjorntorp, 1997; Blanchette et al., 2005; Blouin et al., 2005) and animals (Lacasa et al., 2001; Mendes et al., 1985; Monjo et al., 2003; Rondinone et al., 1992; Shirling et al., 1981) have been extensively studied, this is the first report to take into account and document pathways of progesterone inactivation within adipose tissue. In the present study, using SC and OM adipose tissue from women, we examined the local metabolic pathways of progesterone in isolated mature adipocytes and cultured adipocytes. Incubations with radiolabelled progesterone for 24h were carried out to identify the various steroid products in isolated mature adipocytes, cultured preadipocytes and lipid-storing differentiated adipocytes. We found that preadipocytes generate a complex mixture of 5α/5β, 20α and 3α/β-reduced metabolites. However, overall metabolite formation increased in differentiated adipocytes, with 20α-hydroxyprogesterone as the main metabolite. These findings further validate the detection of 20α-HSD, 3α/β-HSD and 5α-reductase activity in preadipocytes, and support the notion of a complex regulation of steroid action through locally-expressed steroid-converting enzymes. On the other hand, progesterone had no consistent effect on fat cell differentiation in the present study, which is rather consistent with the patterns of progesterone observed.

In our previous work on abdominal adipose tissue obtained in women, we reported 20α-HSD mRNA and activity in SC and OM adipose tissue (Blanchette et al., 2005), and a positive correlation was observed between adiposity and activity of steroid aldo-keto reductases 1C, namely type 3 3α-HSD, type 5 17β-HSD and 20α-HSD (Blouin et al., 2005). Several lines of experimental data showed that progesterone may interact with glucocorticoid receptors (Pedersen et al., 1992), and that progesterone may generate anti-glucocorticoid effects in vitro (Schmidt et al., 1998; Xu et al., 1990). In ovariectomized (OVX) rats, increasing body lipid and protein content were restrained by estradiol replacement (Richard, 1986), whereas progesterone had no effect on energy intake and weight gain in this model (Richard, 1986; Wade and Gray, 1979). Conversely, progesterone increased adipocyte determination and differentiation 1/sterol regulatory element-binding protein 1c gene expression of rat preadipocytes in a dose-dependent manner (Lacasa et al., 2001), and it stimulated steroid-mediated differentiation of 3T3-L1 preadipocytes (Rondinone et al., 1992; Wiper-Bergeron et al., 2003). The receptor mediating these effects has not been clearly identified. Our preliminary experiments on PR expression in whole tissue are consistent with previous studies reporting low PR in adipose tissue, mature adipocytes or preadipocytes (Mayes and Watson, 2004). Low PR expression and significant 20α-HSD activity mediating the conversion of

progesterone into inactive 20α-hydroxyprogesterone may contribute to the inconsistent effect of progesterone observed on adipocyte differentiation.

Through the reduction by aldo-keto reductase 1C (AKR1C) enzymes and 5α/5β reductase activity, progesterone was converted into a complex mixture of metabolites in preadipocytes. These results are consistent with previous studies in other cell types (Quinkler et al., 1999; Zhang et al., 2000). The finding of a slight 5β-reductase activity in adipose cells is novel. Our finding of increased progesterone metabolism in differentiated cells suggests that the differentiation of preadipocytes into lipid-storing mature adipocytes has effects on the pattern of progesterone metabolism. The modulation of progesterone inactivation rates or the formation of some specific progesterone metabolites in preadipocytes vs. mature adipose cells may reflect cell differentiation-related changes in steroid-converting enzymes, particularly AKR1C1.

Hormonal steroids bind to specific receptors to exert their actions and the effect is determined by both the local concentration of the hormone and the receptor number (Vanderbilt et al., 1987; Webb et al., 1992). We have examined progesterone receptor (PR) expression in whole SC and OM adipose tissue samples and found that expression levels were very low. Wiebe reported that receptors of progesterone metabolites (such as 5α-pregnane-3,20-dione and 3α-hydroxy-4-pregnen-20-one) were observed on the cell membrane and involved in cell-signaling pathways related to protein kinase C, phospholipase C, or IP3-induced Ca^{2+} mobilization and Ca^{2+} channels. Their findings suggested a selective nongenomic mechanism (Wiebe, 1997; Wiebe, 2006). Progesterone may induce the acceleration of Xenopus oocyte maturation (Bayaa et al., 2000; Tian et al., 2000), stimulate acrosome reaction in sperm and modulate neurotransmitter and neural excitability (McEwen, 1991), and involve in the activation of the Src/Ras/MAPK pathway in breast cancer cells (Migliaccio et al., 1998). Whether such rapid, membrane-initiated effects of progesterone (Leonhardt et al., 2003) are present in adipocytes remains unclear at this time.

Non-conjugated metabolites of progesterone such as allopregnanolone (5α-pregnane-3α-ol-20-one) and pregnanolone (5β-pregnane-3α-ol-20-one) were generated in preadipocytes. These steroids are considered as neurosteroids and potent positive modulators of gamma-aminobutyric acid type A (GABAA) receptors (Lambert et al., 2001; Majewska et al., 1986). GABA type A receptor is regarded as the target of important compounds, such as anxiolytic benzodiazepines (BZs), barbiturates, neurosteroids, and certain volatile

anaesthetics (Davies, 2003; Lambert et al., 2003; Sieghart, 1995; Watanabe et al., 2002). Metabolites of progesterone as well as deoxycorticosterone (DOC) may actually represent endogenous ligands for this receptor (Darlison et al., 2005). Allopregnanolone effects on GABA receptors include mood alterations such as pubertal mood swings, prementstrual syndrome (PMS), postpartum blues, and perimenopause (Smith et al., 2007). Several studies on animals also reported that allopregnanolone inhibits learning and memory and increases appetite, as well as anxiety (Chen et al., 1996; Gulinello et al., 2001; Johansson et al., 2002). Several lines of investigation on GABAA receptor agonists including allopregnanolone reported that treatment with low dose of this compound generates loss of impulse control, negative mood, and aggression/irritability (Ferrari et al., 1997; Fish et al., 2001; Masia et al., 2000; Miczek et al., 2003). However, sedation, hypnosis, and anxiolysis, and antiepileptic effects are induced when treating with high doses (Herzog, 1991; Sundstrom et al., 1998; Wieland et al., 1991). In the present study, we found that the production of progesterone metabolites was significantly increased in differentiated adipocytes, and a trend for increased allopregnanolone production was found in differentiated adipocytes. It remains to be clearly established whether adipose tissue-derived progesterone metabolites play a role in modulating central nervous system responses. At the local level, adipose tissue has been shown to have benzodiazepine binding sites, and locally produced allopregnanolone may be a ligand for these receptors (Gonzalez Solveyra et al., 1988).

It should be noted that medication used by the patients was not considered in the statistical analysis. Given the relative consistency of our observations from patient to patient, we suggest that a confounding effect of a specific drug is unlikely. Future studies could investigate whether effects are generated by exogenous factors on progesterone metabolism in adipocytes.

In conclusion, the differentiation of preadipocytes into mature adipocytes affects the pattern of progesterone metabolism. Preadipocytes generate a complex mixture of 5α/5β, 20α and 3α/β-reduced metabolites. Metabolite formation increased in mature adipocytes, and 20α-hydroxy-progesterone was the main metabolite. The efficient conversion of progesterone to inactive metabolites in adipose tissue and the low expression of progesterone receptor are concordant with the inconsistent effect of progesterone on fat cell differentiation in humans. The modulation of progesterone inactivation or the formation of some specific progesterone metabolites in preadipocytes vs. mature adipose cells may reflect cell differentiation-related changes in steroid-converting enzymes and differential sensitivity to progesterone action.

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REFERENCES

Anderson LA, McTernan PG, Barnett AH, Kumar S, 2001. The effects of androgens and estrogens on preadipocyte proliferation in human adipose tissue: influence of gender and site. J Clin Endocrinol Metab. 86, 5045-5051.

Bayaa M, Booth RA, Sheng X, Liu XJ, 2000. The classical progesterone receptor mediates Xenopus oocyte maturation through a nongenomic mechansim. Proc.Natl.Acad.Sci.U.S.A. 97, 12607-12612.

Belanger C, Luu-The V, Dupont P, Tchernof A, 2002. Adipose tissue intracrinology: potential importance of local androgen/estrogen metabolism in the regulation of adiposity. Horm Metab Res. 34, 737-745.

Bjorntorp P, 1997. Hormonal control of regional fat distribution. Hum Reprod 12, 21-25.

Blanchette S, Blouin K, Richard C, Dupont P, Luu-The V, Tchernof A, 2005. Expression and activity of 20alpha-hydroxysteroid dehydrogenase (AKR1C1) in abdominal subcutaneous and omental adipose tissue in women. J Clin Endocrinol Metab. 90, 264-270.

Blouin K, Blanchette S, Richard C, Dupont P, Luu-The V, Tchernof A, 2005. Expression and activity of steroid aldoketoreductases 1C in omental adipose tissue are positive correlates of adiposity in women. Am J Physiol Endocrinol Metab. 288, E398-404.

Blouin K, Richard C, Belanger C, Dupont P, Daris M, Laberge P, Luu-The V, Tchernof A, 2003. Local androgen inactivation in abdominal visceral adipose tissue. J Clin Endocrinol Metab 88, 5944-5950.

Chen SW, Rodriguez L, Davies MF, Loew GH, 1996. The hyperphagic effect of 3 alpha-hydroxylated pregnane steroids in male rats. Pharmacol Biochem Behav 53, 777-782.

Darlison MG, Pahal I, Thode C, 2005. Consequences of the evolution of the GABA(A) receptor gene family. Cell Mol Neurobiol 25, 607-624.

Davies M, 2003. The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. J Psychiatry Neurosci 28, 263-274.

Ferrari PF, Parmigiani S, Rodgers RJ, Palanza P, 1997. Differential effects of chlordiazepoxide on aggressive behavior in male mice: the influence of social factors. Psychopharmacology (Berl) 134, 258-265.

Fish EW, Faccidomo S, DeBold JF, Miczek KA, 2001. Alcohol, allopregnanolone and aggression in mice. Psychopharmacology (Berl). 153, 473-483.

Gonzalez Solveyra C, Romeo HE, Rosenstein RE, Estevez AG, Gardinali DP, 1988. Benzodiazepine binding sites in rat interscapular brown adipose tissue: effect of cold environment, denervation and endocrine ablations. Life Sci 42, 393-402.

Gulinello M, Gong QH, Li X, Smith SS, 2001. Short-term exposure to a neuroactive steroid increases alpha4 GABA(A) receptor subunit levels in association with increased anxiety in the female rat. Brain Res 910, 55-66.

H Shimade, K Miura, Y Imamura, 2006. Characteristics and inhibition by flavonoids of 20α-hydroxysteroid dehydrogenase activity in mouse tissues. Life Sci 78, 2931-2936.
Hamosh M, Hamosh P, 1975. The effect of estrogen on the lipoprotein lipase activity of rat adipose tissue. J Clin Invest. 55, 1132-1135.

Hauner H, Skurk T, Wabitsch M, 2001. Cultures of human adipose precursor cells. Methods Mol Biol 155, 239-247.

Herzog AG, 1991. Reproductive endocrine considerations and hormonal therapy for women with epilepsy. Epilepsia 32, S27-S33.

Imamura Y, Ohtaguro M, Shimada H, 2007. Several distinct enzymes catalyze 20alpha-hydroxysteroid dehydrogenase activity in mouse liver and kidney. J Steroid Biochem Mol Biol. 107, 120-126.

Johansson IM, Birzniece V, Lindblad C, Olsson T, Backstrom T, 2002. Allopregnanolone inhibits learning in the Morris water maze. Brain Res 934, 125-131.

Lacasa D, Le Liepvre X, Ferre P, Dugail I, 2001. Progesterone stimulates adipocyte determination and differentiation 1/sterol regulatory element-binding protein 1c gene expression. J Biol Chem. 276, 11512-11516.

Lambert JJ, Belelli D, Peden DR, Vardy AW, Peters JA, 2003. Neurosteroid modulation of GABAA receptors. Prog Neurobiol 71, 67-80.

Lambert JJ, Harney SC, Belelli D, Peters JA, 2001. Neurosteroid modulation of recombinant and synaptic GABAA receptors. Int Rev Neurobiol 46, 177-205.

Leonhardt SA, Boonyaratanakornkit V, Edwards DP, 2003. Progesterone receptor transcription and non-transcription signaling mechanisms. Steroids 68, 761-770.

Luu-The V, Paquet N, Calvo E, Cumps J, 2005. Improved real-time RT-PCR method for high-throughput measurements using second derivative calculation and double correction. Biotechniques 38, 287-293.

M Mori, W.G.Wiest, 1979. Purification of rat ovary 20α-hydroxysteroid dehydrogenase by affinity chromatography. J Steroid Biochem 11, 1443-1449.

Majewska MD, Harrison NL, Schwartz RD, Barker JL, Paul SM, 1986. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232, 1004-1007.

Masia SL, Perrine K, Westbrook L, Alper K, Devinsky O, 2000. Emotional outbursts and post-traumatic stress disorder during intracarotid amobarbital procedure. Neurology 54, 1691-1693.

Mayes, J.S., Watson, G.H., 2004. Direct effects of sex steroid hormones on adipose tissues and obesity. Obes Res , 197-216.

McEwen BS, 1991. Non-genmomic and genomic effects of steroids on neural activity. TIPS Rev 12, 141-147.

Mendes AM, Madon RJ, Flint DJ, 1985. Effects of cortisol and progesterone on insulin binding and lipogenesis in adipocytes from normal and diabetic rats. J Endocrinol 106, 225-231.

Miczek KA, Fish EW, Ed Bold JF, 2003. Neurosteroids, GABAA receptors, and escalated aggressive behavior. Horm Behav 44, 242-257.

Migliaccio A, Piccolo D, Castoria G, Di Domenico M, Bilancio A, Lombardi M, Gong W, Beato M, Auricchio F, 1998. Activation of the Src/p21ras/Erk pathway by progesterone receptor via cross-talk with estrogen receptor. EMBO J 17, 2008-2018.

Monjo M, Rodriguez AM, Palou A, Roca P, 2003. Direct effects of testosterone, 17 beta-estradiol, and progesterone on adrenergic regulation in cultured brown adipocytes: potential mechanism for gender-dependent thermogenesis. Endocrinology. 144, 4923-4930.

Pedersen SB, Borglum JD, Moller-Pedersen T, Richelsen B, 1992. Characterization of nuclear corticosteroid receptors in rat adipocytes. Regional variations and modulatory effects of hormones. Biochim Biophys Acta 1134, 303-308.

Pedersen SB, Kristensen K, Richelsen B, 2003. Anti-glucocorticoid effects of progesterone in vivo on rat adipose tissue metabolism. Steroids 68, 543-550.

Penning TM, 1997. Molecular endocrinology of hydroxysteroid dehydrogenases. Endocr Rev 18, 281-305.

Penning TM, Burczynski ME, Jez JM, Hung CF, Lin HK, Ma H, Moore M, Palackal N, Ratnam K, 2000. Human 3alpha-hydroxysteroid dehydrogenase isoforms (AKR1C1-AKR1C4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. Biochem J 351, 67-77.

Price TM, O'Brien SN, Welter BH, George R, Anandjiwala J, Kilgore M, 1998. Estrogen regulation of adipose tissue lipoprotein lipase--possible mechanism of body fat distribution. Am J Obstet Gynecol 178, 101-107.

Quinkler M, Diederich S, Bahr V, Oelkers W, 2004. The role of progesterone metabolism and androgen synthesis in renal blood pressure regulation. Horm Metab Res 36, 381-386.

Quinkler, M., Johanssen, S., Grossmann, C., Bahr, V., Muller, M., Oelkers, W., Diederich, S., 1999. Progesterone metabolism in the human kidney and inhibition of 11beta-hydroxysteroid dehydrogenase type 2 by progesterone and its metabolites. J Clin Endocrinol Metab 84, 4165-4171.

R.B.Wilcox, W.G.Wiest, 1966. Further studies of rat ovarian 20α-hydroxysteroid dehydrogenase. Steroids 7, 395-413.

Ramirez-Zacarias JL, Castro-Munozledo F, Kuri-Harcuch W, 1992. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. Histochemistry 97, 493-497.

Richard D, 1986. Effects of ovarian hormones on energy balance and brown adipose tissue thermogenesis. Am J Physiol. 250, R245-R249.

Rizner TL, Smuc T, Rupreht R, Sinkovec J, Penning TM, 2006. AKR1C1 and AKR1C3 may determine progesterone and estrogen ratios in endometrial cancer. Mol Cell Endocrinol 248, 126-135.

Rodbell M, 1964. Metabolism of isolated fat cells. J Biol Chem. 239, 375-380.

Roncari DA, Van RL, 1978. Promotion of human adipocyte precursor replication by 17beta-estradiol in culture. J Clin Invest. 62, 503-508.

Rondinone CM, Baker ME, Rodbard D, 1992. Progestins stimulate the differentiation of 3T3-L1 preadipocytes. J Steroid Biochem Mol Biol. 42, 795-802.

Schmidt M, Renner C, Loffler G, 1998. Progesterone inhibits glucocorticoid-dependent aromatase induction in human adipose fibroblasts. J Endocrinol 158, 401-407.

Shirling D, Ashby JP, Baird JD, 1981. Effect of progesterone on lipid metabolism in the intact rat. J Endocrinol 90, 285-294.

Sieghart W, 1995. Structure and pharmacology of gamma-aminobutyric acidA receptor subtypes. Pharmacol Rev 47, 181-234.

Smith SS, Shen H, Gong QH, Zhou X, 2007. Neurosteroid regulation of GABA(A) receptors: Focus on the alpha4 and delta subunits. Pharmacol Ther 21

Sottile V, Seuwen K, 2001. A high-capacity screen for adipogenic differentiation. Anal Biochem 293, 124-128.

Sundstrom I, Andersson A, Nyberg S, Ashbrook D, Purdy RH, Backstrom T, 1998. Patients with premenstrual syndrome have a different sensitivity to a neuroactive steroid during the menstrual cycle compared to control subjects. Neuroendocrinology 67, 126-138.

Tian J, Kim S, Heilig E, Ruderman JV, 2000. Identification of XPR-1, a progesterone receptor required for Xenopus oocyte activation. Proc Natl Acad Sci U S A 97, 14358-14363.

TM Penning, 1997. Molecular endocrinology of hydroxysteroid dehydrogenases. Endocr Rev 18, 281-305.

Vanderbilt JN, Miesfeld R, Maker BA, Yamamoto KR, 1987. Intercellular receptor concentration limits gluco-corticoid-dependent enhancer activity. Molecular Biology 1, 68-74.

W.G.Wiest, R.B.Wilcox, 1961. Purification and properties of rat ovarian 20α-hydroxysteroid dehydrogenase. J Biol Chem 239, 2425-2428.

Wade GN, Gray JM, 1979. Gonadal effects on food intake and adiposity: a metabolic hypothesis. Physiol Behav 22, 583-593.

Warrington JA, Nair A, Mahadevappa M, Tsyganskaya M, 2000. Comparison of human adult and fetal expression and identification of 535 housekeeping/maintenance genes. Physiol Genomics 2, 143-147.

Watanabe M, Maemura K, Kanbara K, Tamayama T, hayasaki H, 2002. GABA and GABA receptors in the central nervous system and other organs. Int Rev Cytol 213, 1-47.

Webb P, Lopez GN, Greene GL, Baxter JD, Kushner PJ, 1992. The limits of the cellular capacity to mediate an estrogen response. Molecular Endocrinology 6, 157-167.

Weinstein Y, 1977. 20alpha-hydroxysteroid dehydrogenase: a T lymphocyte-associated enzyme. J Immunol. 119, 1223-1229.

Wiebe JP, 1997. Nongenomic actions of steroids on gonadotropin release. Recent Prog Horm Res 52, 71-99.

Wiebe JP, 2006. Progesterone metabolites in breast cancer. Endocr Relat Cancer 13, 717-738.

Wiebe JP, Muzia D, Hu J, Szwajcer D, Hill SA, Seachrist JL, 2000. The 4-pregnene and 5alpha-pregnane progesterone metabolites formed in nontumorous and tumorous breast tissue have opposite effects on breast cell proliferation and adhesion. Cancer Res 60, 936-943.

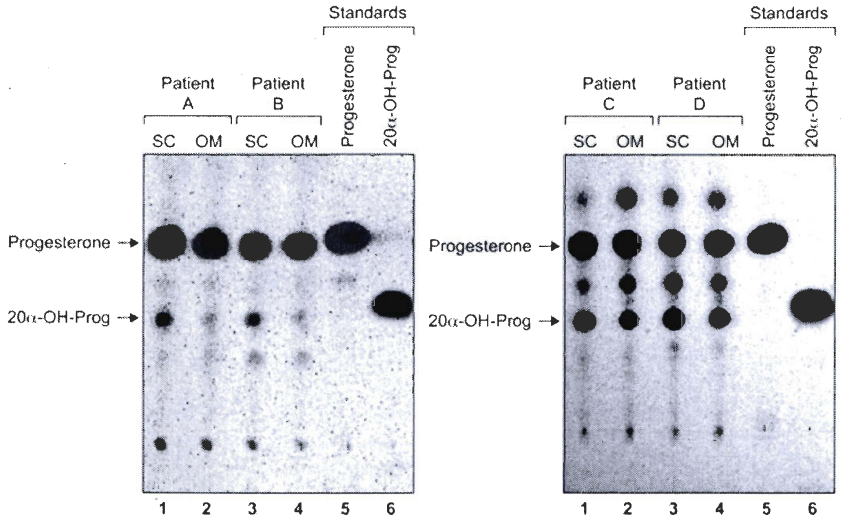
Wieland S, Lan NC, Mirasedeghi S, Gee KW, 1991. Anxiolytic activity of the progesterone metabolite 5 alpha-pregnan-3 alpha-o1-20-one. Brain Res 565, 263-268.

Wiper-Bergeron N, Wu D, Pope L, Schild-Poulter C, Hache RJ, 2003. Stimulation of preadipocyte differentiation by steroid through targeting of an HDAC1 complex. EMBO J. 22, 2135-2145.

Xu XF, Hoebeke J, Bjorntorp P, 1990. Progestin binds to the glucocorticoid receptor and mediates antiglucocorticoid effect in rat adipose precursor cells. J Steroid Biochem 36, 465-471.

Zhang Y, Dufort I, Rheault P, Luu-The V, 2000. Characterization of a human 20alpha-hydroxysteroid dehydrogenase. J Mol Endocrinol 25, 221-228.

