Molecular mapping of the *fatty* (formerly *corpulent*) gene in the LA/N rat obesity model

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ABSTRACT

The LA/N-*corpulent* (*cp*) rat obesity model was a congenic rat strain developed at the National Institutes of Health by C. T. Hansen (1983)[1]. The LA/N-*cp* rat originated from breeding the Koletsky rat, possessing the *corpulent* gene, with the normotensive LA/N inbred rat. The autosomal recessive single gene mutation, *cp*, produces obesity with hyperphagia, hyperinsulinemia and elevated activities of liver and skeletal muscle lipogenic enzymes in the LA/N-*cp* rat model. The Zucker-*fatty* mutation, which was mapped to rat chromosome 5^2, resulted in a comparable, but not identical obesity syndrome. Breeding experiments by Yen et al (1977)[3] have suggested the *cp* and *fa* genes to be homologous and possibly allelic. To verify this hypothesis, we established interstrain crosses, LA/N-*cp* (*cp/+*) with 13M/Vc (*fa/+*), which yielded 10 obese and 32 lean offspring, a 1:3.2 ratio (χ^2=0.030, p>0.95). Our results support the *cp - fa* gene homology. For molecular analysis, crosses segregating the *cp* gene were constructed using a genetically distant counterstrain, Brown Norway (BN)/Crl (*+/+*) rats. Fourteen crosses between (LA/N-*cp* x BN) (*cp/+*) F_1 offspring yielded 31 obese and 80 lean F_2 progeny, a 1:2.6 ratio (χ^2=0.230, p>0.50). We have mapped the chromosomal location of *cp* in 30 obese (LA/N-*cp* x BN) F_2 progeny using microsatellite markers for two genes flanking the *fatty* locus: glucose transporter 1 (*Glut1*) and phosphoglucomutase 1 (*Pgm1*). PCR-amplified sequence polymorphisms detected four Brown Norway alleles (representing 4 crossovers) at the *Glut1* locus and 1 BN allele at the *Pgm1* locus in 60 meioses. Our results place the *cp* mutation on rat chromosome 5, flanked by *Glut1* and *Pgm1*: *Pgm1* --- 1.67 centiMorgan (cM) --- *fa* --- 6.67 cM --- *Glut1*. With the proposed homology verified through our complementation studies, our physical mapping supports *cp* and *fa* as mutations in the same gene (*lepr*)[4]. Thus, the new symbol *fa* is now the proper designation for the obese syndrome, *corpulent* (*cp*).

INTRODUCTION

Obesity is the most common nutritional disorder in the United States afflicting nearly 60 million Americans and costing approximately 100 billion dollars per year.

According to the most recent study by the National Center for Health Statistics of the Centers for Disease Control and Prevention, 22.5% of the American population is obese [body mass index (BMI) > 30.0], and 54.4% are classified as overweight (BMI > 25.0) (Figure 1)[5]. Results from the third National Health and Nutrition Examination Survey (NHANES III) show an increase of 8.0 percentage points in the prevalence of obesity in just 14 years. The number of Americans considered overweight and obese has continued to rise since 1960 in all groups measured, irrespective of race, ethnicity, or gender (Figure 2)[5]. The disturbing trend has also been observed in pre-teen (ages 6-11) children. Boys classified as overweight has increased (15.2 - 22.3%) between 1963 and 1991; overweight girls rose to 22.7% in 1991 from 15.8% in 1980. In African-American children and adolescents, obesity has risen considerably in boys (from 2.0% to 13.4%) and girls (5.3% to 16.2%). Evidence of an increasing world-wide epidemic is observed through reported rising trends in obesity in the United Kingdom, Brazil, Canada, and Mauritius, Africa[6].

Economically, obesity and its related disorders have contributed substantially to the burgeoning health care budget. Obesity, defined as a preponderance of body fat or adipose tissue to lean body mass, costs employers $4 billion annually in lost productivity of their afflicted employees. In 1995, the total financial cost attributable to obesity was estimated to be $99.2 billion dollars; $52 billion dollars of that as direct medical costs. This represents nearly 6% of the National Health Expenditure in the United States[7,8].