A



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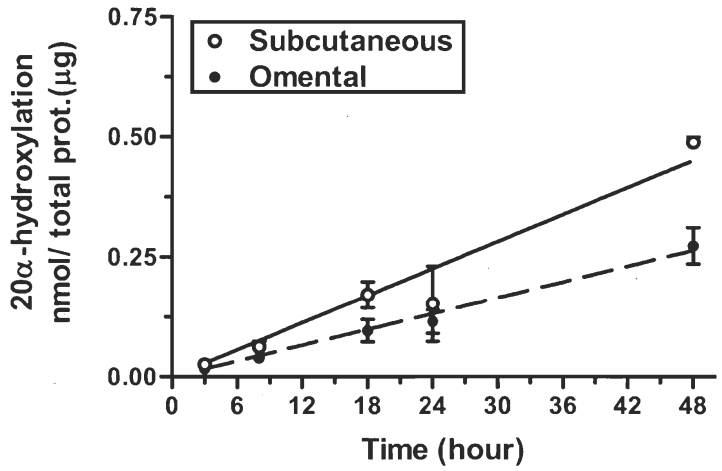
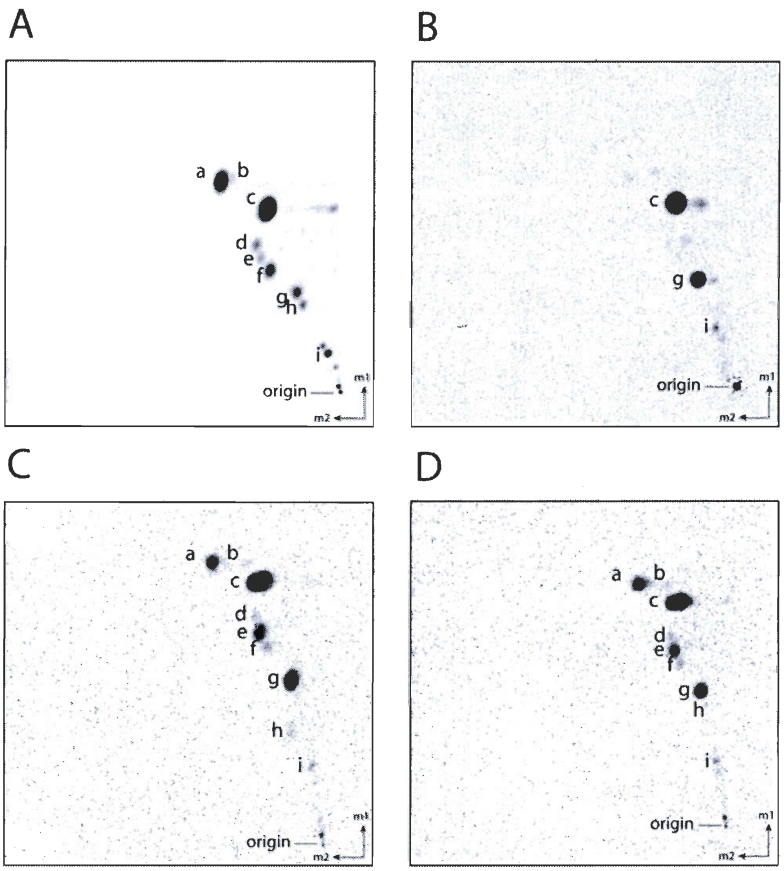


Figure 1: A) Thin layer chromatograms showing steroid products obtained when incubating SC or OM isolated mature adipocytes from obese women with radiolabelled ^{3}H-progesterone (left panel), and incubating SC or OM preadipocytes from obese women with radiolabelled ^{14}C-progesterone (right panel). The chromatograms were photographed under ultraviolet light for the identification of the 20α-hydroxyprogesterone standard (20α-OH-Prog) and this image was superimposed to the autoradiogram by image analysis. B) 20α-reduced metabolite production in subcutaneous (SC) and omental (OM) preadipocyte primary cultures at different time points. Preadipocytes from 2 subcutaneous and 3 omental samples were treated with ^{14}C-labeled progesterone (0.44µM) for the various times indicated. Data presented as mean ± SEM.



Legend :

a. **5α-pregnanedione**
b. 5β-pregnanedione
c. **progesterone**
d. **5α-pregnane-3α/β-ol-20-one**
e. 5α-pregnane-20α-ol-3-one
f. 5β-pregnane-20α-ol-3-one
g. **20α-hydroxyprogesterone**
h. 5β-pregnane-3α/β-ol-20-one
i. 5β-pregnane-3α/β, 20α-diol

m1: migration 1 m2: migration 2

Figure 2: Bidimensional thin layer chromatogram showing progesterone metabolites obtained when incubating SC preadipocytes with radiolabelled ^{14}C-progesterone (A), or with radiolabelled ^{14}C-progesterone and the dual inhibitor of 5α-reductase type 1 and 2 (17β-N,N-diethylcarbamoyl-4-methyl-4-aza-5α-androstan-3-one (4-MA)) (B), and when incubating SC (C) or OM (D) preadipocytes from obese subjects.

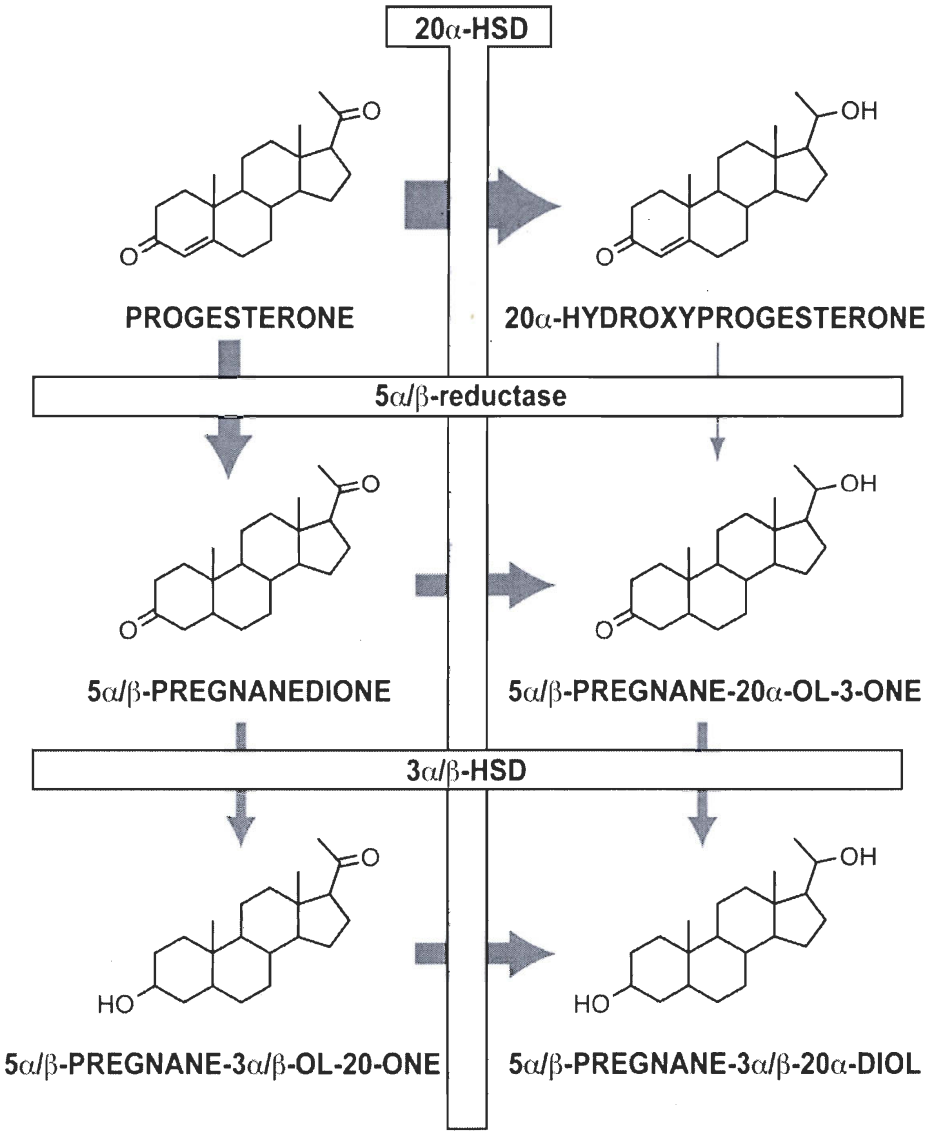


Figure 3: Postulated pathways of progesterone metabolism in human preadipocytes. (HSD: hydroxysteroid dehydrogenase).

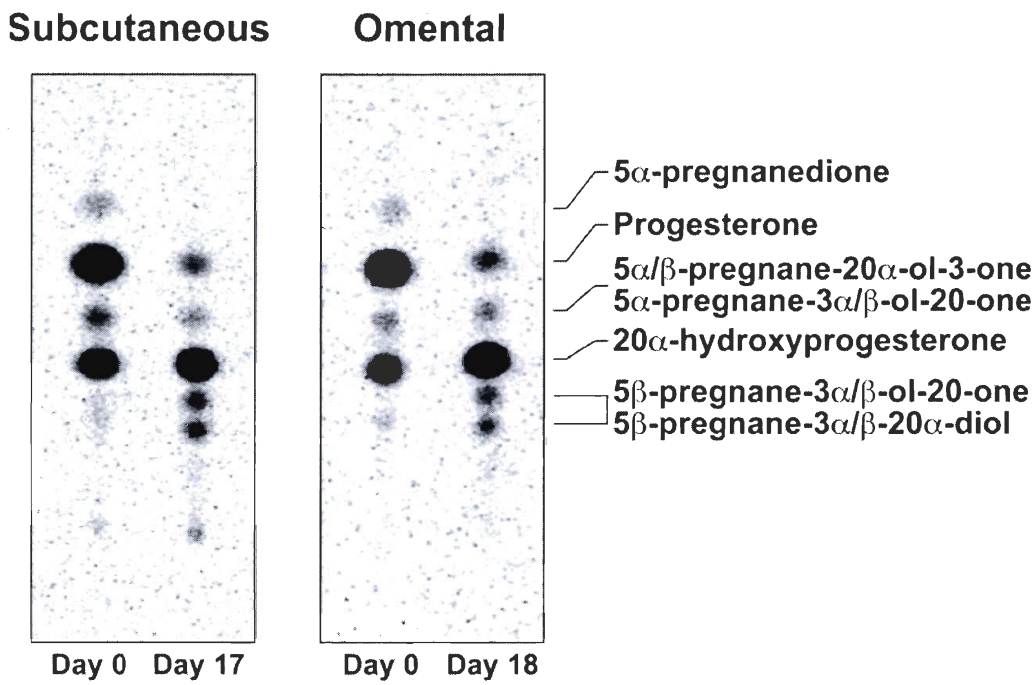


Figure 4. Thin layer chromatograms showing steroid products obtained when incubating preadipocytes before and after hormone-induced fat cell differentiation.

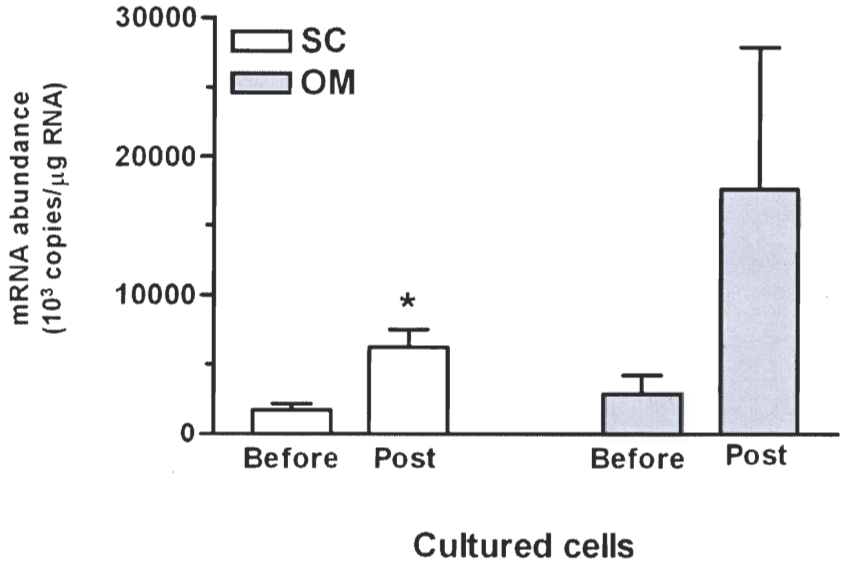


Figure 5. Expression levels of AKR1C1 (20α-HSD) mRNA in preadipocytes (before differentiation) or post-differentiated adipose cells from subcutaneous (SC) (n=6) and omental (OM) (n=4) adipose tissue. Data presented as mean ± SEM. * $p < 0.05$, post-differentiated fat cells vs. preadipocytes (before differentiation) cultured fat cells. In SC samples, average G3PDH activity reached after differentiation was 64.03 ± 45.8 mU/µg protein, and average lipid staining increased by 216.1 ± 85.9% in differentiated adipocytes vs. preadipocytes. In OM samples, average G3PDH activity reached after differentiation was 1.45 ± 0.84 mU/µg protein, and average lipid staining increased by 143.1 ± 30.3% in differentiated adipocytes vs. preadipocytes.

Table 1. Progesterone metabolites generated by preadipocytes and mature adipocytes from two OM fat samples and four SC fat samples incubated with radiolabelled progesterone for 24 hours.

Metabolites	Preadipocytes (n=6)	Differentiated adipocytes (n=6)	Significance
5α-pregnanedione	6.59 ± 4.3	2.24 ± 2.4	NS ($P = 0.1$)
Progesterone	68.3 ± 4.0	10.01 ± 4.6	$P < 0.0001$
5α/β-pregnane-20α-ol-3-one and 5α-pregnane-3α/β-ol-20-one	5.62 ± 2.7	3.93 ± 1.6	NS ($P = 0.14$)
20α-hydroxyprogesterone	16.2 ± 9.3	72.72 ± 9.9	$P = 0.0001$
5β-pregnane-3α/β-ol-20-one and 5β-pregnane-3α/β, 20α-diol	2.80 ± 1.7	9.2 ± 2.0	$P = 0.0007$
Total metabolites	31.2 ± 4.0	88.1 ± 5.5	$P < 0.0001$

Values are presented in percentage of total radioactivity. Samples from the OM and SC depots were pooled since no depot difference was found. Average G3PDH activity reached after differentiation was 0.19 ± 0.14 mU/μg protein, and average lipid staining increased by 195.7 ± 71.8% in differentiated adipocytes vs. preadipocytes. Mean ± SD are shown.
NS: not significant.

Part D: Abdominal adipose tissue metabolism and gene expression in ovariectomized and control cynomolgus monkeys fed an identical hypocaloric regimen

Abdominal adipose tissue metabolism and gene expression in ovariectomized and control cynomolgus monkeys fed an identical hypocaloric regimen

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ABSTRACT

We investigated the acute impact of ovariectomy on abdominal adipose tissue metabolism and gene expression in female cynomolgus monkeys. Fourteen animals were divided into intact (INT; n=7) and ovariectomized (OVX; n=7) groups. All monkeys were strictly fed an identical hypocaloric diet. Abdominal subcutaneous (SC) and mesenteric (ME) fat samples were collected seven days after the beginning of menses in the INT group and 14 days after ovariectomy in the OVX group. Lipoprotein lipase (LPL) activity and lipolysis were measured using standard procedures. Gene expression profiles of pooled ME adipose tissue samples from both groups were measured using Affymetrix HG-U133A microarrays. Significant and similar reductions in body weight were observed in both groups with similar pattern by the end of study. Adipocyte cell size of ME and SC fat was not significantly different in OVX vs. INT monkeys. ME fat LPL activity was significantly lower ($p<0.05$) in the OVX group vs. INT animals. ME basal lipolytic activity was significantly higher in OVX than in INT monkeys. Several genes related to adipose tissue metabolism and lipid synthesis were down-regulated in ME fat of OVX monkeys, including acyl-CoA synthetase long-chain family member 1, stearoyl-CoA desaturase (delta-9-desaturase) and fatty acid synthase. Our findings suggest that ovariectomy is not acutely associated with increased abdominal adipose tissue lipid storage and adipogenesis in cynomolgus monkeys fed an identical weight loss-inducing diet.

Keywords: Ovariectomy; Cynomolgus monkeys; Adipose tissue; Gene expression

INTRODUCTION

Ovariectomy and menopause have been shown to increase food intake and modulate abdominal adipose tissue metabolism in both rodents and humans. Menopause is also known to be associated with increased central body fat accumulation in women [1,2]. Several studies have reported that ovariectomized (OVX) female rodents become obese through increased daily food intake [3,4]. Peripheral fuel partitioning [5] has also been shown to be affected by OVX in rats. The impact of ovarian hormones on fuel partitioning was shown to be independent of food intake in pair-feeding studies [6].

Excessive intra-abdominal or visceral accumulation of fat on anatomical structures such as the greater omentum and mesentery has been recognized as an independent predictor of cardiometabolic risk in humans [7]. In fact, accumulation of visceral fat is not only thought to contribute to the insulin-resistant state of abdominally obese individuals, but may also be a marker of defective fat partitioning between the adipose tissue, skeletal muscle, liver and heart [7]. In this context, functional characteristics of visceral adipocytes, such as LPL activity, the release of free fatty acid (FFA) through lipolysis, the secretion of proinflammatory adipokines and lower adiponectin release are viewed as critical [8,9].

Old world primates like cynomolgus monkeys are largely similar to humans in terms of metabolism, endocrine and reproductive function and have a menstrual cycle of 28-days [10]. OVX female cynomolgus monkeys are good models for many post-menopausal conditions in women, and studies have shown that OVX generates a much increased risk for atherosclerotic lesions and cardiovascular disease (CVD) in this model [6,11]. In the present study, we have studied the acute impact of OVX on abdominal adipose tissue metabolism and gene expression in female cynomolgus monkeys (Macaca Fascicularis) under a strict feeding regimen. We hypothesized that OVX would be related to adipocyte measures and gene expression profiles which are indicative of the expected increase in visceral fat accumulation.

METHODS

Experimental design and collection of samples. Female cynomolgus monkeys (Macaca Fascicularis) were selected from the Molecular Endocrinology and Oncology Research Center monkey colony. The age range of the monkeys was 4-10 years old while body weight varied between 3.0 and 6.0 kg. Animals were healthy as established by a complete veterinary examination, complete blood count and serum biochemistry. Fourteen monkeys were randomly divided in 2 groups of 7 animals each. Group 1 remained intact (INT), whereas group 2 underwent bilateral OVX on study day -1 to eliminate steroids of gonadal origin. The body weights were measured at the beginning of the experiment and on the date of necropsy in INT and OVX groups of female cynomolgus monkeys.

Feeding regimen. All animals were fed an identical regimen. Throughout the study period, the animals were strictly fed eight cookies per day divided in two separate meals. Animals were fed one fruit and one vegetable twice a week (same fruit and same vegetable for all animals on a given day). The regimen was hypocaloric based on daily caloric requirements of 75-120 kcal for these animals and on the significant weight loss observed both in the INT and OVX groups (Figure 1). The fact that the weight loss pattern was similar suggests that it was not related to the surgical procedure in OVX animals.

Fat sampling and measurement of adipocyte cell size. Abdominal subcutaneous (SC) and mesenteric (ME) fat samples were collected at necropsy seven days after the beginning of the menses in the INT group and 14 days after surgery in the OVX group. Freshly isolated adipocytes were prepared by collagenase digestion [12]. Mean cell diameters were determined from digitalized images through the measurement of 250 cells for each sample using the Scion Image Software (Scion Corporation, Frederick, MA, USA).

Lipolytic activity and lipoprotein lipase (LPL) activity. Lipolysis experiments were performed by incubating isolated cell suspensions for 2h at 37°C. Basal, isoproterenol- (10^{-5} to 10^{-10}M) and forskolin (10^{-5}M) -stimulated lipolysis were measured [13]. The glycerol content of the medium was analyzed by bioluminescence using a reduced nicotinamide adenine dinucleotide-linked bacterial luciferase assay and was used as an index of lipolysis [14]. Data were expressed as fold change over basal lipolysis.

LPL activity was measured in heparin eluates recovered from thawed tissue samples and incubated with excess unlabeled and ^{14}C-labeled triolein in a Tris-albumin buffer emulsified by ultrasound. Excess apo-CII from porcine plasma was used to stimulate LPL activity. The resulting free fatty acids released were isolated by the Belfage extraction procedure and measured by liquid scintillation counting [13].

RNA extraction and microarray data analysis. Total RNA was isolated by Trizol (Invitrogen) and 20μg of total RNA was *in vitro* transcribed to produce a biotinylated cRNA target using the labeling protocol as recommended by Affymetrix. Four Affymetrix HG-U133A microarrays containing about 14,500 genes for analysis of over 22,500 probesets per chip were used to assess the expression profile of pooled mesenteric adipose tissue samples from INT (two replicates) and OVX (two replicates) animals. Expression levels were analyzed in the R environment for statistical computing using the Limma package (BioConductor Project) and GeneSprint v7.2 software (Agilent). The background subtraction and normalization of probeset intensities was performed using the method of Robust Multiarray Analysis (RMA). To identify differentially-expressed genes, gene expression intensities were compared using a moderated t-test and by using empirical Bayers methods followed by a false discovery rate (FDR) filtering. Annotations of probe sets were collected from different available databases using the NetAffx online tool (http://www.affymetrix.com/analysis/index.affx), and their identity was verified using the BLAST tool (http://www.ncbi.nlm.nih.gov). Cellular pathways related to these transcripts were identified using the Kegg database (http://www.genome.ad.jp/kegg/) and genecards (http://www.genecards.org/). Nonhuman primates could not be examined with whole genome species-specific microarrays since they did not yet exist when the present experiments were conducted. However, because of the high degree of sequence similarity between human and simian genomic DNA, previous investigators [15] suggested that human genome sequence-based DNA microarrays may be used effectively to study gene expression in nonhuman primates.

Statistical analyses. Unpaired t tests were used to compare fat cell size, lypolysis and LPL activity in SC and ME adipose tissue of INT and OVX monkeys. Paired t tests were used to examine regional differences in measures of adipocyte metabolism between SC and ME fat in the entire sample of monkeys. Statistical tests were performed on $\log_{10}$-transformed or Box-Cox-transformed data when the variables were not normally distributed as tested using the Shapiro-Wilk W test.

RESULTS

As shown in Figure 1, average body weight losses over the course of the study were -5% in INT animals ($p<0.005$) and -6% in OVX monkeys ($p<0.0001$).

Average adipocyte size of ME (Figure 2A) and SC (Figure 2B) adipose tissue samples obtained at necropsy were compared in INT vs. OVX female cynomolgus monkeys. Average cell size of ME adipose tissue was not different in OVX (n=7) compared with INT (n=6) animals (102 ± 8 μm vs. 101 ± 11 μm, p=0.95). Moreover, no significant difference was found in the SC adipocyte size in the OVX (n=4) vs. INT (n=6) group (119 ± 17 μm vs. 112 ± 13 μm, p=0.75). ME and SC fat LPL activities were significantly lower (p=0.05 for both ME and SC adipose tissue) in OVX animals than in INT animals (Figure 2C and D).

No significant difference was found in adipocyte size (p=0.14) and LPL activity (p=0.20) when comparing the ME vs. SC compartments by paired t-test in the INT group. In OVX animals, a significantly higher adipocyte size was found in SC vs. ME fat samples (p=0.03), but no significant difference was observed in LPL activities (p=0.32) between the ME and SC fat samples of this group. When combining both groups, a significantly higher adipocyte size (p=0.009) was observed in SC vs. ME fat samples. No significant difference, however, was found in LPL activity.

Adipocyte lipolytic capacities of ME and SC fat samples in INT and OVX female cynomolgus monkeys are illustrated in Figure 3. Basal lipolytic rates were not different in SC adipocytes from OVX vs. INT animals. ME basal lipolysis was significantly higher in OVX than in INT monkeys. With isoproterenol stimulation, although trends were observed for increased lipolytic responsiveness in adipocytes from both ME and SC fat in OVX vs. INT animals, only forskolin-stimulated lipolysis in ME adipocytes of OVX vs. INT monkeys was significantly higher.

In the transcriptome comparison of whole ME adipose tissue samples in OVX vs. INT animals, 276 significant-differentially expressed probe sets ($p<0.05$) corresponding to 234 different genes were identified (Appended Table 1). Of these transcripts, 93 probe sets corresponding to 81 different genes were down-regulated and 183 probe sets corresponding to 153 different genes were up-regulated in ME fat of OVX female cynomolgus monkeys. According to Gene Ontology, www.geneontology.org, these

significantly modulated genes were found in the categories of binding, catalytic activity, signal transducer activity, structural molecule activity, enzyme regulator activity, transporter activity, transcription regulator activity (Figure 4). Genes that were down-regulated included, among others, the leptin receptor (LEPR), CCAAT/enhancer binding protein (C/EBP) alpha (CEBPA), insulin-like growth factor 1 (IGF1), insulin-induced gene 1 (INSIG1), lipin 1 (LPIN1), and peroxisome proliferator-activated receptor gamma (PPARG). Among these genes, we found several pathways with two or more than two genes differentially expressed (Table 1).

Genes involved in focal adhesion, cytokine-cytokine receptor interaction, regulation of actin cytoskeleton, hematopoietic cell lineage, MAPK signalling pathway and B/T cell receptor signalling pathway were up-regulated. However, genes related to carbon fixation, tyrosine metabolism, pentose phosphate pathway and cysteine metabolism were down-regulated. On the other hand, twenty-four probe sets corresponding to seventeen genes that were considered as adipocyte markers were modulated (Table 2). Of these genes, several were related to adipose tissue metabolism and were down-regulated: acyl-CoA synthetase long-chain family member 1, stearoyl-CoA desaturase (delta-9-desaturase) and fatty acid synthase were found to be down-regulated in ME adipose tissue samples of OVX monkeys.

DISCUSSION

In the present study, we explored adipose tissue metabolism and gene expression in female INT and OVX cynomolgus monkeys. We tested the hypothesis that ovariectomy would lead to adipocyte metabolism measures and expression profiles indicative of increased visceral fat accumulation. However, contrary to our hypothesis, adipose tissue measures showed increased lipolysis and reduced LPL activity in OVX animals Isoproterenol- or forskolin-stimulated lipolysis seemed to be increased in both ME and SC adipocytes from the OVX compared to INT group. These differences were observed despite the fact that no difference was observed on adipocyte diameters of ME and SC fat samples in INT compared to OVX monkeys. In addition, several genes related to adipose tissue metabolism and lipid synthesis were found to be expressed at lower levels in OVX vs. INT animals. These findings are contrary to the expected effects of OVX on fat accumulation. We suggest that the negative energy imbalance due to the hypocaloric regimen prevented the postulated effects of OVX on abdominal fat accumulation in our female cynomolgus monkeys. This is the first study to examine the acute effect of ovariectomy on mesenteric adipose tissue metabolism and gene expression in a primate model.

The effects of menopause or OVX on adipose tissue have been examined in other models. In women, increasing abdominal adiposity at menopause has been observed and attributed to diminished estrogen secretion [16-19]. Evidence that menopause contributes to a preferential increase in abdominal adiposity was provided by cross-sectional [20] and longitudinal studies [1]. A series of investigations indicated that estrogen replacement or cyclic combined estrogen-progesterone therapy could suppress abdominal fat gain due to menopause [21-27]. D'Eon et al [5] suggested that estrogens appear to generate pronounced protective effects on energy homeostasis and lipid metabolism.

As mentioned we found lower ME and SC adipose tissue LPL activity in OVX than in INT monkeys and trends for increased lipolysis measures. These findings are opposite to the expected increase in visceral fat accumulation expected with impaired ovarian function. A specific feature of our study was that animals were on a negative energy balance. A significant reduction was observed in both groups in body weight on the date of necropsy compared with the body weight at the beginning of the experiment. This weight loss may well represent a main determinant of our finding that the negative energy imbalance likely has a greater impact on adipose tissue metabolism in OVX animals. We suggest that this finding is important and relevant since the menopause-associated increase in visceral fat is usually considered as a given. We

show here that there are physiological situations where the negative impact of OVX on abdominal fat metabolism can be prevented. Our findings may also explain why the increase in abdominal fat at menopause is usually difficult to detect, requiring well-controlled longitudinal studies in large samples [19,28]. Variability induced by each woman's energy balance may represent an important confounder in studies on menopause and body fat distribution. From the clinical standpoint, our study suggests that controlling food intake may appear as a relevant approach to prevent abdominal obesity in menopausal women.

Several lines of evidence have shown that OVX induces hyperphagia or increased of food intake in rodents and cats, and suggest that estrogen or oestradiol treatment might reduce hyperphagia and inhibit the rise in food intake following OVX [29-34]. Previous studies have reported that OVX bitches fed *ad libitum* gained weight following increased food intake [35], whereas no weight gain was found in OVX bitches that were fed a fixed amount of commercial dog food and exercised regularly [36]. These findings are concordant with ours. In the present study, the hyperphagic effect of OVX was likely bypassed by the hypocaloric regimen. Under these conditions, we suggest that OVX would promote the use of more lipids as fuel in OVX than in INT monkeys. In addition, our LPL activity data indicate that the suppression of fat storage in abdominal fat compartments is particularly effective in OVX animals.

Gonadal steroids appear to modulate energy balance and adiposity [29] by altering food intake and energy expenditure [31]. We hypothesize that ovarian hormones may modulate energy homeostasis by acting on central appetite and controlling feeding behavior. The loss of ovarian secretion combined with limited food intake may generate particularly strong peripheral signals to compensate caloric insufficiency. Previous reports in humans have indicated that in most studies, weight loss seems to mobilize visceral fat preferentially [37]. In our study, although group differences in lipolytic responsiveness to isoproterenol did not reach significance, the response to forskolin, which bypasses the beta-adrenergic receptors, led to a significant stimulation of glycerol release only in ME adipocytes. This finding is consistent with a preferential mobilization of visceral fat in response to weight loss.

In ME adipose tissue samples of OVX female monkeys, down-regulated genes were identified, including CEBPA, IGF1, INSIG1, LPIN1, PPARG, FASN, ACSL1 and SCD. CEBPA and PPARG are important adipocyte transcription factors. The expression of those genes is induced during adipocyte differentiation

and maintained in mature adipocytes [38,39]. PPARG is considered as the proximal effector of adipogenesis of CEBPA [38]. CEBPA also enhances the expression of the satiety factor leptin [39].

IGF1 has been shown to have insulin-like effects [40]. Our finding of down-regulated IGF1 expression is consistent with increased insulin resistance in ME fat of OVX monkeys. Li et al. [41] found that INSIG1 mRNA rose progressively during 5 weeks of a high-fat diet and declined significantly on a restrictive diet. LIPIN is known as a lipodystrophy and obesity gene [42]. LIPIN deficiency severely impairs adipocyte differentiation and adipogenic gene expression [43]. A previous study [44] has found that systemic and intracerebroventricular treatment of mice with fatty acid synthase inhibitors decreased food intake and induced dramatic weight loss, and suggested that FASN may play an important role in feeding regulation.

In adipose tissue, ACSL1 is a target of PPARγ that increases expression of genes involved in triacylglycerol synthesis as well as in lipid biosynthesis and fatty acid degradation [45]. Fasting decreases ACSL1 mRNA expression in gonadal adipose tissue of rats [46]. Reduced cell proliferation, loss of anchorage-independent growth, and increased sensitivity to ceramide-independent apoptosis have been related to SCD deficiency [47]. Cells with low SCD expression were more sensitive to the cytotoxic effects of exogenous palmitic acids. These findings suggest that SCD plays an essential role in maintaining cellular lipid homeostasis [47]. Our results on the adipose tissue transcriptome closely match what we observed with physiological measures of adipose tissue and adipocyte metabolism. This likely represents the best possible validation of our expression measures.

Estrogens are considered to affect hypothalamic gene expression of neuropeptides and regulate feeding [48] through the direct regulation on ventromedial hypothalamic neuron activities [49]. The reduction in circulating leptin levels may play an important role in the development of temporary hyperphagia and obesity in OVX female animals [30]. Leptin plays a central role in the regulation of feeding behaviour and energy balance with relevant neuroendocrine and reproductive effects [50,51], it is a compound mainly produced and secreted by adipocytes [32]. Leptin is recognized as the afferent signal in the negative feedback loop to maintain constancy of adipose tissue mass [32]. Leptin receptors mediate the effect of leptin [52]. In the present study, using a high throughput microarray technique, we found that the genomic expression of the leptin receptor (LEPR) was significantly down-regulated in ME adipose tissue in OVX

animals. This finding suggests reduced leptin production in ME adipose tissue from OVX monkeys, which may, in turn, significantly impacts on food intake.

In conclusion, results from our cross-sectional comparison suggest that OVX is not acutely associated with increased abdominal adipose tissue adipogenesis and lipid storage in cynomolgus monkeys fed a hypocaloric regimen. This observation is contrary to expected effects of OVX on abdominal fat accumulation. The fact that monkeys were not under *ad libitum* feeding conditions likely explains our finding. Our data provide novel insight on the modulation of energy homeostasis by ovarian hormones. The present study also represents the first investigation of the genome-wide effects of OVX in abdominal fat tissue from female cynomolgus monkeys.

REFERENCES

1. Bjorkelund C, Lissner L, Andersson S, Lapidus L, Bengtsson C: Reproductive history in relation to relative weight and fat distribution. Int J Obes Relat Metab Disord 1996; 20: 213-219.

2. Guthrie JR, Dennerstein L, Taffe JR, Ebeling PR, Randolph JF, Burger HG, Wark JD: Central abdominal fat and endogenous hormones during the menopausal transition. Fertil Steril 2003; 79: 1335-1340.

3. Pedersen SB, Borglum JD, Eriksen EF, Richelsen B: Nuclear estradiol binding in rat adipocytes. Regional variations and regulatory influences of hormones. Biochim Biophys Acta 1991; 1093: 80-86.

4. Pedersen SB, Borglum JD, Moller-Pedersen T, Richelsen B: Effects of in vivo estrogen treatment on adipose tissue metabolism and nuclear estrogen receptor binding in isolated rat adipocytes. Mol Cell Endocrinol 1992; 85: 13-19.

5. D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS: Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. J Biol Chem 2005; 280: 35983-35981.

6. Bellino FL, Wise PM: Nonhuman primate models of menopause workshop. Biol Reprod 2003; 68: 10-18.

7. Despres J.P., Lemieux I.: Abdominal obesity and metabolic syndrome. Nature 2006; 444: 881-887.

8. Drolet R., Belanger C., Fortie M., Huot C., Mailloux J., Legare D., Tchernof A.: Fat depot-specific impact of visceral obesity on adipocyte adiponectin release in women. Obesity 2008; in press:

9. Tchernof A.: Visceral adipocytes and the metabolic syndrome. Nutr Rev 2007; 65: S24-S29.

10. Jewett DA, Dukelow WR: Cyclicity and gestation length of Macaca fascicularis. Primates 1972; 13: 327-330.

11. Wagner JD, Kaplan JR, Burkman RT: Reproductive hormones and cardiovascular disease: mechanism of action and clinical implications. Obstet Gynecol Clin N Am 2002; 29: 475-493.

12. Tchernof A., Belanger C., Morisset A.S., Richard C., Mailloux J., Laberge P., Dupont P.: Regional differences in adipose tissue metabolism in women: minor effect of obesity and body fat distribution. Diabetes 2006; 55: 1353-1360.

13. Tchernof A, Belanger C, Morisset AS, Richard C, Mailloux J, Laberge P, Dupont P: Regional differences in adipose tissue metabolism in women: minor effect of obesity and body fat distribution. Diabetes 2006; 55: 1353-1360.

14. Kather H, Schroder F, Simon B: Microdetermination of glycerol using bacterial NADH-linked luciferase. Clin Chim Acta 1982; 120: 295-300.

15. Walker S.J., Wang Y., Grant K.A., Chan F., Hellmann G.M.: Long versus short oligonucleotide microarrays for the study of gene expression in nonhuman primates. J Neurosci Methods 2006; 152: 179-189.

16. Tchernof A, Desmeules A, Richard C, Laberge P, Daris M, Mailloux J, Rheaume C, Dupont P: Ovarian hormone status and abdominal visceral adipose tissue metabolism. J Clin Endocrinol Metab 2004; 89: 3425-3430.

17. Carr MC: The emergence of the metabolic syndrome with menopause. J Clin Endocrinol Metab 2003; 88: 2404-2411.

18. Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB: The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. Arch Intern Med 2003; 163: 427-436.

19. Guthrie J.R., Dennerstein L.: Studying the menopausal transition: methodological considerations. Climacteric 2003; 6: 354-356.

20. Zamboni M, Armellini F, Milani MP, De Marchi M, Todesco T, Robbi R, Bergamo-Andreis IA, Bosello O: Body fat distribution in pre- and post-menopausal women: metabolic and anthropometric variables and their inter-relationships. Int J Obes Relat Metab Disord 1992; 16: 495-504.

21. Gambacciani M, Ciaponi M, Cappagli B, De Simone L, Orlandi R, Genazzani AR: Prospective evaluation of body weight and body fat distribution in early postmenopausal women with and without hormonal replacement therapy. Maturitas 2001; 39: 125-132.

22. Jensen LB, Vestergaard P, Hermann AP, Gram J, Eiken P, Abrahamsen B, Brot C, Kolthoff N, Sorensen OH, Beck-Nielsen H, Nielsen SP, Charles P, Mosekilde L: Hormone replacement therapy dissociates fat mass and bone mass, and tends to reduce weight gain in early postmenopausal women: a randomized controlled 5-year clinical trial of the Danish Osteoporosis Prevention Study. J Bone Miner Res 2003; 18: 333-342.

23. Kritz-Silverstein D, Barrett-Connor E: Long-term postmenopausal hormone use, obesity, and fat distribution in older women. JAMA 1996; 275: 46-49.

24. Perrone G, Liu Y, Capri O, Critelli C, Barrillaro F, Galoppi P, Zichella L: Evaluation of the body composition and fat distribution in long-term users of hormone replacement therapy. Gynecol Obstet Invest 1999; 48: 52-55.

25. Sumino H, Ichikawa S, Yoshida A, Murakami M, Kanda T, Mizunuma H, Sakamaki T, Kurabayashi M: Effects of hormone replacement therapy on weight, abdominal fat distribution, and lipid levels in Japanese postmenopausal women. Int J Obes Relat Metab Disord 2003; 27: 1044-1051.

26. Sayegh RA, Kelly L, Wurtman J, Deitch A, Chelmow D: Impact of hormone replacement therapy on the body mass and fat compositions of menopausal women: a cross-sectional study. Menopause 1999; 6: 312-315.

27. Reubinoff BE, Wurtman J, Rojansky N, Adler D, Stein P, Schenker JG, Brzezinski A: Effects of hormone replacement therapy on weight, body composition, fat distribution, and food intake in early postmenopausal women: a prospective study. Fertil Steril 1995; 64: 963-968.

28. Bjorkelund C., Lissner L., Andersson S., Lapidus L., Bengtsson C.: Reproductive history in relation to relative weight and fat distribution. Int J Obes Relat Metab Disord 1996; 20: 213-219.

29. Wade GN, Gray JM, Bartness TJ: Gonadal influences on adiposity. Int J Obes 1985; 9: 83-92.

30. Shimomura K, Shimizu H, Tsuchiya T, Abe Y, Uehara Y, Mori M: Is leptin a key factor which develops obesity by ovariectomy? Endocr J 2002; 49: 417-423.

31. Richard D: Effects of ovarian hormones on energy balance and brown adipose tissue thermogenesis. Am J Physiol. 1986; 250: R245-R249.

32. Meli R, Pacilio M, Raso GM, Esposito E, Coppola A, Nasti A, DiCarlo C, Nappi C, Di Carlo R: Estrogen and raloxifene modulate leptin and its receptor in hypothalamus and adipose tissue from ovariectomized rats. Endocrinology 2004; 145: 3115-3121.

33. Liang YQ, Akishita M, Kim S, Ako J, Hashimoto M, Iijima K, Ohike Y, Watanabe T, Sudoh N, Toba K, Yoshizumi M, Ouchi Y: Estrogen receptor beta is involved in the anorectic action of estrogen. Int J Obes Relat Metab Disord 2002; 26: 1103-1109.

34. Cave NJ, Backus RC, Marks SL, Klasing KC: Oestradiol, but not genistein, inhibits the rise in food intake following gonadectomy in cats, but genistein is associated with an increase in lean body mass. J Anim Physiol Anim Nutr (Berl) 2007; 91: 400-410.

35. Houpt KA, Coren B, Hintz HF, Hilderbrant JE: Effect of sex and reproductive status on sucrose preference, food intake, and body weight of dogs. J Am Vet Med Assoc 1979; 174: 1083-1085.

36. Le Roux PH: Thyroid status, oestradiol level, work performance and body mass of ovariectomised bitches and bitches bearing ovarian autotransplants in the stomach wall. J S Afr Vet Assoc 1983; 54: 115-117.

37. Smith S.R., Zachwieja J.J.: Visceral adipose tissue: a critical review of intervention strategies. Int J Obes Relat Metab Disord 1999; 23: 329-335.

38. Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, Spiegelman BM: C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. Genes Dev 2002; 16: 22-26.

39. Miller SG, De Vos P, Guerre-Millo M, Wong K, Hermann T, Staels B, Briggs MR, Auwerx J: The adipocyte specific transcription factor C/EBPalpha modulates human ob gene expression. Proc Natl Acad Sci U S A 1996; 93: 5507-5511.

40. Jones JI, Clemmons DR: Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995; 16: 3-34.

41. Li J, Takaishi K, Cook W, McCorkle SK, Unger RH: Insig-1 "brakes" lipogenesis in adipocytes and inhibits differentiation of preadipocytes. Proc Natl Acad Sci U S A 2003; 100: 9476-9481.

42. Phan J, Reue K: Lipin, a lipodystrophy and obesity gene. Cell Metab 2005; 1: 73-83.

43. Phan J, Peterfy M, Reue K: Lipin expression preceding peroxisome proliferator-activated receptor-gamma is critical for adipogenesis in vivo and in vitro. J Biol Chem 2004; 279: 29558-29564.

44. Loftus TM, Jaworsky DE, Frehywot GL, Townsend CA, Ronnett GV, Lane MD, Kuhajda FP: Reduced food intake and body weight in mice treated with fatty acid synthase inhibitors. Science 2000; 288: 2379-2381.

45. Martin G, Schoonjans K, Lefebvre AM, Staels B, Auwerx J: Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. J Biol Chem. 1997; 272: 28210-28217.

46. Mashek DG, Li LO, Coleman RA: Rat long-chain acyl-CoA synthetase mRNA, protein, and activity vary in tissue distribution and in response to diet. J Lipid Res 2006; 47: 2004-2010.

47. Scaglia N, Igal RA: Stearoyl-CoA desaturase is involved in the control of proliferation, anchorage-independent growth, and survival in human transformed cells. J Biol Chem 2005; 280: 25339-25349.

48. Shimizu H, Ohtani K, Kato Y, Tanaka Y, Mori M: Withdrawal of [corrected] estrogen increases hypothalamic neuropeptide Y (NPY) mRNA expression in ovariectomized obese rat . Neurosci Lett 1996; 204: 81-84.

49. Jones EE, Naftolin F: Estrogen effects on the tuberoinfundibular dopaminergic system in the female rat brain. Brain Res 1990; 510: 84-91.

50. Ahima RS, Flier JS: Leptin. Annu Rev Physiol 2000; 62: 413-437.

51. Wauter M, Considine RV, Van Gaal LF: Human leptin: from an adipocyte hormone to an endocrine mediator. Eur J Endocrinol 2000; 143: 293-311.

52. Chua SC Jr, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, Leibel RL: Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. Science 1996; 271: 994-996.

Figure 1. Body weight changes in intact (INT) and ovariectomized (OVX) female cynomolgus monkeys at the beginning of study and at necropsy. Data are presented as Mean ± SEM.

Figure 2. Comparison of adipocyte size between INT and OVX female cynomolgus monkeys in mesenteric (A) and subcutaneous (B) fat samples, and comparison of adipose tissue lipoprotein lipase (LPL) activity between INT and OVX female cynomolgus monkeys in mesenteric (C) and subcutaneous (D) fat samples. Data are mean ± SEM. In the LPL activity experiments, statistical tests performed on $\log_{10}$-transformed values in mesenteric samples, and Box-Cox-transformed data in subcutaneous adipose tissue; * $p \leq 0.05$, OVX vs. INT animals.

Figure 3. Adipocyte lipolytic capacity after 2h stimulations with isoproterenol (nonselective β-adrenergic agonist) and forskolin 10^{-5}M (postreceptor lipolytic agonist) in INT and OVX female cynomolgus monkeys in mesenteric (A and C) and subcutaneous (B and D) fat samples. Data are presented as mean ± SEM. * $p < 0.05$, OVX vs. INT animals; †, $p = 0.14$; ‡, $p = 0.09$.

Figure 4. According to biological functions, the significantly up-regulated (A) and down-regulated probe sets were clustered hierarchically with the GeneSpring program.

Table 1: Selected pathways with more than two differentially expressed genes in ME adipose tissue, when the comparison was performed between INT and OVX animals. (↑: Up-regulated; ↓: Down-regulated.)

KEGG pathways	Genes
Focal adhesion	COL6A3↑, FIGF↓, RAC2↑, SPP1↑, COL1A2↑, IGF1↓, ITGA7↓
Cytokine-cytokine receptor interaction	IL2RG↑, LTB↑, CCR7↑, CCL21↑, CXCL13↑
Carbon fixation	TKT↓, ALDOC↓, GOT1↓, MDH1↓
Regulation of actin cytoskeleton	CSK↑, RAC2↑, FGF13↑, ITGA7↓
ECM-receptor interaction	COL6A3↑, SPP1↑, COL1A2↑, ITGA7↓
Hematopoietic cell lineage	CD3D↑, CR2↑, CD37↑
Tyrosine metabolism	FAH↓, GOT1↓
MAPK signaling pathway	RAC2↑, FGF13↑
Pentose phosphate pathway	TKT↓, ALDOC↓
Cysteine metabolism	CDO1↓, GOT1↓
Phenylalanine, tyrosine and tryptophan biosynthesi	ENO2↑, GOT1↓
Glycolysis / Gluconeogenesis	ALDOC↓, ENO2↑
B cell receptor signaling pathway	RAC2↑, CR2↑
T cell receptor signaling pathway	CD3D↑, LCK↑

Table 2. Down-regulated and up-regulated genes which related to adipose tissue metabolism and lipid synthesis selected from the comparison of mesenteric fat samples from OVX vs. INT monkeys. (* Fold difference: gene expression level of OVX samples / gene expression level of INT samples.)

Probeset ID	Description	Symbol	Accession	* Fold difference
Downregulated				
201128_s_at	ATP citrate lyase	ACLY	NM_001096	-1.7
210337_s_at	ATP citrate lyase	ACLY	U18197	-1.6
209283_at	Crystalline, alpha B	CRYAB	AF007162	-1.7
212218_s_at	Fatty acid synthase	FASN	AI954041	-1.6
207275_s_at	Acyl-CoA synthetase long-chain family member 1	ACSL1	NM_001995	-1.5
201627_s_at	Insulin induced gene 1	INSIG1	NM_005542	-1.5
201625_s_at	Insulin induced gene 1	INSIG1	BE300521	-1.4
218680_x_at	Huntingtin interacting protein K	HYPK	NM_016400	-1.4
215726_s_at	Cytochrome b-5 type A (microsomal)	CYB5A	M22976	-1.4
202428_x_at	Diazepam binding inhibitor (GABA receptor modulator, acyl-C oenzyme A binding protein)	DBI	NM_020548	-1.3
211708_s_at	Stearoyl-CoA desaturase (delta-9-de saturase)	SCD	BC005807	-1.3
Upregulated				
201852_x_at	Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, a utosomal dominant)	COL3A1	AI813758	1.4
211719_x_at	Fibronectin 1	FN1	BC005858	1.4
210495_x_at	Fibronectin 1	FN1	AF130095	1.4
216442_x_at	Fibronectin 1	FN1	AK026737	1.4
212464_s_at	Fibronectin 1	FN1	X02761	1.6
201438_at	Collagen, type VI, alpha 3	COL6A3	NM_004369	1.4

202403_s_at	Collagen, type I, alpha 2	COL1A2	AA788711	1.5
202404_s_at	Collagen, type I, alpha 2	COL1A2	NM_000089	2.4
202450_s_at	Cathepsin K (pycnodysostosis)	CTSK	NM_000396	1.4
201141_at	Glycoprotein (transmembrane) nmb	GPNMB	NM_002510	1.5
202310_s_at	Collagen, type I, alpha 1	COL1A1	K01228	1.6
202311_s_at	Collagen, type I, alpha 1	COL1A1	AI743621	2.9
202391 _at	Brain abundant, membrane attached signal protein 1	BASP1	NM_006317	2.1

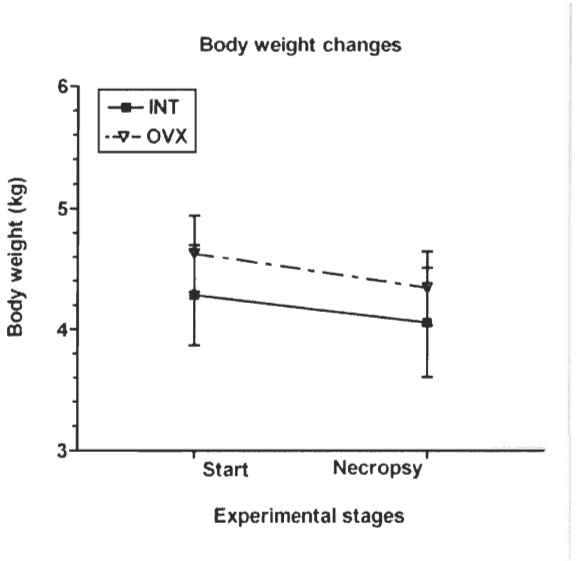

Body weight changes
INT
OVX
Body weight (kg)
6
5
4
3
Start
Necropsy
Experimental stages

Figure 1.