The implications of obesity with regard to morbidity and mortality have been well documented. Examinations by Manson[9] of 115000 women in the Nurses' Health Study showed increasing risks of mortality corresponding to increasing categories of body mass index; obesity contributed to 53% of all deaths of women with a BMI>29. Human obesity has been linked to numerous chronic disorders including diabetes mellitus, coronary heart disease, hypertension, gall bladder disease and various cancers[10, 11, 12]. Diabetes risk is increased 28-fold in women with a body mass index > 30[13]. Men show a 6.7-fold risk increase with a BMI of 29 or more[14] and nearly a quarter of men aged 40-49 who are 25% or more overweight develop diabetes[15]. The prevalence of cardiovascular disease (CHD) is impacted by weight gain as an increase of 10% raises the risk of CHD by 38%[16]. Obesity is predictive of coronary atherosclerosis[9, 17, 18] with obese subjects manifesting left ventricular hypertrophy[19], diastolic dysfunction, and increases in stroke volume and cardiac output[20, 21, 22, 23].

Epidemiological studies have linked obesity to site-specific malignancies. Obese men possessed higher mortality from cancer of the prostate, colon and rectum. Obese women are more likely to die from cancer of the gall bladder, breast, cervix, uterine endometrium, and ovary[10]. Colorectal adenomas are twice as likely to be found in obese men and women compared to lean controls; three obesity-related variables (current BMI, recent weight gain and large weight changes during adulthood) are independently associated with colon and rectal cancer[24, 25, 26]. In women, obesity in conjunction with diabetes greatly increases the risk of endometrial cancer[27]. Extensive case-control analysis has shown a contribution of body weight[28, 29], weight gain[30], and body fat distribution[31] to the development of breast cancer, particularly in postmenopausal women[32]. Recent studies of

men and women in Los Angeles have also shown a four-fold increase in renal cell carcinomas[33].

The physiological manifestation of the obese phenotype is a global organismal balance between energy input and output.

The first law of thermodynamics, the law of conservation of energy, states that the total energy of a system remains constant - stored energy (free energy) equals the difference between energy intake (change in entropy) and work (change in enthalpy). The "set point hypothesis" model has been used extensively to describe this maintenance of energy homeostasis in mammals. Afferent signals indicating the quantity of energy stores are integrated in the central nervous system, with subsequent mediations through efferent pathways to regulate energy expenditure and intake[34, 35, 36]. The involvement of endocrine and neural signals originating from adipose tissue, the hypothalamic integration in the CNS[37, 38, 39, 40], and the resultant endocrine, neurological and gastrointestinal responses have been well documented. The molecules involved (Table 1) in the maintenance of body composition and the mechanisms by which they impact on these processes is under intense investigation[41, 42, 43, 44, 45].

The genetic dissection of the human obese phenotype is a complex endeavor. Human obesity is a "multifactorial trait"[46] involving the integration of genetic, environmental and behavioral factors. Research involving families, adoptees, and twins have confirmed the contribution of genetics to body fatness. Parental obesity is a major risk factor for overweight children, doubling the risk of future adult obesity among children under ten years of age[47, 48]. Adoptees separated from their biological parents soon after birth have

body weights closely correlated with the biological parents[49], are genetically comparable to biological siblings[50], and show no correlation of BMI with their foster parents[51]. Recent work has further strengthened the genetic impact showing the insignificance of environmental factors and childhood family environment on adult adoptees' obesity and body mass indices[52, 53]. Studies of monozygotic and dizygotic twins have shown genetic factors to be responsible for between 66% - 72% of BMI variances[54]; heritability has been implicated in the variability of central abdominal fat[55] and long-term (ten year) changes in BMI in women[56].