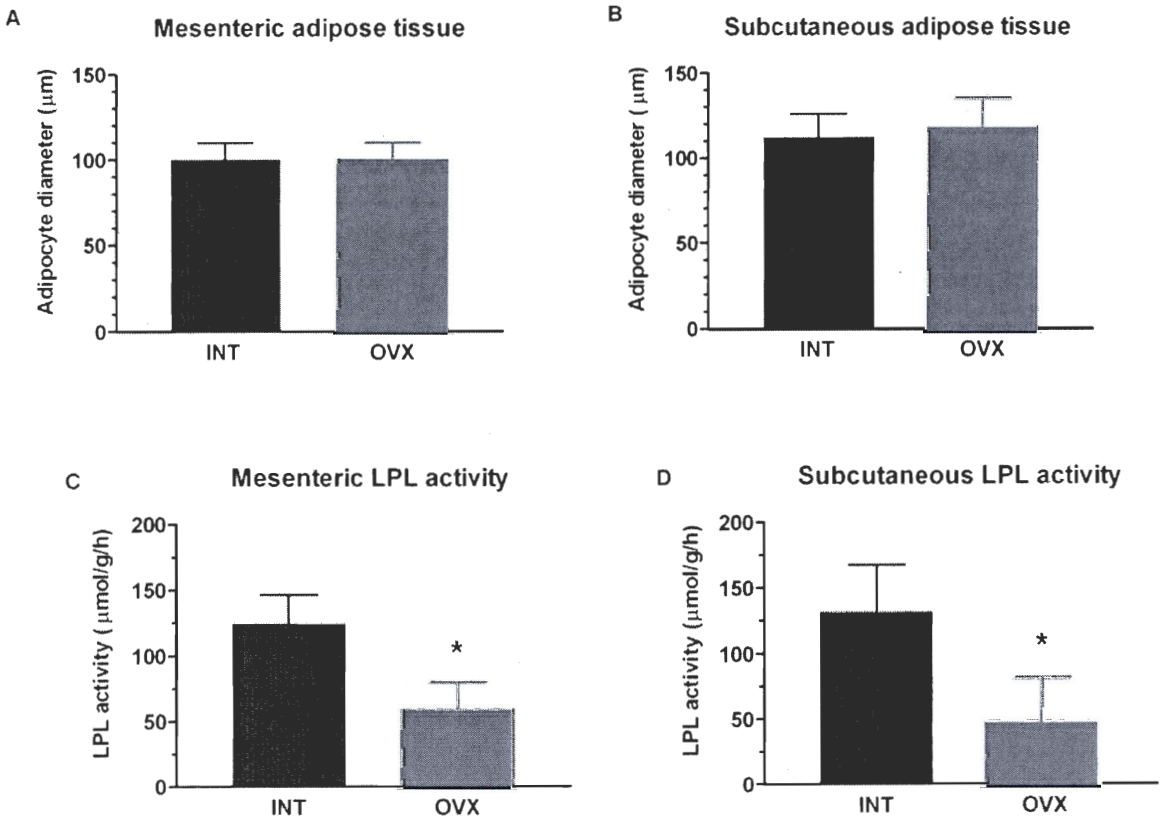

A
Mesenteric adipose tissue
Adipocyte diameter (µm)
150
100
50
0
INT
OVX
B
Subcutaneous adipose tissue
Adipocyte diameter (µm)
150
100
50
0
INT
OVX
C
Mesenteric LPL activity
LPL activity (µmol/g/h)
200
150
100
50
0
*
INT
OVX
D
Subcutaneous LPL activity
LPL activity (µmol/g/h)
200
150
100
50
0
*
INT
OVX

Figure 2.

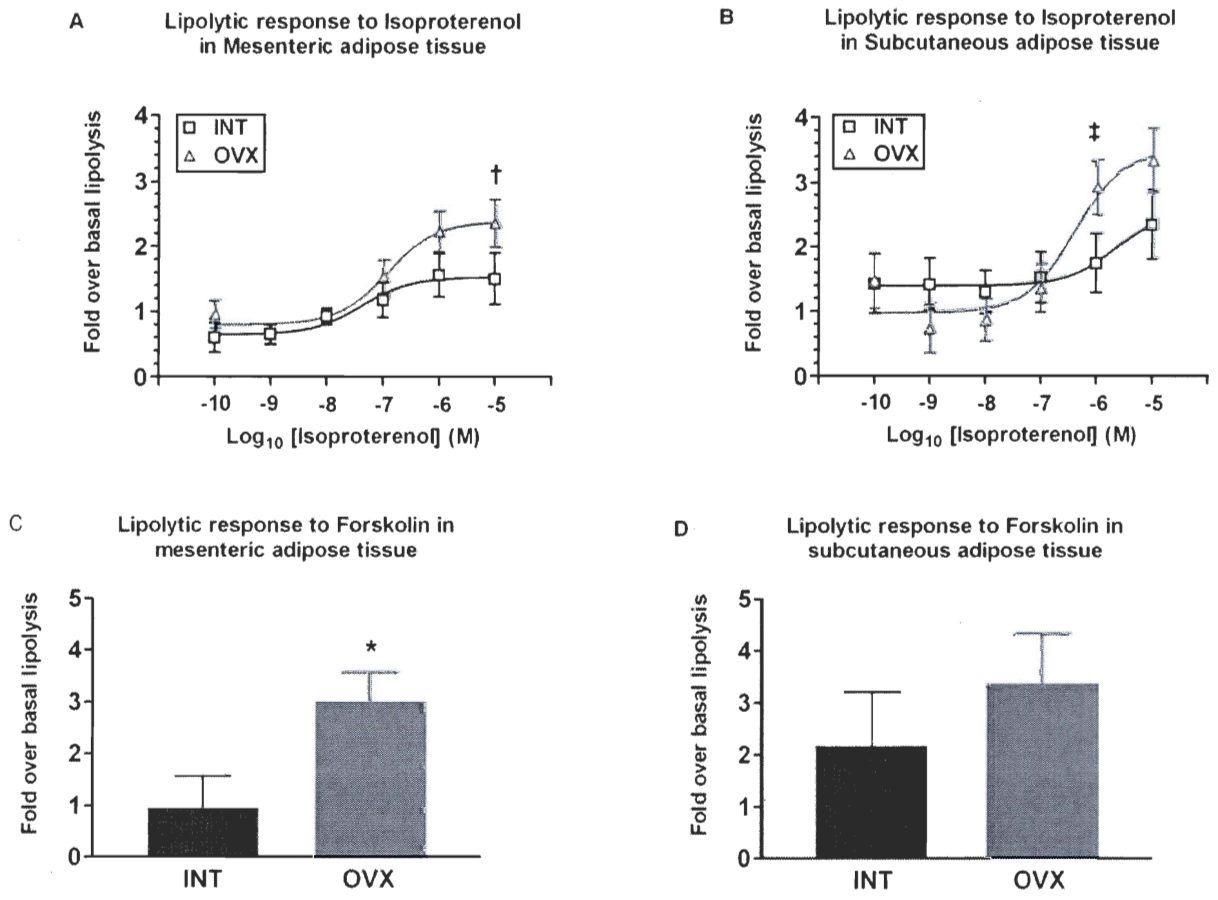
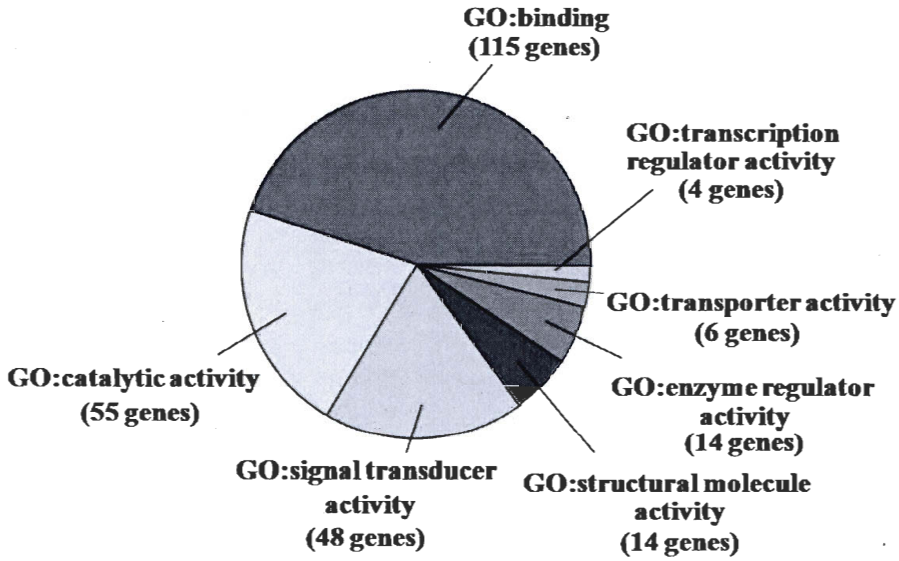

A
Lipolytic response to Isoproterenol in Mesenteric adipose tissue
Fold over basal lipolysis
INT
OVX
†
Log10 [Isoproterenol] (M)
B
Lipolytic response to Isoproterenol in Subcutaneous adipose tissue
Fold over basal lipolysis
INT
OVX
‡
Log10 [Isoproterenol] (M)
C
Lipolytic response to Forskolin in mesenteric adipose tissue
Fold over basal lipolysis
*
INT
OVX
D
Lipolytic response to Forskolin in subcutaneous adipose tissue
Fold over basal lipolysis
INT
OVX

Figure 3

A


GO:binding
(115 genes)
GO:transcription
regulator activity
(4 genes)
GO:transporter activity
(6 genes)
GO:enzyme regulator
activity
(14 genes)
GO:structural molecule
activity
(14 genes)
GO:signal transducer
activity
(48 genes)
GO:catalytic activity
(55 genes)

B

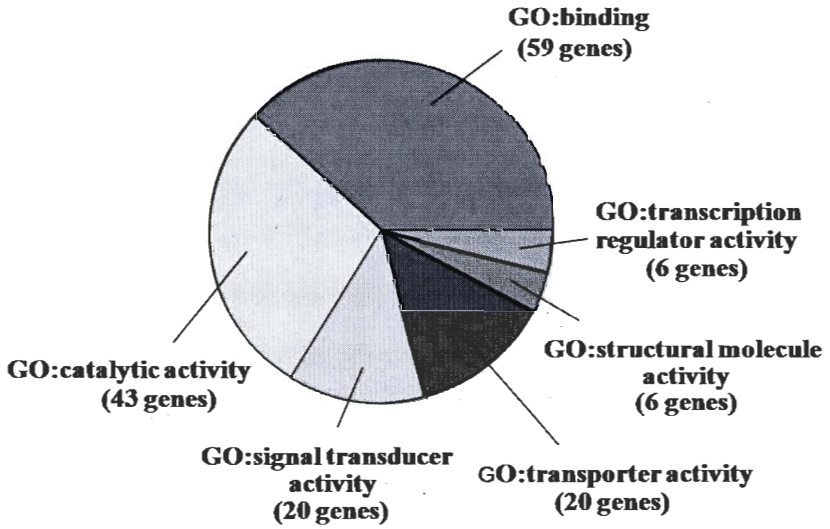

GO:binding
(59 genes)
GO:transcription
regulator activity
(6 genes)
GO:structural molecule
activity
(6 genes)
GO:transporter activity
(20 genes)
GO:signal transducer
activity
(20 genes)
GO:catalytic activity
(43 genes)

Figure 4

Appended Table 1. A total of 276 differentially expressed probesets representing 234 genes were identified from microarray experiments, 93 of them were down-regulated (corresponding to 81 genes), 183 of them were up-regulated (corresponding to 153 genes).

Probe Set ID	Gene Symbol	Description	GeneBank
Down-regulated			
201128_s_at	ACLY	ATP citrate lyase	NM_001096
210337_s_at	ACLY	ATP citrate lyase	U18197
202982_s_at	ACOT2	acyl-CoA thioesterase 2	NM_006821
207275_s_at	ACSL1	acyl-CoA synthetase long-chain family member 1	NM_001995
212175_s_at	AK2	adenylate kinase 2	AL513611
202022_at	ALDOC	aldolase C, fructose-bisphosphate	NM_005165
204998_s_at	ATF5	activating transcription factor 5	NM_012068
208870_x_at	ATP5C1	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	BC000931
213366_x_at	ATP5C1	ATP synthase, H+ transporting, mitochondrial F1 complex, gamma polypeptide 1	AV711183
202427_s_at	BRP44	brain protein 44	NM_015415
219450_at	C4orf19	chromosome 4 open reading frame 19	NM_018302
219054_at	C5orf23	chromosome 5 open reading frame 23	NM_024563
213948_x_at	CADM3	cell adhesion molecule 3	AI564838
208656_s_at	CCNI	cyclin I	AF135162
204154_at	CDO1	cysteine dioxygenase, type I	NM_001801
204039_at	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha	NM_004364
209616_s_at	CES1	carboxylesterase 1 (monocyte/macrophage serine esterase 1)	S73751
210069_at	CPT1B	carnitine palmitoyltransferase 1B (muscle)	U62733
209283_at	CRYAB	crystallin, alpha B	AF007162
215726_s_at	CYB5A	cytochrome b5 type A (microsomal)	M22976
202428_x_at	DBI	diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein)	NM_020548
211150_s_at	DLAT	dihydrolipoamide S-acetyltransferase (E2 component of pyruvate dehydrogenase complex)	J03866
221563_at	DUSP10	dual specificity phosphatase 10	N36770
201694_s_at	EGR1	early growth response 1	NM_001964
204256_at	ELOVL6	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	NM_024090

212339_at	EPB41L1	erythrocyte membrane protein band 4.1-like 1	AL121895
202862_at	FAH	fumarylacetoacetate hydrolase (fumarylacetoacetase)	NM_000137
212218_s_at	FASN	fatty acid synthase	AI954041
206742_at	FIGF	c-fos induced growth factor (vascular endothelial growth factor D)	NM_004469
208813_at	GOT1	glutamic-oxaloacetic transaminase 1, soluble (aspartate aminotransferase 1)	BC000498
220773_s_at	GPHN	gephyrin	NM_020806
205220_at	GPR109B	G protein-coupled receptor 109B	NM_006018
205752_s_at	GSTM5	glutathione S-transferase M5	NM_000851
204018_x_at	HBA1	hemoglobin, alpha 1	NM_000558
209458_x_at	HBA1	hemoglobin, alpha 1	AF105974
211699_x_at	HBA1	hemoglobin, alpha 1	AF349571
211745_x_at	HBA2	hemoglobin, alpha 2	BC005931
214414_x_at	HBA2	Hemoglobin, alpha 2	T50399
204753_s_at	HLF	hepatic leukemia factor	AI810712
218680_x_at	HYPK	Huntingtin interacting protein K	NM_016400
210046_s_at	IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial	U52144
210418_s_at	IDH3B	isocitrate dehydrogenase 3 (NAD+) beta	AF023265
209540_at	IGF1	insulin-like growth factor 1 (somatomedin C)	AU144912
209541_at	IGF1	insulin-like growth factor 1 (somatomedin C)	AI972496
209542_x_at	IGF1	insulin-like growth factor 1 (somatomedin C)	M29644
211577_s_at	IGF1	insulin-like growth factor 1 (somatomedin C)	M37484
218516_s_at	IMPAD1	inositol monophosphatase domain containing 1	NM_017813
201625_s_at	INSIG1	insulin induced gene 1	BE300521
201627_s_at	INSIG1	insulin induced gene 1	NM_005542
209663_s_at	ITGA7	integrin, alpha 7	AF072132
216331_at	ITGA7	integrin, alpha 7	AK022548
211354_s_at	LEPR	leptin receptor	U52913
211355_x_at	LEPR	leptin receptor	U52914
212276_at	LPIN1	lipin 1	D80010
200978_at	MDH1	malate dehydrogenase 1, NAD (soluble)	NM_005917
204059_s_at	ME1	malic enzyme 1, NADP(+)-dependent, cytosolic	NM_002395
217919_s_at	MRPL42	mitochondrial ribosomal protein L42	BE782148
217772_s_at	MTCH2	mitochondrial carrier homolog 2 (C. elegans)	NM_014342
220864_s_at	NDUFA13	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 13	NM_015965
202077_at	NDUFAB1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1, 8kDa	NM_005003

202941_at	NDUFV2	NADH dehydrogenase (ubiquinone) flavoprotein 2, 24kDa	NM_021074
204760_s_at	NR1D1	nuclear receptor subfamily 1, group D, member 1	NM_021724
202600_s_at	NRIP1	nuclear receptor interacting protein 1	AI824012
209279_s_at	NSDHL	NAD(P) dependent steroid dehydrogenase-like	BC000245
200813_s_at	PAFAH1B1	platelet-activating factor acetylhydrolase, isoform Ib, alpha subunit 45kDa	BE256969
203557_s_at	PCBD1	pterin-4 alpha-carbinolamine dehydratase/dimerization cofactor of hepatocyte nuclear factor 1 alpha (TCF1)	NM_000281
200980_s_at	PDHA1	pyruvate dehydrogenase (lipoamide) alpha 1	NM_000284
205960_at	PDK4	pyruvate dehydrogenase kinase, isozyme 4	NM_002612
220741_s_at	PPA2	pyrophosphatase (inorganic) 2	NM_006903
208510_s_at	PPARG	peroxisome proliferator-activated receptor gamma	NM_015869
201490_s_at	PPIF	peptidylprolyl isomerase F (cyclophilin F)	NM_005729
220014_at	PRR16	proline rich 16	NM_016644
203997_at	PTPN3	protein tyrosine phosphatase, non-receptor type 3	NM_002829
200637_s_at	PTPRF	protein tyrosine phosphatase, receptor type, F	AI762627
205577_at	PYGM	phosphorylase, glycogen; muscle (McArdle syndrome, glycogen storage disease type V)	NM_005609
205334_at	S100A1	S100 calcium binding protein A1	NM_006271
202598_at	S100A13	S100 calcium binding protein A13	NM_005979
201819_at	SCARB1	scavenger receptor class B, member 1	NM_005505
211708_s_at	SCD	stearoyl-CoA desaturase (delta-9-desaturase)	BC005807
210010_s_at	SLC25A1	solute carrier family 25 (mitochondrial carrier; citrate transporter), member 1	U25147
204430_s_at	SLC2A5	solute carrier family 2 (facilitated glucose/fructose transporter), member 5	NM_003039
209607_x_at	SULT1A3	sulfotransferase family, cytosolic, 1A, phenol-preferring, member 3	U08032
203888_at	THBD	thrombomodulin	NM_000361
208699_x_at	TKT	transketolase (Wernicke-Korsakoff syndrome)	BF696840
208700_s_at	TKT	transketolase (Wernicke-Korsakoff syndrome)	L12711
202096_s_at	TSPO	translocator protein (18kDa)	NM_000714
201714_at	TUBG1	tubulin, gamma 1	NM_001070
210065_s_at	UPK1B	uroplakin 1B	AB002155
208909_at	UQCRFS1	ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	BC000649
202233_s_at	UQCRH	ubiquinol-cytochrome c reductase hinge protein	NM_006004
211662_s_at	VDAC2	voltage-dependent anion channel 2	L08666
211527_x_at	VEGFA	vascular endothelial growth factor A	M27281

200641_s_at	YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	U28964
Up-regulated			
203300_x_at	AP1S2	adaptor-related protein complex 1, sigma 2 subunit	NM_003916
218870_at	ARHGAP15	Rho GTPase activating protein 15	NM_018460
201288_at	ARHGDIB	Rho GDP dissociation inhibitor (GDI) beta	NM_001175
202207_at	ARL4C	ADP-ribosylation factor-like 4C	BG435404
210971_s_at	ARNTL	aryl hydrocarbon receptor nuclear translocator-like	AB000815
221234_s_at	BACH2	BTB and CNC homology 1, basic leucine zipper transcription factor 2	NM_021813
202391_at	BASP1	brain abundant, membrane attached signal protein 1	NM_006317
219497_s_at	BCL11A	B-cell CLL/lymphoma 11A (zinc finger protein)	NM_022893
219528_s_at	BCL11B	B-cell CLL/lymphoma 11B (zinc finger protein)	NM_022898
207186_s_at	BPTF	bromodomain PHD finger transcription factor	NM_004459
220059_at	BRDG1	BCR downstream signaling 1	NM_012108
202946_s_at	BTBD3	BTB (POZ) domain containing 3	NM_014962
212560_at	C11orf32	chromosome 11 open reading frame 32	AV728268
211639_x_at	C12orf32	Chromosome 12 open reading frame 32	L23518
206707_x_at	C6orf32	chromosome 6 open reading frame 32	NM_015864
204606_at	CCL21	chemokine (C-C motif) ligand 21	NM_002989
213226_at	CCNA2	cyclin A2	AI346350
206337_at	CCR7	chemokine (C-C motif) receptor 7	NM_001838
204192_at	CD37	CD37 molecule	NM_001774
213539_at	CD3D	CD3d molecule, delta (CD3-TCR complex)	NM_000732
209835_x_at	CD44	CD44 molecule (Indian blood group)	BC004372
203416_at	CD53	CD53 molecule	NM_000560
207172_s_at	CDH11	cadherin 11, type 2, OB-cadherin (osteoblast)	NM_001797
207173_x_at	CDH11	cadherin 11, type 2, OB-cadherin (osteoblast)	D21254
205213_at	CENTB1	centaurin, beta 1	NM_014716
204373_s_at	CEP350	centrosomal protein 350kDa	NM_014810
208168_s_at	CHIT1	chitinase 1 (chitotriosidase)	NM_003465
204170_s_at	CKS2	CDC28 protein kinase regulatory subunit 2	NM_001827
202310_s_at	COL1A1	collagen, type I, alpha 1	K01228
202311_s_at	COL1A1	collagen, type I, alpha 1	AI743621
217430_x_at	COL1A1	collagen, type I, alpha 1	Y15916
202403_s_at	COL1A2	collagen, type I, alpha 2	AA788711

202404_s_at	COL1A2	collagen, type I, alpha 2	NM_000089
201852_x_at	COL3A1	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	AI813758
201438_at	COL6A3	collagen, type VI, alpha 3	NM_004369
209083_at	CORO1A	coronin, actin binding protein, 1A	U34690
205544_s_at	CR2	complement component (3d/Epstein Barr virus) receptor 2	NM_001877
202329_at	CSK	c-src tyrosine kinase	NM_004383
204971_at	CSTA	cystatin A (stefin A)	NM_005213
215946_x_at	CTA-246H3.1	similar to omega protein	AL022324
200839_s_at	CTSB	cathepsin B	NM_001908
202295_s_at	CTSH	cathepsin H	NM_004390
202450_s_at	CTSK	cathepsin K	NM_000396
202902_s_at	CTSS	cathepsin S	NM_004079
205242_at	CXCL13	chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)	NM_006419
211919_s_at	CXCR4	chemokine (C-X-C motif) receptor 4	AF348491
204923_at	CXorf9	chromosome X open reading frame 9	AL023653
215785_s_at	CYFIP2	cytoplasmic FMR1 interacting protein 2	AL161999
202437_s_at	CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide 1	NM_000104
201584_s_at	DDX39	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39	NM_005804
221081_s_at	DENND2D	DENN/MADD domain containing 2D	NM_024901
203385_at	DGKA	diacylglycerol kinase, alpha 80kDa	NM_001345
211272_s_at	DGKA	diacylglycerol kinase, alpha 80kDa	AF064771
209560_s_at	DLK1	delta-like 1 homolog (Drosophila)	U15979
205554_s_at	DNASE1L3	deoxyribonuclease I-like 3	NM_004944
219279_at	DOCK10	dedicator of cytokinesis 10	NM_017718
202971_s_at	DYRK2	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	NM_006482
201983_s_at	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)	AW157070
201313_at	ENO2	enolase 2 (gamma, neuronal)	NM_001975
216510_x_at	EXOC7	exocyst complex component 7	AB035175
210889_s_at	FCGR2B	Fc fragment of IgG, low affinity IIb, receptor (CD32)	M31933
205110_s_at	FGF13	fibroblast growth factor 13	NM_004114
210495_x_at	FN1	fibronectin 1	AF130095
211719_x_at	FN1	fibronectin 1	BC005858
212464_s_at	FN1	fibronectin 1	X02761