Previous work has attempted to correlate heredity with characteristics of obesity and excess body weight at the organismal level. Advancements in molecular genetic technologies have permitted a direct analysis of the genes, products and pathways involved. The use of candidate genes and genome scanning techniques provide avenues into the human genome. Candidate genes are single gene mutations identified in animal models based on their phenotypic effect or their role in specific physiological mechanisms of a particular disease[57]. To date, 6 rodent obesity genes have been cloned and human homologues identified. The current work of this thesis has been designed to contribute to the use of a rodent obesity gene, *corpulent* (renamed *lepr*), as a potential candidate in human obesity studies.

Large-scale analysis of entire genomes has elucidated chromosomal regions involved with the obese phenotype. Genome scanning is achieved using polymorphic markers (ex. "alumorphs"[58] or microsatellites) spaced throughout the genome to identify Quantitative Trait Loci (QTL) affecting a particular phenotype[57, 59]. QTLs linked to the human obesity syndrome have been identified on all 23 autosomes, with chromosomes 1, 2, 6, 8, 11, and

20 containing at least three putative loci on both arms[60, 61, 62]. Rodent interstrain crosses have been established to identify obesity-linked loci in mice and rats. By crossing phenotypically divergent strains and genotyping polymorphic loci, 18 QTLs have been identified. Crosses between the diabetic Goto-Kakizaki (GK) rat and non-diabetic strains Fischer 344 and Brown Norway have identified 10 loci including one locus (*Niddm1*) on rat chromosome 1 segregating for glucose and insulin control[63, 64]. This same rat locus has been implicated as a modifier of the leptin receptor gene (formerly *fa*) in (WKY x 13M) rat crosses[65].

Single-gene rodent obesity models serve as excellent candidate genes for human obesity.

Rodents serve as excellent genetic models for obesity, atherosclerosis, hypertension, and human lipid metabolism including diabetes and hyperlipidemia. The fast breeding rate, large average litter size, and highly inbred, genetically homogeneous strains of mice and rats are optimal for biochemical and molecular genetic analysis. Mutations at six genetic loci (*obese, diabetes, fat, tubby, agouti, adipose*) and one in rats (*fatty*) manifest rodent obesity syndromes[66].

The most intensely studied rodent obesity genes are *obese* (*ob*) and *diabetes* (*db*). The *ob* mutation[67], occurred spontaneously at Jackson Laboratories in 1950 and was transferred to the inbred C57BL/6J background. *Ob/ob* mice exhibit an obese syndrome characterized by hyperphagia, infertility, transient hyperglycemia, elevated plasma insulin concentrations associated with increases in the size and number of islet of Langerhans beta-cells and hypertrophic-hyperplastic adiposity[68, 69]. The second mutation, *db*[70], arose

in the C57BL/KsJ strain and manifests a different phenotype from *ob* in that *db/db* mice

are severely diabetic and hyperglycemic leading to a decreased lifespan. Characteristics of

the *db* mutation also include progressive, early-onset hyperinsulinemia reaching a

maximum at 2-3 months of age with beta-cell hyperplasia and hypertrophy, infertility, and

hyperphagia[68, 69]. Classical parabiosis experiments by Coleman have shown that *ob/ob*

mice failed to produce unidentified circulating factor(s) that suppress food intake; *db/db*

mice were able to manufacture the factor, but could not respond to it[71].

The recent cloning of the leptin gene, and the subsequent cloning of its receptor

supported this hypothesis. In 1995, Zhang et al[72] cloned the *obese* gene using positional

cloning techniques. The gene product, named leptin[73], encodes a 16 kiloDalton, highly

conserved 167 amino acid peptide translated from a 4.5 kilobase adipose tissue mRNA.

Leptin is synthesized and secreted from white adipocytes and acts via an undetermined

pathway(s) in the brain to increase energy expenditure and reduce food intake[74]. Tartaglia

et al (1996)[75] cloned the leptin receptor (*lepr*) and attributed the *diabetes* mutation to a

base substitution at this locus yielding a truncated, nonfunctional receptor for leptin[76].

The LA/N-*corpulent* rat serves as a model of human obesity.

Two spontaneous mutations have been identified in the rat. The *fatty* mutation arose

spontaneously in 1961 in the 13M rat strain[77]. Rats (13M) were derived from crosses

between 13C and M (Merck) rats (Figure 3)[78, 81, 82, 83, 84, 85, 86]. The Zucker-*fa* is a model of

human early-onset obesity[79] and similar to Type II diabetes [non-insulin dependent

diabetes mellitus (NIDDM)] with insulin resistance[80].

The second spontaneous obese mutation in rats was discovered in the Koletsky hypertensive strain in the early 1970's[87] and designated *f*. Later, the gene was introduced into a normotensive inbred strain, Lister / Albany (LA) developed at the National Institutes of Health (NIH) by Hansen[1] who changed the designation to *corpulent* (*cp*) (Figure 4)[81, 83, 87, 88, 89]. The LA/N-*cp* rat is an obese rat model exhibiting metabolic characteristics of human type IV hyperlipoproteinemia including normoglycemia or mild hyperglycemia[90], hypertriglyceridemia[91, 92] and basal hyperinsulinemia[90, 92]. The autosomal recessive mutation manifests characteristics similar to human obesity and dysfunctional energy expenditure including increases in adipocyte number and total lipid content in all principal fat depots, significant elevation of total carcass fat, preservation or modest hypertrophy of the lean body mass[93], and impaired glucose tolerance[91]. The hyperinsulinemia is accompanied by hypoglucagonemia with decreased hepatic receptors for both hormones[94, 95]. Even without sustained hyperglycemia, obese rats exhibit beta-cell islet lesions including enlarged islets, decreased insulin secretory response to glucose, increased glucose oxidation and reduced islet-specific transporter protein (*Glut2*)[96].

Corpulent rats have increased weights of the liver, epididymal fat pads[91, 92, 97], heart and kidneys[97], with decreased gastrocnemius muscle weight and overall body water[97]. Previous studies have shown that the obese rat has elevated liver lipogenic[91, 92] and kidney gluconeogenic enzyme activity[91] with these tendencies exaggerated by sucrose feeding[90, 91, 92]. Glycolysis, gluconeogenesis, and lipogenesis are elevated in the obese liver[98]; decreases in protein synthesis and degradation result modify maintenance energy requirements and allow a greater proportion of energy for lipid deposition and storage[93].

Tulp and his group have extensively analyzed thermogenesis and energy expenditure homeostasis in the LA/N-*cp* model. Abnormalities in peripheral thyroid hormone metabolism[93] include decreased obese serum T3 levels and impairment in the T4-5'-deiodinase enzyme activity. Liver, kidney, and skeletal muscle reductions in T3 neogenesis[99] predict impaired capacity for nonshivering or diet-induced thermogenesis[100]. Obese rats manifest lower resting oxygen consumption values, and significantly reduced responses to exogenous sympathomimetic challenge, acute ingestion of chow and carbohydrate meals, and cold challenge exposure[93, 101]. Analysis of interscapular brown adipose tissue (IBAT) shows a greater proportion of thermogenically-less active type IV and V lipid locules in *cp/cp* animals[102] and elevated, compensatory basal deiodinase activity[93, 99]. Manipulations of energy expenditure through exercise partially ameliorate weight and fat gain and alter liver glycolytic and lipogenic enzyme activity[103], but do not prevent obesity.

Recent work in Kahle's lab has demonstrated the significant role of skeletal muscle in the manifestation of obesity in the LA/N-*cp* model. Elevated enzymatic markers for tricarboxylic acid (TCA) flux in predominantly red muscle fibers of the hindlimb of obese rats versus lean littermates has been observed[104]. We have reported increased femoral artery and vein serum triglycerides and free fatty acids in microvessel cannulation studies[105]. Further evaluation showed increased *in vivo* incorporation of tritium from ^3H_2O into *de novo* fatty acid synthesis in red fiber hindlimb and postural muscles of obese rats compared to lean. Recent studies focusing on the ultrastructure of skeletal muscle satellite cells, have shown differences between obese and lean rats using electron microscopy

(differences in cytoplasmic volume, organelle development and cell membrane structure)[106] and confocal microscopy (increased lipid content)[107].