216442_x_at	FN1	fibronectin 1	AK026737
210105_s_at	FYN	FYN oncogene related to SRC, FGR, YES	M14333
216033_s_at	FYN	FYN oncogene related to SRC, FGR, YES	S74774
203706_s_at	FZD7	frizzled homolog 7 (Drosophila)	NM_003507
203066_at	GALNAC4S-6ST	B cell RAG associated protein	NM_014863
209604_s_at	GATA3	GATA binding protein 3	BC003070
204220_at	GMFG	glia maturation factor, gamma	NM_004877
201141_at	GPNMB	glycoprotein (transmembrane) nmb	NM_002510
207651_at	GPR171	G protein-coupled receptor 171	NM_013308
202957_at	HCLS1	hematopoietic cell-specific Lyn substrate 1	NM_005335
214669_x_at	HLA-C	Major histocompatibility complex, class I, C	BG485135
214768_x_at	HLA-C	Major histocompatibility complex, class I, C	BG540628
215176_x_at	HLA-C	Major histocompatibility complex, class I, C	AW404894
216576_x_at	HLA-C	Major histocompatibility complex, class I, C	AF103529
217480_x_at	HLA-C	Major histocompatibility complex, class I, C	M20812
211991_s_at	HLA-DPA1	major histocompatibility complex, class II, DP alpha 1	M27487
201137_s_at	HLA-DPB1	major histocompatibility complex, class II, DP beta 1	NM_002121
212671_s_at	HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1	BG397856
212998_x_at	HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1	AI583173
208894_at	HLA-DRA	major histocompatibility complex, class II, DR alpha	M60334
213619_at	HNRPH1	Heterogeneous nuclear ribonucleoprotein H1 (H)	AV753392
213038_at	IBRDC3	IBR domain containing 3	AL031602
201422_at	IFI30	interferon, gamma-inducible protein 30	NM_006332
216557_x_at	IFI6	interferon, alpha-inducible protein 6	U92706
217022_s_at	IGHA1	immunoglobulin heavy constant alpha 1	S55735
217369_at	IGHG1	immunoglobulin heavy constant gamma 1 (G1m marker)	AJ275383
211430_s_at	IGHG3	immunoglobulin heavy constant gamma 3 (G3m marker)	M87789
209374_s_at	IGHM	immunoglobulin heavy constant mu	BC001872
216491_x_at	IGHM	immunoglobulin heavy constant mu	U80139
211644_x_at	IGKC	immunoglobulin kappa constant	L14458
221651_x_at	IGKC	immunoglobulin kappa constant	BC005332
221671_x_at	IGKC	immunoglobulin kappa constant	M63438
216207_x_at	IGKV1D-13	immunoglobulin kappa variable 1D-13	AW408194
209138_x_at	IGL@	Immunoglobulin lambda locus	M87790

215379_x_at	IGL@	immunoglobulin lambda locus	AV698647
216560_x_at	IGL@	immunoglobulin lambda locus	D87021
214677_x_at	IGLJ3	immunoglobulin lambda joining 3	X57812
217148_x_at	IGLV2-14	immunoglobulin lambda variable 2-14	AJ249377
213193_x_at	IL23A	Interleukin 23, alpha subunit p19	AL559122
204116_at	IL2RG	interleukin 2 receptor, gamma (severe combined immunodeficiency)	NM_000206
211650_x_at	IL8	Interleukin 8	L34164
204698_at	ISG20	interferon stimulated exonuclease gene 20kDa	NM_002201
217258_x_at	IVD	Isovaleryl Coenzyme A dehydrogenase	AF043583
212192_at	KCTD12	potassium channel tetramerisation domain containing 12	AI718937
202503_s_at	KIAA0101	KIAA0101	NM_014736
206478_at	KIAA0125	KIAA0125	NM_014792
217388_s_at	KYNU	kynureninase (L-kynurenine hydrolase)	D55639
213519_s_at	LAMA2	laminin, alpha 2 (merosin, congenital muscular dystrophy)	AI078169
201720_s_at	LAPTM5	lysosomal associated multispanning membrane protein 5	AI589086
201721_s_at	LAPTM5	lysosomal associated multispanning membrane protein 5	NM_006762
221011_s_at	LBH	limb bud and heart development homolog (mouse)	NM_030915
204890_s_at	LCK	lymphocyte-specific protein tyrosine kinase	U07236
204891_s_at	LCK	lymphocyte-specific protein tyrosine kinase	NM_005356
221558_s_at	LEF1	lymphoid enhancer-binding factor 1	AF288571
211637_x_at	LOC652128	Similar to Ig heavy chain V-II region ARH-77 precursor	L23516
213502_x_at	LOC91316	similar to bK246H3.1 (immunoglobulin lambda-like polypeptide 1, pre-B-cell specific)	AA398569
204674_at	LRMP	lymphoid-restricted membrane protein	NM_006152
35974_at	LRMP	lymphoid-restricted membrane protein	4872173_RC
207339_s_at	LTB	lymphotoxin beta (TNF superfamily, member 3)	NM_002341
205668_at	LY75	lymphocyte antigen 75	NM_002349
210754_s_at	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog	M79321
213975_s_at	LYZ	lysozyme (renal amyloidosis)	AV711904
205105_at	MAN2A1	mannosidase, alpha, class 2A, member 1	NM_002372
209166_s_at	MAN2B1	mannosidase, alpha, class 2B, member 1	U68567
206296_x_at	MAP4K1	mitogen-activated protein kinase kinase kinase kinase 1	NM_007181
201668_x_at	MARCKS	myristoylated alanine-rich protein kinase C substrate	AW163148
201670_s_at	MARCKS	myristoylated alanine-rich protein kinase C substrate	M68956
203936_s_at	MMP9	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV	NM_004994

		collagenase)	
204959_at	MNDA	myeloid cell nuclear differentiation antigen	NM_002432
210356_x_at	MS4A1	membrane-spanning 4-domains, subfamily A, member 1	BC002807
201959_s_at	MYCBP2	MYC binding protein 2	AA488899
218589_at	P2RY5	purinergic receptor P2Y, G-protein coupled, 5	NM_005767
213517_at	PCBP2	Poly(rC) binding protein 2	AW103422
203243_s_at	PDLIM5	PDZ and LIM domain 5	NM_006457
203879_at	PIK3CD	phosphoinositide-3-kinase, catalytic, delta polypeptide	U86453
219014_at	PLAC8	placenta-specific 8	NM_016619
213241_at	PLXNC1	plexin C1	AF035307
204284_at	PPP1R3C	protein phosphatase 1, regulatory (inhibitor) subunit 3C	N26005
204060_s_at	PRKX	protein kinase, X-linked	NM_005044
209606_at	PSCDBP	pleckstrin homology, Sec7 and coiled-coil domains, binding protein	L06633
204279_at	PSMB9	proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)	NM_002800
206060_s_at	PTPN22	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)	NM_015967
207238_s_at	PTPRC	protein tyrosine phosphatase, receptor type, C	NM_002838
212587_s_at	PTPRC	protein tyrosine phosphatase, receptor type, C	AI809341
204960_at	PTPRCAP	protein tyrosine phosphatase, receptor type, C-associated protein	NM_005608
207419_s_at	RAC2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	NM_002872
213603_s_at	RAC2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	BE138888
208206_s_at	RASGRP2	RAS guanyl releasing protein 2 (calcium and DAG-regulated)	NM_005825
213566_at	RNASE6	ribonuclease, RNase A family, k6	NM_005615
218499_at	RP6-213H19.1	serine/threonine protein kinase MST4	NM_016542
201476_s_at	RRM1	ribonucleotide reductase M1 polypeptide	AI692974
201477_s_at	RRM1	ribonucleotide reductase M1 polypeptide	NM_001033
212414_s_at	SEPT6	septin 6	D50918
212415_at	SEPT6	septin 6	AW150913
213666_at	SEPT6	septin 6	AK026589
218921_at	SIGIRR	single immunoglobulin and toll-interleukin 1 receptor (TIR) domain	NM_021805
204204_at	SLC31A2	solute carrier family 31 (copper transporters), member 2	NM_001860
217248_s_at	SLC7A8	solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	AL365343

213994_s_at	SPON1	spondin 1, extracellular matrix protein	AI885290
209875_s_at	SPP1	secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)	M83248
208610_s_at	SRRM2	serine/arginine repetitive matrix 2	AI655799
220114_s_at	STAB2	stabilin 2	NM_017564
216920_s_at	TARP	TCR gamma alternate reading frame protein	M27331
203085_s_at	TGFB1	transforming growth factor, beta 1	BC000125
213135_at	TIAM1	T-cell lymphoma invasion and metastasis 1	U90902
204529_s_at	TOX	thymocyte selection-associated high mobility group box	AI961231
210972_x_at	TRAC	T cell receptor alpha constant	M15565
205804_s_at	TRAF3IP3	TRAF3 interacting protein 3	NM_025228
204352_at	TRAF5	TNF receptor-associated factor 5	NM_004619
210915_x_at	TRBC1	T cell receptor beta constant 1	M15564
217826_s_at	UBE2J1	ubiquitin-conjugating enzyme E2, J1 (UBC6 homolog, yeast)	NM_016021
202546_at	VAMP8	vesicle-associated membrane protein 8 (endobrevin)	NM_003761
204620_s_at	VCAN	versican	NM_004385
211571_s_at	VCAN	versican	D32039
221731_x_at	VCAN	versican	BF218922
202664_at	WIPF1	WAS/WASL interacting protein family, member 1	AW058622
213655_at	YWHAE	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide	AA502643
212368_at	ZNF292	zinc finger protein 292	AA972711

CONCLUSION

Our data on the transcriptome analysis and comparison indicated a stable gene expression profile in humans adipose tissue, and a few interesting androgen-responsive genes and molecular pathways were identified in mice fat. Progesterone seems to generate little effects on abdominal fat cell differentiation in women, which is consistent with the high rates of inactivation observed in preadipocytes and lipid-storing adipocytes. Effects of OVX on abdominal adipose tissue development and metabolism may be influenced by other environmental factors such as diet. In general, these findings may provide novel information about the human adipose tissue transcriptome, the effects of sex hormones on adipose tissue gene expression profiles and metabolism in different animal models and cultured cells from humans.

Objective 1.

We used SC and OM adipose tissue samples from 10 obese men for microarray hybridizations, and measured expression levels for ~22,200 probesets to study the interindividual variability of gene expression in both depots. Our findings indicated that interindividual variability in gene expression in both depots in all subjects was rather low. In addition, no difference in the distribution of CVs was observed among fat depots. This provides evidence that gene expression within abdominal OM and SC adipose tissue samples is relatively homogenous; and indirectly suggests that primary characteristics of adipose tissue from both the SC and OM compartments are relatively similar. Several studies have now used microarrays to investigate gene expression profiling of adipose tissue in rodents (Boeuf et al., 2001; Boeuf et al., 2002; Bullen, Jr. et al., 2004; Lopez et al., 2003), and humans (Gomez-Ambrosi et al., 2004; Linder et al., 2004; Vohl et al., 2004). However, no study had examined human adipose tissue gene expression variability. Individual analyses of adipose tissue gene expression within a homogeneous study group or population might help to identify possible new functional links between different genes. This is the largest microarray study of human SC and OM fat performed to date and the first to provide information on the interindividual variability of gene expression in human adipose tissue.

In summary, our data demonstrated that interindividual variability of gene expression in abdominal SC and OM adipose tissue samples from obese men was rather low. Future studies are required to investigate relations between different phenotypes (such as obesity, insulin, blood lipids) and expression of these transcripts.

Objective 2.

This work represented the first microarray investigation on the effects of DHT treatment in retroperitoneal adipose tissue from a rodent model. A significant modulation of the adipose tissue transcriptome was observed in response to DHT in both male and female mice. A number of genes involved in distinct cellular functions were modulated in a sex-specific manner.

In male mice, we found that some genes related to cell cycle, DNA replication and recombination as well as repair, molecular transport, and cell death were up-regulated. Other genes involved in cellular function and maintenance were down-regulated. In female mice, some genes related to connective tissue development and function or tissue morphology were up-regulated, and some genes involved in cell cycle and cell death were down-regulated. Only four genes showed identical regulatory patterns in both male and female mice. These genes were Fkbp5, Cdkn1a, Gadd45g and Mt1. All these genes, when up-regulated, appear to be related to a negative regulation of adipocyte differentiation and adipogenesis.

Several other genes which were significantly modulated in the microarray analysis and positively validated by quantitative real-time RT-PCR in both male and female retroperitoneal fat samples were myogenic in nature. These genes included myosin light polypeptide kinase (Mylk), myosin heavy polypeptide 4 (Myh4), enolase 3 (Eno3), phosphofructokinase (Pfkm) and myosin heavy polypeptide 1 (Myh1). Similar findings of myogenic genes in fat were also reported in other studies (Lee and Kemp, 2006; Sasao et al., 2003; Zuk et al., 2002). Adipose tissue apparently shows mesenchymal plasticity for myogenic differentiation (Lee and Kemp, 2006), and adipocyte precursors and skeletal myoblasts are capable of differentiating along similar developmental lineages (Rink et al., 2006). Previous reports and the present findings support the notion that a strong relationship between myogenic and adipose genes may exist in adipose tissue of both male and female mice.

In this study, we used 6 time points to examine trends of gene modulation in mice from both sexes. Significant modulations were observed with microarray data using criteria described in the methods section. With respect to validation using RT-PCR, most previous studies used only two time points on a limited number of genes. In the present study, a very large number of genes were validated using RT-PCR on all 6 time points and in the vehicle group. Using a rather stringent validation procedure, our analysis indicates that microarray results are moderately reproducible when compared to quantitative real-time RT-

PCR over several time points. Indeed, only 37.0% and 26.5% of transcripts in male and female mice respectively showed identical time courses. Using only peak time vs. control comparisons to validate the direction of fold changes in gene expression, valid findings increased to 74.0% and 63.3% of modulated genes in male and female mice, respectively. Because we used the pooled samples, our analysis may have contributed to yield lower than expected reproducibility rates. The use of several arrays would have allowed us to use repeated measures ANOVA to identify significantly modulated genes. This may have led to higher reproducibility rates when comparing with RT-PCR results. We suggest, however, that the use of multiple time points and a rather stringent filtering procedure decreased the likelihood of identified false positives. Our results nevertheless suggest that careful validation on a different platform remains an absolute necessity (Liu et al., 2005).

In conclusion, our study indicates that microarray technology can be used to identify androgen-responsive genes in retroperitoneal adipose tissue from male and female mice. We found that DHT consistently stimulates genes that are associated with the regulation of adipogenesis in adipose tissue. This study also provides evidence to support the hypothesis that a myogenic response may be triggered by DHT in fat.

Objective 3.

Although the effects of progesterone on adipose tissue and adipocytes from humans (Belanger et al., 2002; Bjorntorp, 1997; Blanchette et al., 2005; Blouin et al., 2005) and animals (Lacasa et al., 2001; Mendes et al., 1985; Monjo et al., 2003; Rondinone et al., 1992; Shirling et al., 1981) have been extensively studied, this is the first report to take into account and document pathways of progesterone inactivation within adipose tissue. In the present study, we found that preadipocytes generate a complex mixture of 5α/5β, 20α and 3α/β-reduced metabolites. However, overall metabolite formation increased in differentiated adipocytes, with 20α-hydroxyprogeserone as the main metabolite. These findings further validate the detection of 20α-HSD, 3α/β-HSD and 5α-reductase activity in preadipocytes, and support the notion of a complex regulation of steroid action through locally-expressed steroid-converting enzymes. On the other hand, progesterone had no consistent effect on fat cell differentiation in the present study, which is rather consistent with the patterns of progesterone observed.

Through the reduction by aldo-keto reductase 1C (AKR1C) enzymes and 5α/5β reductase activity, progesterone was converted into a complex mixture of metabolites in preadipocytes. These results are consistent with previous studies in other cell types (Quinkler et al., 1999; Zhang et al., 2000). The finding

of a slight 5β-reductase activity in adipose cells is novel. Our finding of increased progesterone metabolism in differentiated cells suggests that differentiation of preadipocytes into lipid-storing mature adipocytes has effects on the pattern of progesterone metabolism. The modulation of progesterone inactivation rates or the formation of some specific progesterone metabolites in preadipocytes vs. mature adipose cells may reflect cell differentiation-related changes in steroid-converting enzymes, particularly AKR1C1.

Non-conjugated metabolites of progesterone such as allopregnanolone (5α-pregnane-3α-ol-20-one) and pregnanolone (5β-pregnane-3α-ol-20-one) were generated in preadipocytes. These steroids are considered as neurosteroids and potent positive modulators of gamma-aminobutyric acid type A (GABAA) receptors (Lambert et al., 2001; Majewska et al., 1986). Allopregnanolone effects on GABA receptors include mood alterations such as pubertal mood swings, prementstrual syndrome (PMS), postpartum blues, and perimenopause (Smith et al., 2007). In the present study, we found that the production of progesterone metabolites was significantly increased in differentiated adipocytes, and a trend for increased allopregnanolone production was found in differentiated adipocytes. It remains to be clearly established whether adipose tissue-derived progesterone metabolites play a role in modulating central nervous system responses. At the local level, adipose tissue has been shown to have benzodiazepine binding sites, and locally produced allopregnanolone may be a ligand for these receptors (Gonzalez Solveyra et al., 1988).

In conclusion, the differentiation of preadipocytes into mature adipocytes affects the pattern of progesterone metabolism. Preadipocytes generate a complex mixture of 5α/5β, 20α and 3α/β-reduced metabolites. Metabolite formation increased in mature adipocytes, and 20α-hydroxy-progesterone was the main metabolite. The efficient conversion of progesterone to inactive metabolites in adipose tissue and the low expression of progesterone receptor are concordant with the inconsistent effect of progesterone on fat cell differentiation in humans. The modulation of progesterone inactivation or the formation of some specific progesterone metabolites in preadipocytes vs. mature adipose cells may reflect cell differentiation-related changes in steroid-converting enzymes and differential sensitivity to progesterone action. Preadipocytes and lipid-storing, mature adipocytes efficiently generate progesterone metabolites in women, which is consistent with rather modest effects progesterone on abdominal fat cell differentiation.

Objective 4.

In the present study, we tested the hypothesis that OVX would be related to adipocyte measures and expression profiles which are indicative of the expected increase in visceral fat accumulation. The feeding regimen was hypocaloric, leading to a significant weight loss through the course of our study in both groups with a similarly parallel trend. LPL activity was significantly lower in OVX monkeys than in INT monkeys. In addition, isoproterenol- or forskolin-stimulated lipolysis tended to be increased in both mesenteric and SC adipocytes from the OVX compared to INT group. These differences were observed despite the fact that no difference was observed on adipocyte diameters of mesenteric and SC fat samples in INT vs. OVX monkeys. In addition, several genes related to adipose tissue metabolism and lipid sythesis were identified, and were found to be expressed at lower levels in OVX vs. INT animals. These findings are contrary to the expected effects of ovariectomy on fat accumulation, and we did not observe any indication of increased abdominal fat storage in OVX animals.

Our findings are opposite to the expected increase in visceral fat accumulation expected with impaired ovarian function. A specific feature of our study was that animals were on a negative energy balance. As mentioned, the negative energy imbalance likely has a greater impact on adipose tissue metabolism compared to ovariectomy. We suggest that this finding is important and relevant since the menopause-associated increase in visceral fat is usually considered as a given. We show here that there are physiological situations where the negative impact of ovariectomy on abdominal fat metabolism can be prevented. Our findings may also explain why the increase in abdominal fat at menopause is usually difficult to detect, requiring well-controlled longitudinal studies in large samples (Bjorkelund et al., 1996; Guthrie and Dennerstein, 2003). We suggest that variability induced by each woman's energy balance may represent an important confounder in studies on menopause and body fat distribution. From the clinical standpoint, our study suggests that controlling food intake may appear as a relevant approach to prevent abdominal obesity in menopausal women.

These results show for the first time that a negative energy imbalance will supersede the postulated effects of ovariectomy on abdominal fat accumulation in female cynomolgus monkeys. Our study also provides novel information on the abdominal adipose tissue transcriptome in a primate model.

In conclusion, results from our cross-sectional comparison suggest that ovariectomy was not acutely associated with increased abdominal adipose tissue adipogenesis and lipid storage in cynomolgus monkeys fed an identical hypocaloric regimen. This observation is contrary to expected effects of ovariectomy on abdominal fat accumulation. The fact that monkeys were not under *ad libitum* feeding conditions likely explains our finding. Our data provide novel insight on ovarian hormones modulation in the energy homeostasis. This work also represents the first investigation of the genome-wide effects of OVX in abdominal fat tissue from female cynomolgus monkeys.

PERSPECTIVE

Surveys of transcriptomics examining the gene expression profile in fat samples of humans and animals under different conditions were performed based on DNA microarray technology in my Ph.D. project. A few interesting genes and molecular pathways were identified in these studies to date. In the future, primarily using high-throughput techniques, we will make plans to further investigate and elucidate whether their functions or signals specifically correlate with development and proliferation of adipocytes as well as obesity-related complications. I believe that our genomic studies of the adipose tissue transcriptome may bring significant scientific contributions in obesity research.

REFERENCES

1. Adams,M., Montague,C.T., Prins,J.B., Holder,J.C., Smith,S.A., Sanders,L., Digby,J.E., Sewter,C.P., Lazar,M.A., Chatterjee,V.K., Rahilly,S., 1997, "Activators of peroxisome proliferator-activated receptor gamma have depot-specific effects on human preadipocyte differentiation.", J Clin Invest., vol. 100, 3149-3153.

2. Ahima,R.S. 2006, "Metabolic actions of adipocyte hormones: focus on adiponectin.", Obesity (Silver Spring), vol. 14 Suppl 1, 9S-15S.

3. Ajuwon,K.M. and Spurlock,M.E., 2005, "Adiponectin inhibits LPS-induced NF-kappaB activation and IL-6 production and increases PPARgamma2 expression in adipocytes.", Am J Physiol Regul Integr Comp Physiol, vol. 288, R1220-R1225.

4. Alley,D.E. and Chang,V.W., 2007, "The changing relationship of obesity and disability, 1988-2004.", JAMA, vol. 298, 2020-2027.

5. Anwar,A., McTernan,P.G., Anderson,L.A., Askaa,J., moody,G.C., Barnett,A.H., Eggo,M.C., Kumar,S., 2001, "Site-specific regulation of oestrogen receptor-alpha and -beta by oestradiol in human adipose tissue.", Diabetes Obes Metab, vol. 3, 338-349.

6. Arner,P. 2000, "Hunting for human obesity genes? Look in the adipose tissue!", Int J Obes Relat Metab Disord, vol. 24 Suppl 4, S57-S62.

7. Atzmon,G., Yang,X.M., Muzumdar,R., Ma,X.H., Gabriely,I., Barzilai,N., 2002, "Differential gene expression between visceral and subcutaneous fat depots.", Horm Metab Res, vol. 34, 622-628.

8. Bai,Y., Zhang,S., Kim,K.S., Lee,J.K., Kim,K.H., 1996, "Obese gene expression alters the ability of 30A5 preadipocytes to respond to lipogenic hormones.", J Biol Chem, vol. 271, 13939-13942.

9. Ballor,D.L. and Keesey,R.E., 1991, "A meta-analysis of the factors affecting exercise-induced changes in body mass, fat mass and fat-free mass in males and females.", Int J Obes, vol. 15, 717-726.