The aim of this research is to develop the first molecular genetic analysis of the *corpulent* locus.

The relationship between the obesity syndromes manifested by the *fatty* and *corpulent* mutations has been extensively studied. The *cp* and *fa* mutations cause similar obesity syndromes but not homologous dysfunction in energy metabolism. The LA/N-*cp* model is normoglycemic, more hyperinsulinemic, and develops lean tissue to normal size and mass[92]. Initial breeding data by Zucker (1961)[77] and Koletsky (1973)[87] suggested that obesity in these models resulted from a single gene mutation inherited in a recessive manner. Interstrain crosses by Yen[3] between rats carrying the *fatty* and *corpulent* gene mutations have suggested the genes to be homologous. This region of rat chromosome 5 possesses synteny homology with the region of mouse chromosome 4 containing the *diabetes* receptor (*lepr*) obesity mutation and with the short arm of human chromosome 1 (1p31-34)[108]. Therefore, the discovery of the putative obesity gene in rats will have direct implications on human genetic obesity.

To date, the chromosomal location of *corpulent* has not been determined. We will attempt to map the chromosomal location of the *cp* gene with regard to molecular markers tightly linked to the *fatty* locus. Through crosses between LA/N-*cp* and 13M/Vc Zucker-*fa* rats, we will confirm the established homology of the *cp* and *fa* genes. This cross will reevaluate Yen's hypothesis using a slightly varied derivative of the genetics in his Koletsky strain.

To map the gene, we will segregate *cp* on the nonobese counterstrain, Brown Norway. Outcrosses of LA/N-*cp* on to BN followed by intercrosses of F_1 heterozygotes should produce 25% obese F_2 progeny. The manifestation of the obese phenotype will be observed in the (LA/N-*cp* x BN) offspring. Organismal (overall body weight) and organ (liver, fat pads) weights will indicate the degree of adiposity in this new hybrid. Also plasma levels of glucose, insulin, and a glucocorticoid (corticosterone) will be evaluated as a measurement of glucose homeostasis. The parent LA/N-*cp* is hyperinsulinemic with normal or slightly elevated blood glucose levels[90, 92]. There is also evidence that elevated levels of glucocorticoids interact with insulin to increase food intake[91]. Phenotypic characterization of this newly created obese strain will provide a preliminary observation of the affect of the background genome on the penetrance of the *cp* gene.

The obese F_2 progeny will be utilized to map *cp* at polymorphic molecular markers flanking *fatty*. The previous work of Truett et al[2] and Kershaw et al[113] has placed *fa* approximately 7.5 cM proximal to *Glut1* and 1 cM distal to *Pgm1* on rat chromosome 5. Following these techniques of positional cloning or "reverse genetics," this thesis research aims to map the *corpulent* (*cp*) mutation within the rat genome and establish a genetic relationship between *cp* and the previously mapped *fa* mutation.