10. Belanger,C., Luu-The,V., Dupont,P., Tchernof,A., 2002, "Adipose tissue intracrinology: potential importance of local androgen/estrogen metabolism in the regulation of adiposity.", Horm Metab Res., vol. 34, 737-745.

11. Beltowski,J. 2003, "Adiponectin and resistin--new hormones of white adipose tissue.", Med Sci Monit, vol. 9, RA55-RA61.

12. Berkey,C.S., Rockett,H.R., Field,A.E., Gillman,M.W., Frazier,A.L., Camargo,C.A., Jr., Colditz,G.A., 2000, "Activity, dietary intake, and weight changes in a longitudinal study of preadolescent and adolescent boys and girls.", Pediatrics, vol. 105, E56.

13. Bessesen,D.H. 2008, "Update on obesity.", J Clin Endocrinol Metab, vol. 93, 2027-2034.

14. Birmingham,C.L., Muller,J.L., Palepu,A., Spinelli,J.J., Anis,A.H., 1999, "The cost of obesity in Canada.", CMAJ, vol. 160, 483-488.

15. Bjorkelund,C., Lissner,L., Andersson,S., Lapidus,L., Bengtsson,C., 1996, "Reproductive history in relation to relative weight and fat distribution.", Int J Obes Relat Metab Disord, vol. 20, 213-219.

16. Bjorntorp,P. 1996, "The regulation of adipose tissue distribution in humans.", Int J Obes Relat Metab Disord, vol. 20, 291-302.

17. Bjorntorp,P. 1997, "Hormonal control of regional fat distribution.", Hum Reprod, vol. 12, 21-25.

18. Blanchette,S., Blouin,K., Richard,C., Dupont,P., Luu-The,V., Tchernof,A., 2005, "Expression and activity of 20alpha-hydroxysteroid dehydrogenase (AKR1C1) in abdominal subcutaneous and omental adipose tissue in women.", J Clin Endocrinol Metab., vol. 90, 264-270.

19. Blouin,K., Blanchette,S., Richard,C., Dupont,P., Luu-The,V., Tchernof,A., 2005, "Expression and activity of steroid aldoketoreductases 1C in omental adipose tissue are positive correlates of adiposity in women.", Am J Physiol Endocrinol Metab., vol. 288, E398-404.

20. Blundell,J.E. and King,N.A., 1999, "Physical activity and regulation of food intake: current evidence.", Med Sci Sports Exerc, vol. 31, S573-S583.

21. Boeuf,S., Keijer,J., Franssen-Van Hal,N.L., Klaus,S., 2002, "Individual variation of adipose gene expression and identification of covariated genes by cDNA microarrays.", Physiol Genomics., vol. 11, 31-36.

22. Boeuf,S., Klingenspor,M., Van Hal,N.L., Schneider,T., Keijer,J., Klaus,S., 2001, "Differential gene expression in white and brown preadipocytes.Differential gene expression in white and brown preadipocytes.", Physiol Genomics., vol. 7, 15-25.

23. Boivin,A., Brochu,G., Marceau,P., Hould,F.S., Tchernof,A., 2007, "Regional differences in adipose tissue metabolism in obese men.", Metabolism, vol. 56, 533-540.

24. Bolander, F. F. 1989, *Molecular endocrinology* Academic Press, New York.

25. Bolduc,C., Larose,M., Lafond,N., Yoshioka,M., Rodrigue,M.A., Morissette,J., Labrie,C., Raymond,V., St-Amand,J., 2004a, "Adipose tissue transcriptome by serial analysis of gene expression.", Obes Res., vol. 12, 750-757.

26. Bolduc,C., Larose,M., Yoshioka,M., Ye,P., Belleau,P., Labrie,C., Morissette,J., Raymond,V., Labrie,F., St-Amand,J., 2004b, "Effects of dihydrotestosterone on adipose tissue measured by serial analysis of gene expression.", J Mol Endocrinol., vol. 33, 429-444.

27. Broberger,C. 2005, "Brain regulation of food intake and appetite: molecules and networks.", J Intern Med, vol. 258, 301-327.

28. Bronnegard,M., Ottosson,M., Boos,J., Marcus,C., Bjorntorp,P., 1994, "Lack of evidence for estrogen and progesterone receptors in human adipose tissue.", J Steroid Biochem Mol Biol, vol. 51, 275-281.

29. Bullen,J.W., Jr., Ziotopoulou,M., Ungsunan,L., Misra,J., Alevizos,I., Kokkotou,E., Maratos-Flier,E., Stephanopoulos G, Mantzoros CS, 2004, "Short-term resistance to diet-induced obesity in A/J mice is not associated with regulation of hypothalamic neuropeptides.", Am J Physiol Endocrinol Metab., vol. 287, E662-E670.

30. Campfield,L.A., Smith,F.J., Guisez,Y., Devos,R., Burn,P., 1995, "Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks.", Science, vol. 269, 546-549.

31. Carlson,L.A. 2005, "Nicotinic acid: the broad-spectrum lipid drug. A 50th anniversary review.", J Intern Med, vol. 258, 94-114.

32. Carnell,S. and Wardle,J., 2008, "Appetitive traits and child obesity: measurement, origins and implications for intervention.", Proc Nutr Soc, vol. 20, 1-13.

33. Chan,D.C., Watts,G.F., Sussekov,A.V., Barrett,P.H., Yang,Z., Hua,J., Song,S., 2004, "Adipose tissue compartments and insulin resistance in overweight-obese Caucasian men.", Diabetes Res Clin Pract., vol. 63, 77-85.

34. Christakis,N.A. and Fowler,J.H., 2007, "The spread of obesity in a large social network over 32 years.", N Engl J Med., vol. 357, 370-379.

35. Church,T.S., Earnest,C.P., Skinner,J.S., Blair,S.N., 2007, "Effects of different doses of physical activity on cardiorespiratory fitness among sedentary, overweight or obese postmenopausal women with elevated blood pressure: a randomized controlled trial.", JAMA, vol. 297, 2081-2091.

36. Cianflone,K., Xia,Z., Chen,L.Y., 2003, "Critical review of acylation-stimulating protein physiology in humans and rodents.", Biochim Biophys Acta, vol. 1609, 127-143.

37. Constance,C.M., Morgan,J.I.4., Umek,R.M., 1996, "C/EBPalpha regulation of the growth-arrest-associated gene gadd45.", Mol Cell Biol, vol. 16, 3878-3883.

38. Coppack,S.W., Fisher,R.M., Gibbons,G.F., Humphreys,S.M., McDonough,M.J., Potts,J.L., Frayn,K.N., 1990, "Postprandial substrate deposition in human forearm and adipose tissues in vivo.", Clin Sci (Lond), vol. 79, 339
348.

39. Dahlman,I. and Arner,P., 2007, "Obesity and polymorphisms in genes regulation human adipose tissue.", Int J Obes, vol. 31, 1629-1641.

40. Dahlman,I., Kaaman,M., Jiao,H., Kere,J., Laakso,M., Arner,P., 2005a, "The CIDEA gene V115F polymorphrism is associated with obesity in Swedish subjects.", Diabetes, vol. 54, 3032-3034.

41. Dahlman,I., Linder,K., Arvidsson Nordstrom,E., Andersson,I., Liden,J., Verdich,C., 2005b, "Changes in adipose tissue gene expression with energy-restricted diets in obese women.", Am J Clin Nutr, vol. 81, 1275-1285.

42. de Castro,J.M. 2004, "Genes, the environment and the control of food intake.", Br J Nutr, vol. 92, S59-S62.

43. De Pergola,G. 2001, "The adipose tissue metabolism: role of testosterone and dehydroepiandrosterone.", Int J Obes Relat Metab Disord, vol. 24, 59-63.

44. De Pergola,G., Xu,X., Yang,S., Giorgino R., Bjorntorp,P., 1990, "Up-regulation of androgen receptor binding in male rat fat pad adipose precursor cells exposed to testosterone: study in a whole cell assay system.", J Steroid Biochem Mol Biol, vol. 4, 553-558.

45. Deslypere,J.P., Verdonck,L., Vermeulen,A., 1985, "Fat tissue: a steroid reservoir and site of steroid metabolism.", J Clin Endocrinol Metab, vol. 61, 564-570.

46. Despres,J.P. and Lemieux,I., 2006, "Abdominal obesity and metabolic syndrome.", Nature, vol. 444, 881-887.

47. Despres,J.P., Moorjani,S., Lupien,P.J., Tremblay,A., Nadeau,A., Bouchard,C., 1990, "Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease.", Arteriosclerosis., vol. 10, 497-511.

48. Dhatariya,K.K. and Nair,K.S., 2003, "Dehydroepiandrosterone: is there a role for replacement?", Mayo Clin Proc, vol. 78, 1257-1273.

49. Dieudonne,M.N., Pecquery,R., Boumediene,A., Leneveu,M.C., Giudicelli,Y., 1998, "Androgen receptors in human preadipocytes and adipocytes: regional specificities and regulation by sex steroids.", Am J Physiol, vol. 274, C1645-C1652.

50. Dixon,T.M., Daniel,K.W., Farmer,S.R., Collins,S., 2001, "CCAAT/enhancer-binding protein alpha is required for transcription of the beta 3-adrenergic receptor gene during adipogenesis.", J Biol Chem, vol. 276, 722-728.

51. Djian,P., Roncari,A.K., Hollenberg,C.H., 1983, "Influence of anatomic site and age on the replication and differentiation of rat adipocyte precursors in culture.", J Clin Invest., vol. 72, 1200-1208.

52. Drolet,R., Belanger,C., Fortie,M., Huot,C., Mailloux,J., Legare,D., Tchernof,A., 2008, "Fat depot-specific impact of visceral obesity on adipocyte adiponectin release in women.", Obesity, vol. in press.

53. Eaton,D.K., Kann,L., Kinchen,S., Ross,J., Hawkins,J., Harris,W.A., Lowry,R., McManus,T., Chyen,D., Shanklin,S., Lim,C., Grunbaum,J.A., Wechsler,H., 2006, "Youth risk behavior surveillance--United States, 2005.", J Sch Health, vol. 76, 353-372.

54. Edens,N.K., Fried,S.K., Kral,J.G., Hirsch,J., Leibel,R.L., 1993, "In vitro lipid synthesis in human adipose tissue from three abdominal sites.", Am J Physiol, vol. 265, E374-E379.

55. Eisenmann,J.C., Bartee,R.T., Wang,M.Q., 2002, "Physical activity, TV viewing, and weight in U.S. youth: 1999 Youth Risk Behavior Survey.", Obes Res, vol. 10, 379-385.

56. Ekelund,U., Aman,J., Yngve,A., Renman,C., Westerterp,K., Sjostrom,M., 2002, "Physical activity but not energy expenditure is reduced in obese adolescents: a case-control study.", Am J Clin Nutr, vol. 76, 935-941.

57. El-Jack,A.K., Hamm,J.K., Pilch,P.F., Farmer,S.R., 1999, "Reconstitution of insulin-sensitive glucose transport in fibroblasts requires expression of both PPARgamma and C/EBPalpha.", J Biol Chem, vol. 274, 7946-7951.

58. Elbers,J.M., Asscheman,H., Seidell,J.C., Megens,J.A., Gooren,L.J., 1997, "Long-term testosterone administration increases visceral fat in female to male transsexuals.", J Clin Endocrinol Metab., vol. 82, 2044-2047.

59. Etherton,T.D. 2000, "The biology of somatotropin in adipose tissue growth and nutrient partitioning.", J Nutr, vol. 130, 2623-2625.

60. Falkenstein,E., Tillmann,H.C., Christ,M., Feuring,M., Wehling,M., 2000, "Multiple actions of steroid hormones--a focus on rapid, nongenomic effects.", Pharmacol Rev, vol. 52, 513-556.

61. Fan,W., Yanase,T., Nomura,M., Okabe,T., Goto,K., Sato,T., Kawano,H., Kato,S., Nawata,H., 2005, "Androgen receptor null male mice develop late-onset obesity caused by decreased energy expenditure and lipolytic activity but show normal insulin sensitivity with high adiponectin secretion.", Diabetes, vol. 54, 1000-1008.

62. Farmer,S.R. 2006, "Transcriptional control of adipocyte formation.", Cell Metab, vol. 4, 263-273.

63. Farnier,C., Krief,S., Blache,M., Diot-Dupuy,F., Mory,G., Ferre,P., Bazin,R., 2003, "Adipocyte functions are modulated by cell size change: potential involvement of an integrin/ERK signalling pathway.", Int J Obes Relat Metab Disord, vol. 27, 1178-1186.

64. Ferrerira,I., Snijder,M.B., Twisk,J.W., van Mechelen,W., Kemper,H.C., Seidell,J.C., Stehouwer,C.D., 2004, "Central fat mass versus peripheral fat and lean mass: opposite (adverse versus favorable) associations with arterial stiffness? The Amsterdam Growth and Health Longitudinal Study.", J Clin Endocrinol Metab, vol. 89, 2632-2639.

65. Folkerd,E.J. and James,V.H., 1982, "Studies on the activity of 17 beta-hydroxysteroid dehydrogenase in human adipose tissue.", J Steroid Biochem, vol. 16, 539-543.

66. Fonseca-Alaniz,M.H., Takada,J., Cardoso Alonso-Vale,M.I., Lima,F.B., 2007, "Adipose tissue as an endocrine organ: from theory to practice.", J Pediatr, vol. 83, S192-S203.

67. Food and Nutrition Board,I.o.M.o.t.N.A. 2002, "Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids.", Washington (DC), vol. National Academies Press.

68. Foretz,M., Guichard,C., Ferre,P., Foufelle,F., 1999, "Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes.", Proc Natl Acad Sci U S A, vol. 96, 12737-12742.

69. Foreyt,J.P., Goodrick,G.K., Reeves,R.S., Raynaud,A.S., 1993, "Response to free-living adults to behavioral treatment of obesity: attrition and compliance to exercise.", Behav Ther, vol. 24, 659-669.

70. Formiguera,X. and Canton,A., 2004, "Obesity: epidemiology and clinical aspects.", Best Pract Res Clin Gastroenterol, vol. 18, 1125-1146.

71. Franck,N., Stenkula,K.G., Ost,A., Lindstrom,T.S.P., Nystrom,F.H., 2007, "Insulin-induced GLUT4 translocation to the plasma membrane is blunted in large compared with small primary fat cells isolated from the same individual.", Diabetologia., vol. 50, 1716-1722.

72. Frayling,T.M., Timpson,N.J., Weedon,M.N., Zeggini,E., Freathy,R.M., Lindgren,C.M., Perry,J.R., Elliott,K.S., Lango,H., Rayner,N.W., Shields,B., Harries,L.W., Barrett,J.C., Ellard,S., Groves,C.J., Knight,B., Patch,A.M., et al, 2007, "A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity.", Science, vol. 316, 889-894.

73. Frayn,K.N. 2002, "Adipose tissue as a buffer for daily lipid flux.", Diabetologia, vol. 45, 1201-1210.

74. Frayn,K.N., Fielding,B.A., Karpe,F., 2005, "Adipose tissue fatty acid metabolism and cardiovascular disease.", Curr Opin Lipidol, vol. 16, 409-415.

75. Frayn,K.N., Karpe,F., Fielding,B.A., Macdonald,I.A., Coppack,S.W., 2003, "Integrative physiology of human adipose tissue.", Int J Obes Relat Metab Disord., vol. 27, 875-888.

76. Freedman,D.S., Khan,L.K., Serdula,M.K., Ogden,C.L., Dietz,W.H., 2006, "The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study.", Obesity (Silver Spring), vol. 14, 301-308.

77. French,S. and Robinson,T., 2003, "Fats and food intake.", Curr Opin Clin Nutr Metab Care, vol. 6, 629-634.

78. Friedman,J.M. and Halaas,J.L., 1998, "Leptin and the regulation of body weight in mammals.", Nature, vol. 395, 763-770.

79. Fruhbeck,G. and Gomez-Ambrosi,J., 2003, "Control of body weight: a physiologic and transgenic perspective.", Diabetologia., vol. 46, 143-172.

80. Fruhbeck,G., Gomez-Ambrosi,J., Muruzabal,F.J., Burrell,M.A., 2001, "The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation.", Am J Physiol Endocrinol Metab, vol. 280, E827-E847.

81. Fukuhara,A., Matsuda,M., Nishizawa,M., Segawa,K., Tanaka,M., Kishimoto,K., Matsuki,Y., Murakami,M., Ichisaka,T., Murakami,H., Watanabe,E., Takagi,T., Akiyoshi,M., Ohtsubo,T., Kihara,S., Yamashita,S., Makishima,M., Funahashi,T., Yamanaka,S., Hiramatsu,R., Matsuzawa,Y., Shimomura,I., 2005, "Visfatin: a protein secreted by visceral fat that mimics the effects of insulin.", Science, vol. 307, 426-430.

82. Gabrielsson,B.G., Johansson,J.M., Lonn,M., Jernas,M., Olbers,T., Peltonen,M., Larsson,I., Lonn,L., Sjostrom,L., Carlsson,B., Carlsson,L.M., 2003, "High expression of complement components in omental adipose tissue in obese men.", Obes Res., vol. 11, 699-708.

83. Garaulet,M., Perez-Llamas,F., Fuente,T., Zamora,S., Tebar,F.J., 2000, "Anthropometric, computed tomography and fat cell data in an obese population: relationship with insulin, leptin, tumor necrosis factor-alpha, sex hormone-binding globulin and sex hormones.", Eur J Endocrinol, vol. 143, 657-666.

84. Garrow,J.S. and Summerbell,C.D., 1995, "Meta-analysis: effect of exercise, with or without dieting, on the body composition of overweight subjects.", Eur J Clin Nutr, vol. 49, 1-10.

85. Ge,K. 2006, "The status and trends of obesity in China.", Int J Vitam Nutr Res, vol. 76, 247-252.

86. Gesta,S., bluher,M., Yamamoto,Y., Norris,A.W., Berndt,J., Kralisch,S., Boucher,J., Lewis,C., Kahn,C.R., 2006, "Evidence for a role of developmental genes in the origin of obesity and body fat distribution.", Proc Natl Acad Sci U S A., vol. 25, 6676-6681.

87. Gesta,S., Tseng,Y.H., Kahn,C.R., 2007, "Developmental origin of fat: tracking obesity to its source.", Cell, vol. 131, 242-256.

88. Gomez-Ambrosi,J., Catalan,V., Diez-Caballero,A., Martnez-Cruz,L.A., Gil,M.J., Garcia-Foncillas,J., Cienfuegos,J.A., Salvador,J., Mato,J.M., Fruhbeck,G., 2004, "Gene expression profile of omental adipose tissue in human obesity.", FASEB J., vol. 18, 215-217.

89. Gonzalez Solveyra,C., Romeo,H.E., Rosenstein,R.E., Estevez,A.G., Gardinali,D.P., 1988, "Benzodiazepine binding sites in rat interscapular brown adipose tissue: effect of cold environment, denervation and endocrine ablations.", Life Sci, vol. 42, 393-402.

90. Gonzalez,C.A. 2006, "Nutrition and cancer: the current epidemiological evidence.", Br J Nutr, vol. 96, S42-S45.

91. Gout,J., Tirard,J., Thevenon,C., Riou,J.P., Begeot,M., Naville,D., 2006, "CCAAT/enhancer-binding proteins (C/EBPs) regulate the basal and cAMP-induced transcription of the human 11beta-hydroxysteroid dehydrogenase encoding gene in adipose cells.", Biochimie, vol. 88, 1115-1124.

92. Graves,B.J., Johnson,P.F., McKnight,S.L., 1986, "Homologous recognition of a promoter domain common to the MSV LTR and the HSV tk gene.", Cell, vol. 44, 565-576.

93. Gray,J.M. and Wade,G.N., 1979, "Cytoplasmic progestin binding in rat adipose tissues.", Endocrinology, vol. 104, 1377-1382.

94. Greenberg,A.S., Egan,J.J., Wek,S.A., Garty,N.B., Blanchette-mackie,E.J., Londos,C., 1991, "Perilipin, a major hormonally regulated adipocyte-specific phosphoprotein associated with the periphery of lipid storage droplets.", J Biol Chem, vol. 266, 11341-11346.

95. Gregg,E.W., Cheng,Y.J., Cadwell,B.L., Imperatore,G., Williams,D.E., Flegal,K.M., Narayan,K.M., Williamson,D.F., 2005, "Secular trends in cardiovascular disease risk factors according to body mass index in US adults.", JAMA, vol. 293, 1868-1874.

96. Gupta,V., Bhasin,S., Guo,W., Singh,R., Miki,R., Choong,K., Tchkonia,T., Lebrasseur,N.K., Flanagan,J.N., Hamilton,J.A., Viereck,J.C., Narula,N.S., Kirkland,J.L., Jasuja,R., 2008, "Effects of dihydrotestosterone on differentiation and proliferation of human mesenchymal stem cells and preadipocytes.", Mol Cell Endocrinol in press.

97. Guthrie,J.R. and Dennerstein,L., 2003, "Studying the menopausal transition: methodological considerations.", Climacteric, vol. 6, 354-356.

98. Halaas,J.L., Gajiwala,K.S., Maffei,M., Cohen,S.L., Chait,B.T., Rabinowitz,D., Lallone,R.L., Burley,S.K., Friedman,J.M., 1995, "Weight-reducing effects of the plasma protein encoded by the obese gene.", Science, vol. 269, 543-546.

99. Hamosh,M. and Hamosh,P., 1975, "The effect of estrogen on the lipoprotein lipase activity of rat adipose tissue.", J Clin Invest., vol. 55, 1132-1135.

100. Hauner,H. and Entenmann,G., 1991, "Regional variation of adipose differentiation in cultured stromal-vascular cells from the abdominal and femoral adipose tissue of obese women.", Int J Obes., vol. 15, 121-126.

101. Hauner,H., Skurk,T., Wabitsch,M., 2001, "Cultures of human adipose precursor cells.", Methods Mol Biol, vol. 155, 239-247.

102. Hemati,N., Ross,S.E., Erickson,R.L., Groblewski,G.E., MacDougald,O.A., 1997, "Signaling pathways through which insulin regulates CCAAT/enhancer binding protein alpha (C/EBPalpha) phosphorylation and gene expression in 3T3-L1 adipocytes. Correlation with GLUT4 gene expression.", J Biol Chem, vol. 272, 25913-25919.

103. Henry,B.A. and Clarke,I.J., 2008, "Adipose tissue hormones and regulation of food intake.", J Neuroendocrinol, vol. 20, 842-849.

104. Henry,B.A., Goding,J.W., Alexander,W.S., Tilbrook,A.J., Canny,B.J., Dunshea,F., Rao,A., Mansell,A., Clarke,I.J., 1999, "Central administration of leptin to ovariectomized ewes inhibits food intake without affecting the secretion of hormones from the pituitary gland: evidence for a dissociation of effects on appetite and neuroendocrine function.", Endocrinology, vol. 140, 1175-1182.

105. Hernandez-Morante,J.J., Milagro,F., Gabaldon,J.A., Martinez,J.A., Zamora,S., Garaulet,M., 2006, "Effect of DHEA-sulfate on adiponectin gene expression in adipose tissue from different fat depots in morbidly obese humans.", Eur J Endocrinol, vol. 155, 593-600.

106. hernandez-Morante,J.J., Perez-de-Heredia,F., Lujan,J.A., Zamora,S., Garaulet,M., 2008, "Role of DHEA-S on body fat distribution: Gender- and depot-specific stimulation of adipose tissue lipolysis.", Steroids, vol. 73, 209-215.

107. Heymsfield,S.B., Greenberg,A.S., Fujioka,K., Dixon,R.M., Kushner,R., Hunt,T., Lubina,J.A., Patane,J., Self,B., Hunt,P., McCamish,M., 1999, "Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial.", JAMA, vol. 282, 1568-1575.

108. Hill,J.O. and Peters,J.C., 1998, "Environmental contributions to the obesity epidemic", Science, vol. 280, 1371-1374.

109. Hillebrand,J.J., de Wied,D., Adan,R.A., 2002, "Neuropeptides, food intake and body weight regulation: a hypothalamic focus.", Peptides, vol. 23, 2283-2306.

110. Hinney,A., Nguyen,T.T., Scherag,A., Friedel,S., Bronner,G., Muller,T.D., Grallert,H., Illig,T., Wichmann,H.E., Rief,W., Schafer,H., Hebebrand,J., 2007, "Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants.", PLos ONE, vol. 2, e1361.

111. Hollenberg,A.N., Susulic,V.S.M.J.P., Zhang,B., Moller,D.E., Tontonoz,P., Sarraf,P., Spiegelman,B.M., Lowell,B.B., 1997, "Functional antagonism between CCAAT/Enhancer binding protein-alpha and peroxisome proliferator-activated receptor-gamma on the leptin promoter.", J Biol Chem, vol. 272, 5283-5290.

112. Horowitz,J.F. 2003, "Fatty acid mobilization from adipose tissue during exercise.", Trends Endocrinol Metab, vol. 14, 386-392.

113. Hossain,P., Kawar,B., El Nahas,M., 2007, "Obesity and diabetes in the developing world--a growing challenge.", N Engl J Med., vol. 356, 213-215.

114. Hotamisligil,G.S. and Spiegelman,B.M., 1994, "Tumor necrosis factor alpha: a key component of the obesity-diabetes link.", Diabetes, vol. 43, 1271-1278.

115. Hu,E., Zhu,Y., Fredrickson,T., Barnes,M., Kelsell,D., Beeley,L., Brooks,D., 1998, "Tissue restricted expression of two human Frzbs in preadipocytes and pancreas.", Biochem Biophys Res Commun, vol. 247, 287-293.

116. Hwang,C.S., Mandrup,S., MacDougald,O.A., Geiman,D.E., Lane,M.D., 1996, "Transcriptional activation of the mouse obese (ob) gene by CCAAT/enhancer binding protein alpha.", Proc Natl Acad Sci U S A, vol. 93, 873-877.

117. Ibrahimi,A., Bertrand,B., Bardon,S., Amri,E.Z., Grimaldi,P., Ailhaud,G., Dani,C., 1993, "Cloning of alpha 2 chain of type VI collagen and expression during mouse development.", Biochem J, vol. 289, 141-147.

118. Jernas,M., Palming,J., Sjoholm,K., Jennische,E., Svensson,P.A., Gabrielsson,B.G., Levin,M., Sjogren,A.R.M., Lystig,T.C., Carlsson,B., Carlsson,L.M., Lonn,M., 2006, "Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression.", FASEB J, vol. 20, 1540-1542.

119. Jitrapakdee,S., Slawik,M., Medina-Gomez,G., Campbell,M., Wallace,J.C., Sethi,J.K., O'Rahilly,S., Vidal-Puig,A.J., 2005, "The peroxisome proliferator-activated receptor-gamma regulates murine pyruvate carboxylase gene expression in vivo and in vitro.", J Biol Chem, vol. 280, 27466-27476.

120. Jolliffe,D. 2004, "Extent of overweight among US children and adolescents from 1971 to 2000.", Int J Obes Relat Metab Disord, vol. 28, 4-9.

121. Juhan-Vague,I., Morange,P.E., Alessi,M.C., 2002, "The insulin resistance syndrome: implications for thrombosis and cardiovascular disease.", Pathophysiol Haemost Thromb, vol. 32, 269-273.

122. Jump,D.B., Clarke,S.D., Thelen,A., Liimatta,M., 1994, "Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids.", J Lipid Res, vol. 35, 1076-1084.

123. Katzmarzyt,P.T. and Ardern,C.I., 2004, "Overweight and obesity mortality trends in Canada, 1985-2000.", Can J Public Health, vol. 95, 16-20.

124. Katzmarzyt,P.T. and Janssen,I., 2004, "The economic costs associated with physical inactivity and obesity in Canada: an update.", Can J Appl Physiol, vol. 29, 90-115.

125. Katzmarzyt,P.T., Janssen,I., Ardern,C.I., 2003, "Physical inactivity, excess adiposity and premature mortality.", Obes Res, vol. 4, 259-290.

126. Kawabe,H., Murata,K., Shibata,H., Hirose,H., Tsujioka,M., Saito,I., Saruta,T., 2000, "Participation in school sports clubs and related effects on cardiovascular risk factors in young males.", Hypertens Res, vol. 23, 227-232.

127. Kelly,M.J. and levin,E.R., 2001, "Rapid actions of plasma membrane estrogen receptors.", Trends Endocrinol Metab, vol. 12, 152-156.

128. Kershaw,E.E. and Flier,J.S., 2004, "Adipose tissue as an endocrine organ.", J Clin Endocrinol Metab., vol. 89, 2548-2556.

129. Kershaw,E.E., Morton,N.M., Dhillon,H., Ramage,L., Seckl,J.R., Flier,J.S., 2005, "Adipocyte-specific glucocorticoid inactivation protects against diet-induced obesity.", Diabetes, vol. 54, 1023-1031.

130. Kersten,S. 2001, "Mechanisms of nutritional and hormonal regulation of lipogenesis.", EMBO Rep, vol. 2, 282-286.

131. Kim,H.J. and Kalkhoff,R.K., 1975, "Sex steroid influence on triglyceride metabolism.", J Clin Invest, vol. 56, 888-896.

132. Kirkland,J.L., Hollenberg,C.H., Gillon,W.S., 1990, "Age, anatomic site, and the replication and differentiation of adipocyte precursors.", Am J Physiol., vol. 258, C206-C210.

133. Kishi,T. and Elmquist,J.K., 2005, "Body weight is regulated by the brain: a link between feeding and emotion.", Mol Psychiatry, vol. 10, 132-146.

134. Koenig,S.M. 2001, "Pulmonary complications of obesity.", Am J Med Sci, vol. 321, 249-279.

135. Kuk,J.L., Lee,S., Heymsfield,S.B., Ross,R., 2005, "Waist circumference and abdominal adipose tissue distribution: influence of age and sex.", Am J Clin Nutr., vol. 81, 1330-1334.

136. Kumanyika,S., Jeffery,R.W., Morabia,A., Ritenbaugh,C., Antipatis,V.J., Public Health Approaches to the Prevention of Obesity (PHAPO) Working Group of the International Obesity Task Force (IOTF), 2002, "Obesity prevention: the case for action.", Int J Obes Relat Metab Disord, vol. 26, 425-436.

137. Kumanyika,S.K., Obarzanek,E., Stettler,N., Bell,R., Field,A.E., Fortmann,S.P., Franklin,B.A., Gillman,M.W., Lewis,C.E., Poston,W.C.2., Stevens,J., Hong,Y., American Heart Association Council on Epidemiology and Prevention, Interdisciplinary Committee for Prevention, 2008, "Population-based prevention of obesity: the need for comprehensive promotion of healthful eating, physical activity, and energy balance: a scientific statement from American Heart Association Council on Epidemiology and Prevention, Interdisciplinary Committee for Prevention (formerly the expert panel on population and prevention science).", Circulation, vol. 118, 428-464.

138. Kussmann,M. and Affolter,M., 2006, "Proteomic methods in nutrition.", Curr Opin Clin Nutr Metab Care, vol. 9, 575-583.

139. Lacasa,D., Le Liepvre,X., Ferre,P., Dugail,I., 2001, "Progesterone stimulates adipocyte determination and differentiation 1/sterol regulatory element-binding protein 1c gene expression.", J Biol Chem., vol. 276, 11512-11516.

140. Lafontan,M., Moro,C., Berlan,M., Crampes,F., Sengenes,C., Galitzky,J., 2008, "Control of lipolysis by natriuretic peptides and cyclic GMP.", Trends Endocrinol Metab, vol. 19, 130-137.

141. Lambert,J.J., Harney,S.C., Belelli,D., Peters,J.A., 2001, "Neurosteroid modulation of recombinant and synaptic GABAA receptors.", Int Rev Neurobiol, vol. 46, 177-205.

142. Landschulz,W.H., Johnson,P.F., Adashi,E.Y., Graves,B.J., McKnight,S.L., 1988, "Isolation of a recombinant copy of the gene encoding C/EBP.", Genes Dev, vol. 2, 780-800.

143. Langin,D. and Arner,P., 2006, "Importance of TNFalpha and neutral lipases in human adipose tissue lipolysis.", Trends Endocrinol Metab, vol. 17, 314-320.

144. Langin, D. & lafontan, M. 2004, "Lipolysis and lipid mobilization in human adipose tissue," in *Handbook of Obesity. Etiology and Pathophysiology*, 2nd edn, G. A. Bray & C. Bouchard, eds., Marcel Dekker, pp. 515-532.

145. Lazzer,S., Boirie,Y., Bitar,A., Montaurier,C., Vernet,J., Meyer,M., Vermorel,M., 2003, "Assessment of energy expenditure associated with physical activities in free-living obese and nonobese adolescents.", Am J Clin Nutr, vol. 78, 471-479.

146. Lea-Currie,Y.R., Wen,P., McIntosh,M.K., 1997, "Dehydroepiandrosterone-sulphate (DHEA-S) reduces adipocyte hyperplasia associated with feeding rats a high-fat diet.", Int J Obes Relat Metab Disord, vol. 21, 1058-1064.

147. Lea-Currie,Y.R., Wen,P., McIntosh,M.K., 1998, "Dehydroepiandrosterone reduces proliferation and differentiation of 3T3-L1 preadipocytes.", Biochem Biophys Res Commun, vol. 248, 497-504.

148. Lean,M., Lara,J., Hill,J.O., 2006, "ABC of obesity. Strategies for preventing obesity.", BMJ, vol. 333, 959-962.

149. Lee,G.H. and Kemp,D.M., 2006, "Human adipose-derived stem cells display myogenic potential and perturbed function in hypoxic conditions.", Biochem Biophys Res Commun., vol. 341, 882-888.

150. Leibowitz,S.F. and Wortley,K.E., 2004, "Hypothalamic control of energy balance: different peptides, different functions.", Peptides, vol. 25, 473-504.

151. Lekstrom-Himes,J. and Xanthopoulos,K.G., 1998, "Biological role of the CCAAT/enhancer-binding protein family of transcription factors.", J Biol Chem, vol. 273, 28545-28548.

152. Lemieux,I., Pascot,A., Prud'homme,D., Almeras,N., Bogaty,P., Nadeau,A., Bergeron,J., Despres,J.P., 2001, "Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity.", Arterioscler Thromb Vasc Biol., vol. 21, 961-967.

153. Lemieux,S., Prud'homme,D., Bouchard,C., Tremblay,A., Despres,J.P., 1993, "Sex differences in the relation of visceral adipose tissue accumulation to total body fatness.", Am J Clin Nutr., vol. 58, 463-467.

154. Levine,J.A. 2008, "Obesity in China: causes and solutions.", Chin Med J (Engl), vol. 121, 1043-1050.

155. Levine,J.A., Schleusner,S.J., Jensen,M.D., 2000, "Energy expenditure of nonexercise activity.", Am J Clin Nutr, vol. 72, 1451-1454.

156. Li,M. and Bjorntorp,P., 1995, "Effects of testosterone on triglyceride uptake and mobilization in different adipose tissues in male rats in vivo. ", Obes Res., vol. 3, 113-119.

157. Linder,K., Arner,P., Flores-Morales,A., Tollet-Egnell,P., Norstedt,G., 2004, "Differentially expressed genes in visceral or subcutaneous adipose tissue of obese men and women.", J Lipid Res., vol. 45, 148-154.

158. Linhart,H.G., Ishimura-Oka,K., DeMayo,F., Kibe,T., Repka,D., Poindexter,B., Bick,R.J., Darlington,G.J., 2001, "C/EBPalpha is required for differentiation of white, but not brown, adipose tissue.", Proc Natl Acad Sci U S A, vol. 98, 12532-12537.

159. Liu,L., Ikeda,K., Chen,M., Yin,W., Mizushima,S., Miki,T., Nara,Y., Yamori,Y., 2004, "Obesity, emerging risk in China: trend of increasing prevalence of obesity and its association with hypertension and hypercholesterolaemia among the Chinese.", Clin Exp Pharmacol Physiol, vol. 31 Suppl 2, S8-S10.

160. Liu,Y.Z., Dvornyk,V., Lu,Y., Shen,H., Lappe,J.M., Recker,R.R., Deng,H.W., 2005, "A novel pathophysiological mechanism for osteoporosis suggested by an in vivo gene expression study of circulating monocytes.", J Biol Chem, vol. 280, 29011-29016.

161. Longcope,C. and Fineberg,S.E., 1985, "Production and metabolism of dihydrotestosterone in peripheral tissues.", J Steroid Biochem, vol. 23, 415-419.

162. Loos,R.J. and Bouchard,C., 2008, "FTO: the first gene contributing to common forms of human obesity.", Obes Rev, vol. 9, 246-250.

163. Lopez,I.P., Marti,A., Milagro,F.I., Zulet Md Mde,L., Moreno-Aliaga,M.J., Martinez,J.A., De Miguel,C., 2003, "DNA microarray analysis of genes differentially expressed in diet-induced (cafeteria) obese rats.", Obes Res., vol. 11, 188-194.

164. Macek,M., Bell D., Rutenfranz,J., Vavra,J., Masopust,J., Neidhart,B., Schmidt,K.H., 1989, "A comparison of coronary risk factors in groups of trained and untrained adolescents.", Eur J Appl Physiol Occup Physiol, vol. 58, 577-582.

165. Maffei,M., Fei,H., Lee,G.H., Dani,C., Leroy,P., Zhang,Y., Proenca,R., Negrel,R., Ailhaud,G., Friedman,J.M., 1995, "Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus.", Proc Natl Acad Sci U S A., vol. 92, 6957-6960.

166. Majewska,M.D., Harrison,N.L., Schwartz,R.D., Barker,J.L., Paul,S.M., 1986, "Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor.", Science, vol. 232, 1004-1007.

167. Marin,P., Oden,B., Bjorntorp,P., 1995, "Assimilation and mobilization of triglycerides in subcutaneous abdominal and femoral adipose tissue in vivo in men: effects of androgens.", J Clin Endocrinol Metab, vol. 80, 239-243.

168. Marin,P., Rebuffe-Scrive,M., Bjorntorp,P., 1990, "Uptake of triglyceride fatty acids in adipose tissue in vivo in man.", Eur J Clin Invest, vol. 20, 158-165.

169. Mattsson,C. and Olsson,T., 2007, "Estrogens and glucocorticoid hormones in adipose tissue metabolism.", Curr Med Chem, vol. 14, 2918-2924.

170. Mayes,J.S., McCann,J.P., Ownbey,T.C., Watson,G.H., 1996, "Regional differences and up-regulation of progesterone receptors in adipose tissues from oestrogen-treated sheep.", J Endocrinol, vol. 148, 19-25.

171. Mayes,J.S. and Watson,G.H., 2004, "Direct effects of sex steroid hormones on adipose tissues and obesity.", Obes Res 197-216.

172. McCann,J.P., Mayes,J.S., Hendricks,G.R., Harjo,J.B., Watson,G.H., 2001, "Subcellular distribution and glycosylation pattern of androgen receptor from sheep omental adipose tissue.", J Endocrinol, vol. 169, 587-593.

173. McClean,K.M., Kee,F., Young,I.S., Elbom,J.S., 2008, "Obesity and the lung: 1. Epidemiology.", Thorax, vol. 63, 649-654.

174. Meier,U. and Gressner,A.M., 2004, "Genes, the environment and the control of food intake.", Clin Chem, vol. 50, 1511-1525.

175. Mendes,A.M., Madon,R.J., Flint,D.J., 1985, "Effects of cortisol and progesterone on insulin binding and lipogenesis in adipocytes from normal and diabetic rats.", J Endocrinol, vol. 106, 225-231.

176. Meyer,J.E. and Pudel,V., 1972, "Experimental studies on food-intake in obese and normal weight subjects.", J Psychosom Res, vol. 16, 305-308.

177. Middleton,F.A., Ramos,E.J., Xu,Y., Diab,H., hao,X., as,U.N., eguid,M., 2004, "Application of genomic technologies: DNA microarrays and metabolic profiling of obesity in the hypothalamus and in subcutaneous fat.", Nutrition, vol. 20, 14-25.

178. Miller,G.D., Nicklas,B.J., Davis,C.C., Ambrosius,W.T., Loeser,R.F., Messier,S.P., 2004, "Is serum leptin related to physical function and is it modifiable through weight loss and exercise in older adults with knee osteoarthritis?", Int J Obes Relat Metab Disord, vol. 28, 1383-1390.

179. Miller,W.C., Koceja,D.M., Hamilton,E.J., 1997, "A meta-analysis of the past 25 years of weight loss research using diet, exercise or diet plus exercise intervention.", Int J Obes Relat Metab Disord, vol. 21, 941-947.

180. Mizutani,T., Nishikawa,Y., Adachi,H., Enomoto,T., Ikegami,H., Kurachi,H., Nomura,T., Miyake,A., 1994, "Identification of estrogen receptor in human adipose tissue and adipocytes.", J Clin Endocrinol Metab, vol. 78, 950-954.

181. Mokdad,A.H., Marks,J.S., Stroup,D.F., Gerberding,J.L., 2004, "Actual causes of death in the United States, 2000.", JAMA., vol. 291, 1238-1245.

182. Monjo,M., Rodriguez,A.M., Palou,A., Roca,P., 2003, "Direct effects of testosterone, 17 beta-estradiol, and progesterone on adrenergic regulation in cultured brown adipocytes: potential mechanism for gender-dependent thermogenesis.", Endocrinology., vol. 144, 4923-4930.

183. Moore,L.L., Gao,D., Bradlee,M.L., Cupples,L.A., Sundarajan-Ramamurti,A., Proctor,M.H., Hood,M.Y., Singer,M.R., 2003, "Does early physical activity predict body fat change throughout childhood?", Prev Med, vol. 37, 10-17.

184. Morisset,A.S., Huot,C., Legare,D., Tchernof,A., 2008, "Circulating IL-6 concentrations and abdominal adipocyte isoproterenol-stimulated lipolysis in women.", Obesity (Silver Spring), vol. 16, 1487-1492.

185. National Institutes of Health 2008, "1998 Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: the evidence report.", Obes Res, vol. 6, 51S-209S.

186. Nawata,H., Yanase,T., Goto,K., Okabe,T., Ashida,K., Watanabe,T., 2004, "Adrenopause.", Horm Res, vol. 62, 110-114.

187. Neel,J.V. 1962, "Diabetes mellitus: a "thrifty" genotype rendered detrimental by "progress"?", Am J Hum Genet, vol. 14, 353-362.

188. Nielsen,S., Guo,Z., Johnson,C.M., Hensrud,D.D., Jensen,M.D., 2004, "Splanchnic lipolysis in human obesity.", J Clin Invest, vol. 113, 1582-1588.

189. Nilsson-Ehle,P. 1981, "Impaired regulation of adipose tissue lipoprotein lipase in obesity.", Int J Obes, vol. 5, 695-699.

190. O'Brien,S.N., Welter,B.H., Mantzke,K.A., Price,T.M., 1998, "Identification of progesterone receptor in human subcutaneous adipose tissue.", J Clin Endocrinol Metab, vol. 83, 509-513.

191. Ogden,C.L., Carroll,M.D., Curtin,L.R., McDowell,M.A., Tabak,C.J., Flegal,K.M., 2006, "Prevalence of overweight and obesity in the United States, 1999-2004.", JAMA, vol. 295, 1549-1555.

192. Ogden,C.L., Yanovski,S.Z., Carroll,M.D., Flegal,K.M., 2007, "The epidemiology of obesity.", Gastroenterology, vol. 132, 2087-2102.

193. Okura,T., Nakata,Y., Yamabuki,K., Tanaka,K., 2004, "Regional body composition changes exhibit opposing effects on coronary heart disease risk factors.", Arterioscler Thromb Vasc Biol, vol. 24, 923-929.

194. Ouchi,N., Kihara,S., Funahashi,T., Nakamura,T., Nishida,M., Kumada,M., Okamoto,Y., Ohashi,K., Nagaretani,H., Kishida,K., nishizawa,H., maeda,N., Kobayashi,H., Hiraoka,H., Matsuzawa,Y., 2003, "Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue.", Circulation, vol. 107, 671
674.

195. Pate,R.R., Pratt,M., Blair,S.N., Haskell,W.L., Macera,C.A., Bouchard,C., Buchner,D., Ettinger,W., Heath,G.W., King,A.C., et al, 1995, "Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine.", JAMA, vol. 273, 402-407.

196. Patel,M.S. and Srinivasan,M., 2002, "Metabolic programming: causes and consequences.", J Biol Chem, vol. 277, 1629-1632.

197. Payne,V.A., Au,W.S., Gray,S.L., Nora,E.D., Rahman,S.M., Sanders,R., Hadaschik,D., Friedman,J.E., O'Rahilly,S., Rochford,J.J., 2007, "Sequential regulation of diacylglycerol acyltransferase 2 expression by CAAT/enhancer-binding protein beta (C/EBPbeta) and C/EBPalpha during adipogenesis.", J Biol Chem, vol. 282, 21005-21014.

198. Pedersen,S.B., Borglum,J.D., Moller-Pedersen,T., Richelsen,B., 1992, "Effects of in vivo estrogen treatment on adipose tissue metabolism and nuclear estrogen receptor binding in isolated rat adipocytes.", Mol Cell Endocrinol, vol. 85, 13-19.

199. Pedersen,S.B., Fuglsig,S., Sjogren,P., Richelsen,B., 1996, "Identification of steroid receptors in human adipose tissue.", Eur J Clin Invest, vol. 26, 1051-1056.

200. Peelman,F., Waelput,W., Iserentant,H., Lavens,D., Eyckerman,S., Zabeau,L., Tavernier,J., 2004, "Leptin: linking adipocyte metabolism with cardiovascular and autoimmune diseases.", Prog Lipid Res, vol. 43, 283-301.

201. Peeters,A., Barendregt,J.J., Willekens,F., Mackenbach,J.P., Al Mamun,A., Bonneux,L., NEDCOM,t.N.E.a.D.C.o.M.R.G., 2003, "Obesity in adulthood and its consequences for life expectancy: a life-table analysis.", Ann Intern Med, vol. 138, 24-32.

202. Peeters,A., Beckers,S., Verrijken,A., Roevens,P., Peeters,P., van Gaal,L., Van Hul,W., 2008, "Variants in the FTO gene are associated with common obesity in the Belgian population.", Mol Genet Metab, vol. 93, 481-484.

203. Perez de heredia,F., Cerezo,D., Zamora,S., Garaulet,M., 2007, "Effect of dehydroepiandrosterone on protein and fat digestibility.", Br J Nutr, vol. 97, 464-470.

204. Piche,M.E., Lapointe,A., Weisnagel,S.J., Corneau,L., Nadeau,A., Bergeron,J., Lemieux,S., 2008, "Regional body fat distribution and metabolic profile in postmenopausal women.", Metabolism, vol. 57, 1101-1107.

205. Pietras,R.J., Nemere,I., Szego,C.M., 2001, "Steroid hormone receptors in target cell membranes.", Endocrine, vol. 14, 417-427.

206. Pond,C.M. 1992, "An evolutionary and functional view of mammalian adipose tissue.", Proc Nutr Soc, vol. 51, 367-377.

207. Proctor,M.H., Moore,L.L., Gao,D., Cupples,L.A., Bradlee,M.L., Hood,M.Y., Ellison,R.C., 2003, "Television viewing and change in body fat from preschool to early adolescence: The Framingham Children's Study.", Int J Obes Relat Metab Disord, vol. 27, 827-833.

208. Puche,C., Jose,M., Cabero,A., Meseguer,A., 2002, "Expression and enzymatic activity of the P450c17 gene in human adipose tissue.", Eur J Endocrinol, vol. 146, 223-229.

209. Qi,Y., Takahashi,N., Hileman,S.M., Patel,H.R., Berg,A.H., Pajvani,U.B., Scherer,P.E., Ahima,R.S., 2004, "Adiponectin acts in the brain to decrease body weight.", Nat Med, vol. 10, 524-529.

210. Qiao,L., Maclean,P.S., Schaack,J., Orlicky,D.J., Darimont,C., Pagliassotti,M., Friedman,J.E., Shao,J., 2005, "C/EBPalpha regulates human adiponectin gene transcription through an intronic enhancer.", Diabetes, vol. 54, 1744-1754.