Figure 1. Increasing trends of Americans classified as obese (BMI $\geq$ 30) between 1960-1994. Values are a compilation of the three classes of obesity: I (BMI 30.0 - 34.9), II (BMI 34.9 - 39.9), and III (BMI $\geq$ 40.0) and stated as percentages of the entire sample population. Survey periods include: NHES I (1960-1962), NHANES I (1971-1974), NHANES II (1976-1980), and NHANES III (1988-1994). Data compiled from the National Center for Health Statistics of the Centers for Disease Control and Prevention by Flegal et al (1997).[5]
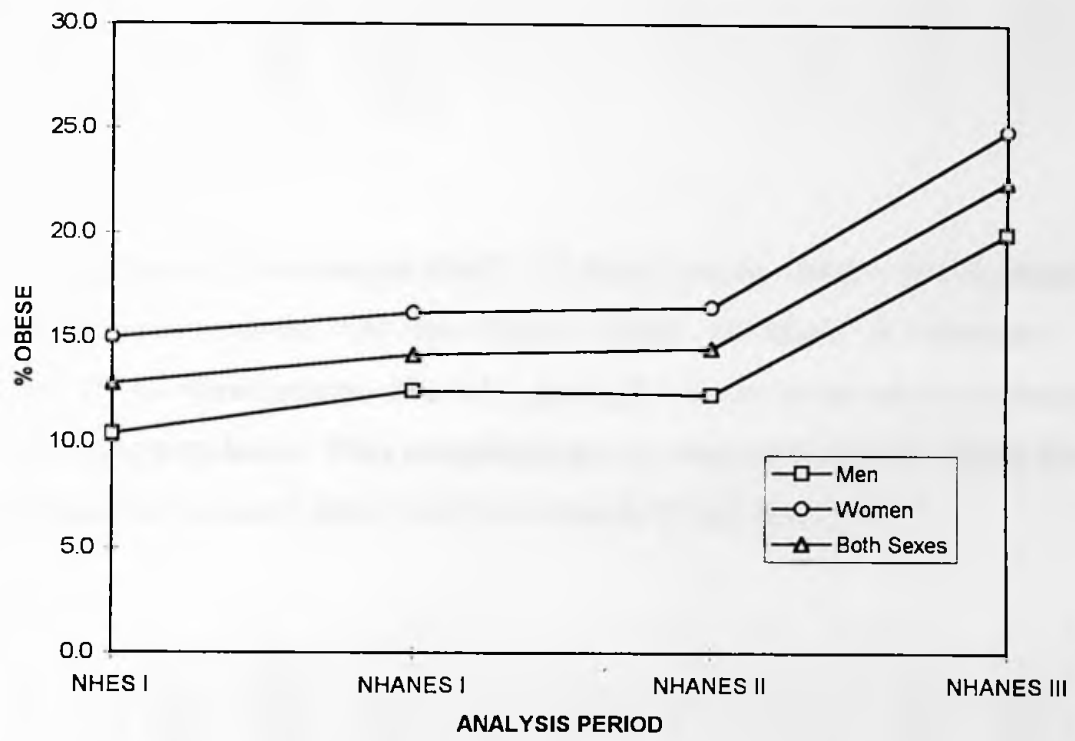
Figure 2. Prevalence of overweight (BMI > 25.0) and obesity (BMI > 30.0) in Americans by race-ethnic group and sex. (A) Non-Hispanic White. (B) Black. (C) Mexican-American. (D) All ethnic groups. Shaded region indicates the obese subset within the total overweight population. Data compiled from the National Center for Health Statistics of the Centers for Disease Control and Prevention by Flegel et al (1997)[5].

(A)

NON-HISPANIC WHITE

(B)

BLACK

(C)

MEXICAN-AMERICAN

(D)

ALL GROUPS
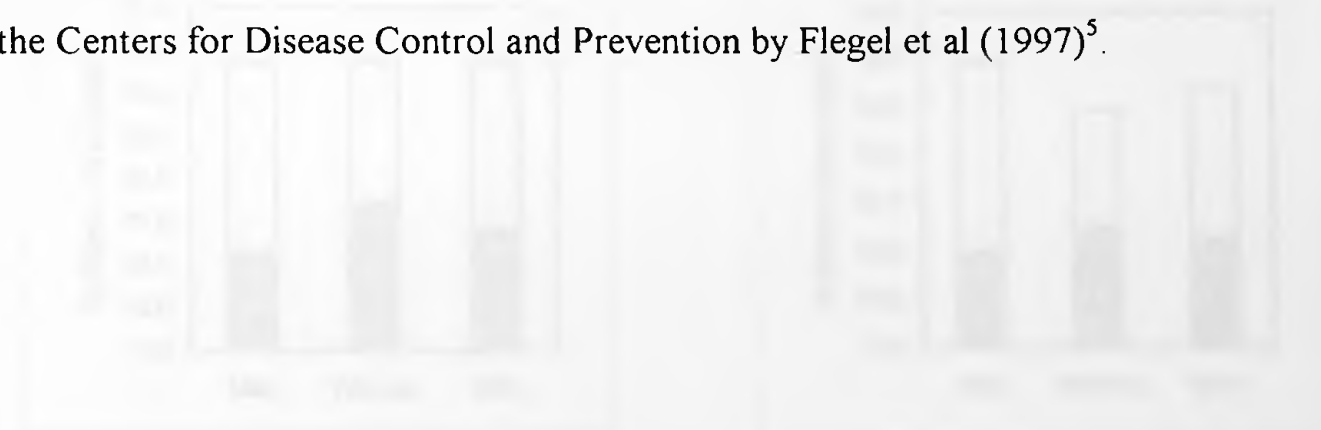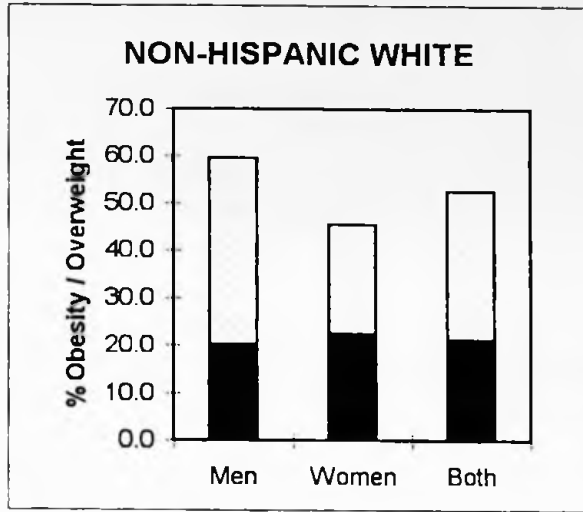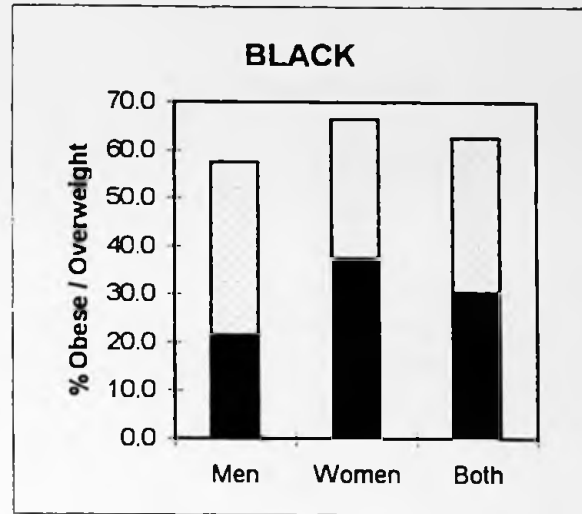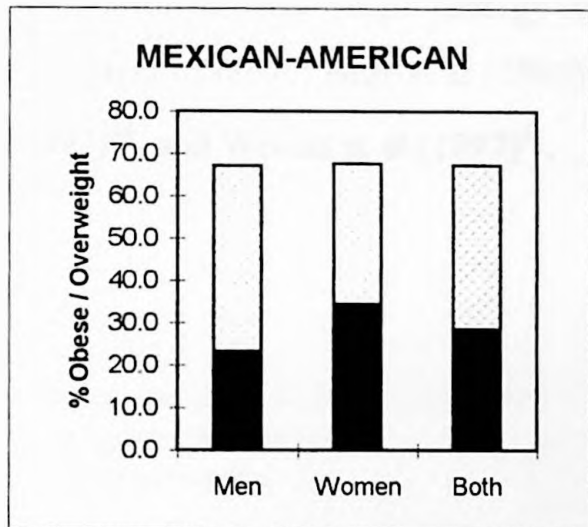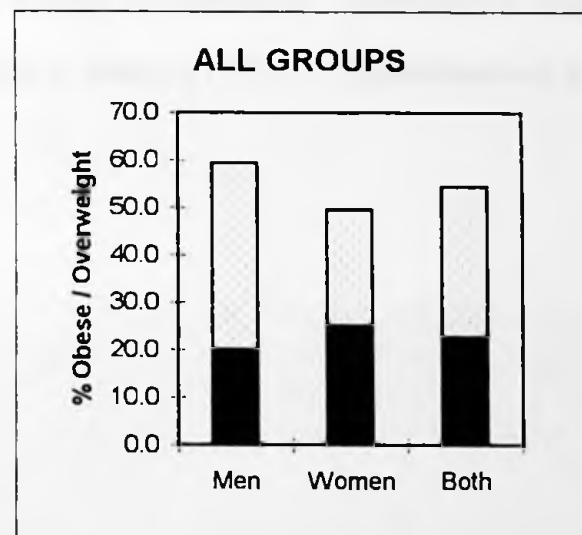
Table 1. List of the molecules and systems currently investigated for their role in the modulation of body weight (energy intake and energy expenditure). Information compiled from Bray (1986)[41], Bray et al (1989)[42], Lardy and Shago (1990)[43], Rosenbaum et al (1997)[44], and Woods et al (1997)[45].