211. Quinkler,M., Johanssen,S., Grossmann,C., Bahr,V., Muller,M., Oelkers,W., Diederich,S., 1999, "Progesterone metabolism in the human kidney and inhibition of 11beta-hydroxysteroid dehydrogenase type 2 by progesterone and its metabolites.", J Clin Endocrinol Metab, vol. 84, 4165-4171.

212. Raitakari,O.T., Taimela,S., Porkka,K.V., Telama,R., Valimaki,I., Akerblom,H.K., Viikari,J.S., 1997, "Associations between physical activity and risk factors for coronary heart disease: the Cardiovascular Risk in Young Finns Study.", Med Sci Sports Exerc, vol. 29, 1055-1061.

213. Rebuffe-Scrive,M., Basdevant,A., Guy-Grand,B., 1983, "Effect of local application of progesterone on human adipose tissue lipoprotein lipase.", Horm Metab Res, vol. 15, 566.

214. Rebuffe-Scrive,M., Bronnegard,M., Nilsson,A., Eldh,J., Gustafsson,J.A., Bjorntorp,P., 1990, "Steroid hormone receptors in human adipose tissues.", J Clin Endocrinol Metab, vol. 71, 1215-1219.

215. Reinehr,T., Roth,C., Menke,T., Andler,W., 2004, "Adiponectin before and after weight loss in obese children.", J Clin Endocrinol Metab, vol. 89, 3790-3794.

216. Reynisdottir,S., dauzats,M., Thome,A., Langin,D., 1997, "Comparison of hormone-sensitive lipase activity in visceral and subcutaneous human adipose tissue.", J Clin Endocrinol Metab, vol. 82, 4162-4166.

217. Reynolds,K., Gu,D., Whelton,P.K., Wu,X., Duan,X., Mo,J., He,J., InterASIA Collaborative Group, 2007, "Prevalence and risk factors of overweight and obesity in China.", Obesity (Silver Spring), vol. 15, 10-18.

218. Richelsen,B., Pedersen,S.B., Moller-Pedersen,T., Bak,J.F., 1991, "Regional differences in triglyceride breakdown in human adipose tissue: effects of catecholamines, insulin, and prostaglandin E2.", Metabolism, vol. 40, 990-996.

219. Rink,C., Roy,S., Khanna,S., Rink,T., Bagchi,D., Sen,C.K., 2006, "Transcriptome of the subcutaneous adipose tissue in response to oral supplementation of type 2 Leprdb obese diabetic mice with niacin-bound chromium.", Physiol Genomics, vol. 27, 370-379.

220. Romao,I. and Roth,J., 2008, "Genetic and environmental interactions in obesity and type 2 diabetes.", J Am Diet Assoc, vol. 108, S24-S28.

221. Romon,M., Duhamel,A., Collinet,N., Weill,J., 2005, "Influence of social class on time trends in BMI distribution in 5-year-old French children from 1989 to 1999.", Int J Obes (Lond), vol. 29, 54-59.

222. Rondinone,C.M., Baker,M.E., Rodbard,D., 1992, "Progestins stimulate the differentiation of 3T3-L1 preadipocytes.", J Steroid Biochem Mol Biol., vol. 42, 795-802.

223. Rosen,E.D., Hsu,C.H., Wang,X., Sakai,S., Freeman,M.W., Gonzalez,F.J., Spiegelman,B.M., 2002, "C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway.", Genes Dev, vol. 16, 22-26.

224. Rosen,E.D. and MacDougald,O.A., 2006, "Adipocyte differentiation from the inside out.", Nat Rev Mol Cell Biol, vol. 7, 885-896.

225. Rosen,E.D. and Spiegelman,B.M., 2000, "Molecular regulation of adipogenesis.", Annu Rev Cell Dev Biol., vol. 16, 145-171.

226. Ross,R., Shaw,K.D., Rissanen,J., Martel,Y., de Guise,J., Avruch,L., 1994, "Sex differences in lean and adipose tissue distribution by magnetic resonance imaging: anthropometric relationships.", Am J Clin Nutr., vol. 59, 1277-1285.

227. Rowlands,A.V., Eston,R.G., Ingledew,D.K., 1999, "Relationship between activity levels, aerobic fitness, and body fat in 8- to 10-yr-old children.", J Appl Physiol, vol. 86, 1428-1435.

228. Saltiel,A.R. and Kahn,C.R., 2001, "Insulin signalling and the regulation of glucose and lipid metabolism.", Nature, vol. 414, 799-806.

229. Sasao,N., Hirayama,E., Kim,J., 2003, "Characterization of heterokaryons between skeletal myoblasts and preadipocytes: myogenic potential of 3T3-L1 preadipocytes.", Eur J Cell Biol, vol. 82, 97-103.

230. Sato,T., Matsumoto,T., Yamada,T., Watanabe,T., Kawano,H., Kato,S., 2003, "Late onset of obesity in male androgen receptor-deficient (AR KO) mice. ", Biophys.Res.Commun., vol. 300, 167-171.

231. Schachter,S., Goldman,R., Gordon,A., 1968, "Effects of fear, food deprivation, and obesity on eating.", J Pers Soc Psychol, vol. 10, 91-97.

232. Schena,M., Shalon,D., Davis,R.W., Brown,P.O., 1995, "Quantitative monitoring of gene expression patterns with a complementary DNA microarray.", Science, vol. 270, 467-470.

233. Schmidt,B.M., Gerdes,D., Feuring,M., Falkenstein,E., Christ,M., Wehling,M., 2000, "Rapid, nongenomic steroid action: a new age?", Front Neuroendocrinol, vol. 21, 57-94.

234. Schmitz,K.H., Jacobs,D.R., Jr., Hong,C.P., Steinberger,J., Moran,A., Sinaiko,A.R., 2002, "Association of physical activity with insulin sensitivity in children.", Int J Obes Relat Metab Disord, vol. 26, 1310-1316.

235. Schwartz,M.W., Woods,S.C., Porte,D., Jr., Seeley,R.J., Baskin,D.G., 2000, "Central nervous system control of food intake.", Nature, vol. 404, 661-671.

236. Scuteri,A., Sanna,S., Chen,W.M., Uda,M., Albai,G., Strait,J., Najjar,S., Nagaraja,R., Orru,M., Usala,G., et al, 2007, "Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits.", PloS Genet, vol. 3, e115.

237. Seckl,J.R. and Walker,B.R., 2001, "Minireview: 11beta-hydroxysteroid dehydrogenase type 1- a tissue-specific amplifier of glucocorticoid action.", Endocrinology, vol. 142, 1371-1376.

238. Seidell,J.C., Nooyens,A.J., Visscher,T.L., 2005, "Cost-effective measures to prevent obesity: epidemiological basis and appropriate target groups.", Proc Nutr Soc, vol. 64, 1-5.

239. Sethi,J.K. and Vidal-Puig,A.J., 2007, "Thematic review series: adipocyte biology. Adipose tissue function and plasticity orchestrate nutritional adaptation.", J Lipid Res, vol. 48, 1253-1262.

240. Shields,M. 2006, "Overweight and obesity among children and youth.", Health Rep, vol. 17, 27-42.

241. Shimomura,I., Funahashi,T., Takahashi,M., Maeda,K., Kotani,K., Nakamura,T., Yamashita,S., Miura,M., Fukuda,Y., Takemura,K., Tokunage,K., Matsuzawa,Y., 1996, "Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity.", Nat Med, vol. 2, 800-802.

242. Shirling,D., Ashby,J.P., Baird,J.D., 1981, "Effect of progesterone on lipid metabolism in the intact rat.", J Endocrinol, vol. 90, 285-294.

243. Siegrist-Kaiser,C.A., Pauli,V., Juge-Aubry,C.E., Boss,O., Pernin,A., Chin,W.W., Cusin,I., Rohner-Jeanrenaud,F., Burger,A.G., Zapf,J., Meier,C.A., 1997, "Direct effects of leptin on brown and white adipose tissue.", J Clin Invest, vol. 100, 2858
2864.

244. Siiteri,P.K. 1987, "Adipose tissue as a source of hormones.", Am J Clin Nutr, vol. 45 (1 Suppl), 277-282.

245. Sikand,G., Kondo,A., Foreyt,J.P., Jones,P.H., Gotto,A.M., Jr., 2008, "Two-year follow-up of patients treated with a very-low-calorie diet and exercise training.", J Am Diet Assoc, vol. 88, 487-488.

246. Singh,R., Artaza,J.N., Taylor,W.E., Brage,M., Yuan,X., Gonzalez-Cadavid,N.F., Bhasin,S., 2006, "Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: nuclear translocation of androgen receptor complex with beta-catenin and T-cell factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors.", Endocrinology, vol. 147, 141-154.

247. Small,C.J. and Bloom,S.R., 2004, "Gut hormones and the control of appetite.", Trends Endocrinol Metab, vol. 15, 259-263.

248. Smith,J., Al-Amri,M., Dorairaj,P., Sniderman,A., 2006a, "The adipocyte life cycle hypothesis.", Clin Sci (Lond), vol. 110, 1-9.

249. Smith,J., Al-Amri,M., Sniderman,A., Cianflone,K., 2006b, "Visfatin concentration in Asian Indians is correlated with high density lipoprotein cholesterol and apolipoprotein A1.", Clin Endocrinol (Oxf), vol. 65, 667-672.

250. Smith,S.R. 1996, "The endocrinology of obesity.", Endocrinol Metab Clin North Am, vol. 25, 921-942.

251. Smith,S.S., Shen,H., Gong,Q.H., Zhou,X., 2007, "Neurosteroid regulation of GABA(A) receptors: Focus on the alpha4 and delta subunits.", Pharmacol Ther, vol. 21.

252. Sniderman,A.D., Maslowska,M., Cianflone,K., 2000, "Of mice and men (and women) and the acylation-stimulating protein pathway.", Curr Opin Lipidol, vol. 11, 291-296.

253. Snijder,M.B., Henry,R.M., Visser,M., Dekker,J.M., Seidell,J.C., Ferreira,I., Bouter,L.M., Yudkin,J.S., Westerhof,N., Stehouwer,C.D., 2004, "Regional body composition as a determinant of arterial stiffness in the elderly: The Hoorn Study.", J Hypertens, vol. 22, 2339-2347.

254. Snijder,M.B., Visser,M., Dekker,J.M., Goodpaster,B.H., Harris,T.B., Kritchevsky,S.B., De Rekeneire,N., Kanaya,A.M., Newman,A.B., Tylavsky,F.A., Seidell,J.C., Health ABC Stduy, 2005, "Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC Study.", Diabetologia, vol. 48, 301-308.

255. Spiegelman,B.M. and Flier,J.S., 1996, "Adipogenesis and obesity: rounding out the big picture.", Cell, vol. 87, 377-389.

256. Steingrimsdottir,L., Brasel,J., Greenwood,M.R., 1980, "Hormonal modulation of adipose tissue lipoprotein lipase may alter food intake in rats.", Am J Physiol, vol. 239, E162-E167.

257. Steppan,C.M., Bailey,S.T., Bhat,S., Brown,E.J., Banerjee,R.R., Wright,C.M., Patel,H.R., Ahima,R.S., Lazar,M.A., 2001, "The hormone resistin links obesity to diabetes.", Nature, vol. 409, 307-312.

258. Stunkard,A. and Kaplan,D., 1977, "Eating in public places: a review of reports of the direct observation of eating behavior.", Int J Obes, vol. 1, 89-101.

259. Stunkard,A.J. 1996, "Current views on obesity.", Am J Med, vol. 100, 230-236.

260. Stunkard,A.J. and Fox,S., 1971, "The relationship of gastric motility and hunger. A summary of the evidence.", Psychosom Med, vol. 33, 123-134.

261. Suter,E. and Hawes,M.R., 1993, "Relationship of physical activity, body fat, diet, and blood lipid profile in youths 10-15 yr.", Med Sci Sports Exerc, vol. 25, 748-754.

262. Tagliaferro,A.R., Ronan,A.M., Payne,J., Meeker,L.D., Tse,S., 1995, "Increased lipolysis to beta-adrenergic stimulation after dehydroenpiandrosterone treatment in rats.", Am J Physiol, vol. 268, 1374-1380.

263. Tan,G.D., Goossens,G.H., Humphreys,S.M., Vidal,H., Karpe,F., 2004, "Upper and lower body adipose tissue function: a direct comparison of fat mobilization in humans.", Obes Res, vol. 12, 114-118.

264. Tang-Christensen,M., Havel,P.J., Jacobs,R.R., Larsen,P.J., Cameron,J.L., 1999, "Central administration of leptin inhibits food intake and activates the sympathetic nervous system in rhesus macaques.", J Clin Endocrinol Metab, vol. 84, 711-717.

265. Tchernof, A. 2008, "Sex differences in energy balance, body composition and body fat distribution.," in *Diabetes in women.*

266. Tchernof,A., Belanger,C., Morisset,A.S., Richard,C., Mailloux,J., Laberge,P., Dupont,P., 2006, "Regional differences in adipose tissue metabolism in women: minor effect of obesity and body fat distribution.", Diabetes, vol. 55, 1353-1360.

267. Tchernof,A., Calles-Escandon,J., Sites,C.K., Poehlman,E.T., 1998, "Menopause, central body fatness, and insulin resistance: effects of hormone-replacement therapy.", Coron Artery Dis, vol. 9, 503-511.

268. Tchernof,A. and Despres,J.P., 2000, "Sex steroid hormones, sex hormone-binding globulin, and obesity in men and women.", Horm Metab Res., vol. 32, 526-536.

269. Tchernof,A., Labrie,F., Belanger,A., Prud'homme,D., Bouchard,C., Tremblay,A., Nadeau,A., Despres,J.P., 1997, "Androstane-3alpha,17beta-diol glucuronide as a steroid correlate of visceral obesity in men.", J Clin Endocrinol Metab, vol. 82, 1528-1534.

270. Tchkonia,T., Giorgadze,N., Pirtskhalava,T., Tchoukalova,Y., Karagiannides,I., Forse,R.A., DePonte,M., Stevenson,M., Guo,W., Han,J., Waloga,G., Lash,T.L., Jensen,M.D., Kirkland,J.L., 2002, "Fat

depot origin affects adipogenesis in primary cultured and cloned human preadipocytes.", Am J Physiol., vol. 282, R1286-R1296.

271. Tchkonia,T., Tchoukalova,Y.D., Giorgadze,N., Pirtskhalava,T., Karagiannides,I., Forse,R.A., Koo,A., Stevenson,M., Chinnappan,D., Cartwright,A., Jensen,M.D., Kirkland,J.L., 2005, "Abundance of two human preadipocyte subtypes with distinct capacities for replication, adipogenesis, and apoptosis varies among fat depots.", Am J Physiol., vol. 288, E267-E277.

272. Thakur,V., Pritchard,M.T., McMullen,M.R., Nagy,L.E., 2006, "Adiponectin normalizes LPS-stimulated TNF-alpha production by rat Kupffer cells after chronic ethanol feeding.", Am J Physiol Gastrointest Liver Physiol, vol. 290, G998-G1007.

273. Tjepkema,M. 2006, "Adult obesity.", Health Rep, vol. 17, 9-25.

274. Trayhurn,P. and Beattie,J.H., 2001, "Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ.", Proc Nutr Soc, vol. 60, 329-339.

275. Tremblay,M.S. and Willms,J.D., 2003, "Is the Canadian childhood obesity epidemic related to physical inactivity?", Int J Obes Relat Metab Disord, vol. 27, 1100-1105.

276. Tsai,S.P., Donnelly,R.P., Wendt,J.K., 2006, "Obesity and mortality in a prospective study of a middle-aged industrial population.", J Occup Environ Med, vol. 48, 22-27.

277. Vague,J. 1947, "La differention sexuelle. Facteur determinant des formes de l'obesit?", Presse Med., vol. 55, 339.

278. Vague,J. 1956, "The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease.", Am J Clin Nutr, vol. 4, 20-34.

279. Velculescu,V.E., Zhang,L., Vogelstein,B., Kinzler,K.W., 1995, "Serial analysis of gene expression.", Science, vol. 270, 484-487.

280. Villena,J.A., Kim,K.H., Sul,H.S., 2002, "Pref-1 and ADSF/resistin: two secreted factors inhibiting adipose tissue development.", Horm Metab Res, vol. 34, 664-670.

281. Vohl,M.C., Sladek,R., Robitaille,J., Gurd,S., Marceau,P., Richard,D., Hudson,T.J., Tchernof,A., 2004, "A survey of genes differentially expressed in subcutaneous and visceral adipose tissue in men.", Obes Res., vol. 12, 1217-1222.

282. von Eyben,F.E., Kroustrup,J.P., Larsen,J.E., Celis,J., 2004, "Comparison of gene expression in intra-abdominal and subcutaneous fat: a study of men with morbid obesity and nonobese men using microarray and proteomics.", Ann NY Acad Sci, vol. 1030, 508-536.

283. Wadden,T.A. 1993, "Treatment of obesity by moderate and severe caloric restriction. Results of clinical research trials.", Ann Intern Med, vol. 119, 688-693.

284. Wadden,T.A., Vogt,R.A., Andersen,R.E., Bartlett,S.J., Foster,G.D., Kuehnel,R.H., Wilk,J., Weinstock,R., Buckenmeyer,P., Berkowitz,R.I., Steen,S.N., 1997, "Exercise in the treatment of obesity: effects of four interventions on body composition, resting energy expenditure, appetite, and mood.", J Consult Clin Psychol, vol. 65, 269-277.

285. Wade,G.N. and Gray,J.M., 1978, "Cytoplasmic 17 beta-[3H]estradiol binding in rat adipose tissues.", Endocrinology, vol. 103, 1695-1701.

286. Wajchenberg,B.L. 2000, "Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome.", Endocr Rev., vol. 21, 697-738.

287. Walsh,M.C., Brennan,L., malthouse,J.P., Roche,H.M., Gibney,M.J., 2006, "Effect of acute dietary standardization on the urinary, plasma, and salivary metabolomic profiles of healthy humans.", Am J Clin Nutr, vol. 84, 531-539.

288. Wang,H., Du,S., Zhai,F., Popkin,B.M., 2007a, "Trends in the distribution of body mass index among Chinese adults, aged 20-45 years (1989-2000).", Int J Obes (Lond), vol. 31, 272-278.

289. Wang,M.Y., Lee,Y., Unger,R.H., 1999, "Novel form of lipolysis induced by leptin.", J Biol Chem, vol. 274, 17541-17544.

290. Wang,N.D., Finegold,M.J., Bradley,A., Ou,C.N., Abdelsayed,S.V., Milde,M.D., Taylor,L.R., Wilson,D.R., Darlington,G.J., 1995, "Impaired energy homeostasis in C/EBP alpha knockout mice.", Science, vol. 269, 1108-1112.

291. Wang,P., Mariman,E., Renes,J., Keijer,J., 2007b, "The secretory function of adipocytes in the physiology of white adipose tissue.", J Cell Physiol, vol. 216, 3-13.

292. Wang,P., van Greevenbroek,M.M., Bouwman,F.G., Brouwers,M.C., van der Kallen,C.J., Smit,E., Keijer,J., Mariman,E.C., 2007c, "The circulating PBEF/NAMPT/visfatin level is associated with a beneficial blood lipid profile.", Pflugers Arch, vol. 454, 971-976.

293. Wang,Y., Mi,J., Shan,X.Y., Wang,Q.J., Ge,K.Y., 2007d, "Is China facing an obesity epidemic and the consequences? The trends in obesity and chronic disease in China.", Int J Obes (Lond), vol. 31, 177-188.

294. Warldle,J. and Boniface,D., 2008, "Changes in the distributions of body mass index and waist circumference in English adults, 1993/1994 to 2002/2003.", Int J Obes (Lond), vol. 32, 527-532.

295. Watson,G.H., Manes,J.L., Mayes,J.S., McCann,J.P., 1993, "Biochemical and immunological characterization of oestrogen receptor in the cytosolic fraction of gluteal, omental and perirenal adipose tissues from sheep.", J Endocrinol, vol. 139, 107-115.

296. Wing,R.R. 1999, "Physical activity in the treatment of the adulthood overweight and obesity: current evidence and research issues.", Med Sci Sports Exerc, vol. 31(11 Suppl), S547-S552.

297. Wiper-Bergeron,N., Wu,D., Pope,L., Schild-Poulter,C., Hache,R.J., 2003, "Stimulation of preadipocyte differentiation by steroid through targeting of an HDAC1 complex.", EMBO J., vol. 22, 2135-2145.

298. World Health Organization 1997, I*nternational Statistical Classification of Disease and Related Health Problems.* WHO, Geneva.

299. World Health Organization 2000, O*besity: preventing and managing the global epidemic. WHO Technical Report Series* Geneva, Swetzerland, No.894.

300. World Health Organization 2006, "Obesity and overweight.", Fact Sheet No 311.

301. Wu,Y. 2006, "Overweight and obesity in China.", BMJ, vol. 333, 362-363.

302. Wu,Y.F., Ma,G.S., Hu,Y.H., Li,Y.P., Li,X., Cui,Z.H., Chen,C.M., Kong,L.Z., 2005, "[The current prevalence status of body overweight and obesity in China: data from the China National Nutrition and Health Survey]", Zhonghua Yu Fang Yi Xue Za Zhi, vol. 39, 316-320.

303. Wu,Z., Rosen,E.D., Brun,R., Hauser,S., Adelmant,G., Troy,A.E., McKeon,C., Darlington,G.J., Spiegelman,B.M., 1999, "Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity.", Mol Cell, vol. 3, 151-158.

304. Xu,X., De Pergola,G., Bjorntorp,P., 1990, "The effects of androgens on the regulation of lipolysis in adipose tissue precursor cells.", Endocrinology, vol. 126, 1229-1234.

305. Yamauchi,T., Kamon,J., Waki,H., Terauchi,Y., Kubota,N., Hara,K., Mori,Y., Ide,T., Murakami,K., Tsuboyama-Kasaoka,N., Ezaki,O., Akanuma,Y., Gavrilova,O., Vinson,C., Reitman,M.L., Kagechika,H., Shudo,K., Yoda,M., Nakano,Y., Tobe,K., Nagai,R., Kimura,S., Tomita,M., Froguel,P., Kadowaki,T., 2001, "The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity.", Nat Med, vol. 7, 941-946.

306. Yang,Y.S., Song,H.D., Shi,W.J., Hu,R.M., Han,Z.G., Chen,J.L., 2002, "Chromosome localization analysis of genes strongly expressed in human visceral adipose tissue.", Endocrine., vol. 18, 57-66.

307. Yusuf,S., Hawken,S., Ounpuu,S., Dans,T., Avezum,A., Lanas,F., McQueen,M., Budaj,A., Pais,P., Varigos,J., Lisheng,L., INTERHEART Study Investigators, 2004, "Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study.", Lancet., vol. 364, 937-952.

308. Zhang,Y., Dufort,I., Rheault,P., Luu-The,V., 2000, "Characterization of a human 20alpha-hydroxysteroid dehydrogenase.", J Mol Endocrinol, vol. 25, 221-228.

309. Zhang,Y., Proenca,R., Maffei,M., Barone,M., Leopold,L., Friedman,J.M., 1994, "Positional cloning of the mouse obese gene and its human homologue.", Nature, vol. 372, 425-432.

310. Zierath,J.R., Livingston,J.N., Thorne,A., Bolinder,J., Reynisdottir,S., Lonnqvist,F., Arner,P., 1998, "Regional difference in insulin inhibition of non-esterified fatty acid release from human adipocytes:

relation to insulin receptor phosphorylation and intracellular signalling through the insulin receptor substrate-1 pathway.", Diabetologia., vol. 41, 1343-1354.

311. Zinder,O. and Shapiro,B., 1971, "Effect of cell size on epinephrine- and ACTH-induced fatty acid release from isolated fat cells.", J Lipid Res, vol. 12, 91-95.

312. Zuk,P.A., Zhu,M., Ashjian,P., De Ugarte,D.A., Huang,J.I., Mizuno,H., Alfonso,Z.C., Fraser,J.K., Benhaim,P., Hedrick,M.H., 2002, "Human Adipose Tissue Is a Source of Multipotent Stem Cells", Mol Biol Cell, vol. 13, 4279-4295.