INDICATION

AFFERENTS
(indicate nutritional status)

GASTROINTESTINAL

- glucagon
- cholecystokinin
- bombesin
- opioids
- neurotensin
- growth hormone releasing hormone
- somatostatin

PERIPHERAL / CENTRAL NERVOUS SYSTEM

- norepinephrine
- dopamine
- gamma-aminobutyric acid
- galanin

ENDOCRINE

- epinephrine
- insulin
- peptide YY
- glucocorticoids
- estrogens
- androgens
- progesterone

ADIPOSE TISSUE

- leptin
- adipsin
- uncoupling proteins 1,2,3

BLOOD

- cachectin

INTEGRATION

HYPOTHALAMUS

- neuropeptide Y
- serotonin
- melanocyte-concentrating hormone
- glucagon-like peptide 1
- beta-endorphin

- corticotropin-releasing hormone
- orexins A and B
- dynorphin
- agouti-related protein

REGULATION

EFFERENTS
(modulate energy intake vs. energy expenditure

- sympathetic vs. parasympathetic nervous system
  - thyroid hormones

Figure 3. Derivation of *fatty* rat strains. Information compiled from references 81-86.
[a]WK (Wistar-Kyoto) outbred stock obtained from Dr. Hitoshi Ikeda at the Kyoto
University School of Medicine in Japan. The Wistar rat line was originally derived from
the Wistar Institute, Philadelphia, Pennsylvania[83]. [b]WKY/N strain was obtained from the
National Institutes of Health, Bethesda, Maryland via Harlan-Sprague Dawley, Inc.,
Indianapolis, Indiana. WKY/N was derived at the NIH from the outbred WK line of
Kyoto University[85].

Connecticut Agricultural Experiment Center

Osborne-Mendel Stock
albino stock, large size
established about 1909
unknown origin

transferred about 1919

Columbia University
Dept. of Chemistry
Dr. H. C. Sherman

Sherman stock

Merck Institute for
Therapeutic Research
Rahway, NJ

Sherman stock albino females
x
local wild males

M (Merck)

13C Stock

Laboratory of Comparative Pathology
Stow, MA

13C (albino) x M (black)

13M (outbred, agouti with variable white patches)

spontaneous mutation

Zucker-*fatty*

Indiana University SOM
Diabetes Research Training Center

Takeda Chemical Industries, Ltd.
Osaka, Japan

F8 WK[a] (inbred) x 13M Zucker-*fa* (*fa*/+)

WDF/Ta-*fa*
(backcrossed, inbred)

Vassar College
Obesity Research Center
Animal Model Core Lab.

13M / Vc

(received breeding stock in 1974)
(closed / outbred)

A
WKY/N[b] x Zucker-*fa*
(backcrossed-congenic)

WKY/N Drt-*fa*

B
WDF/Ta-*fa* (inbred from Wistar *fatty*)

WDF/Ta Drt-*fa*

C
ZDF/Drt-*fa*
(Zucker-*fatty* selectively inbred for
hyperglycemia and glucose intolerance)

Figure 4. Derivation of the *fatty* rat strains. Data compiled from references 81, 83, and 87-89. [a]Sprague-Dawley strain was established about 1925 from an unknown origin by Robert D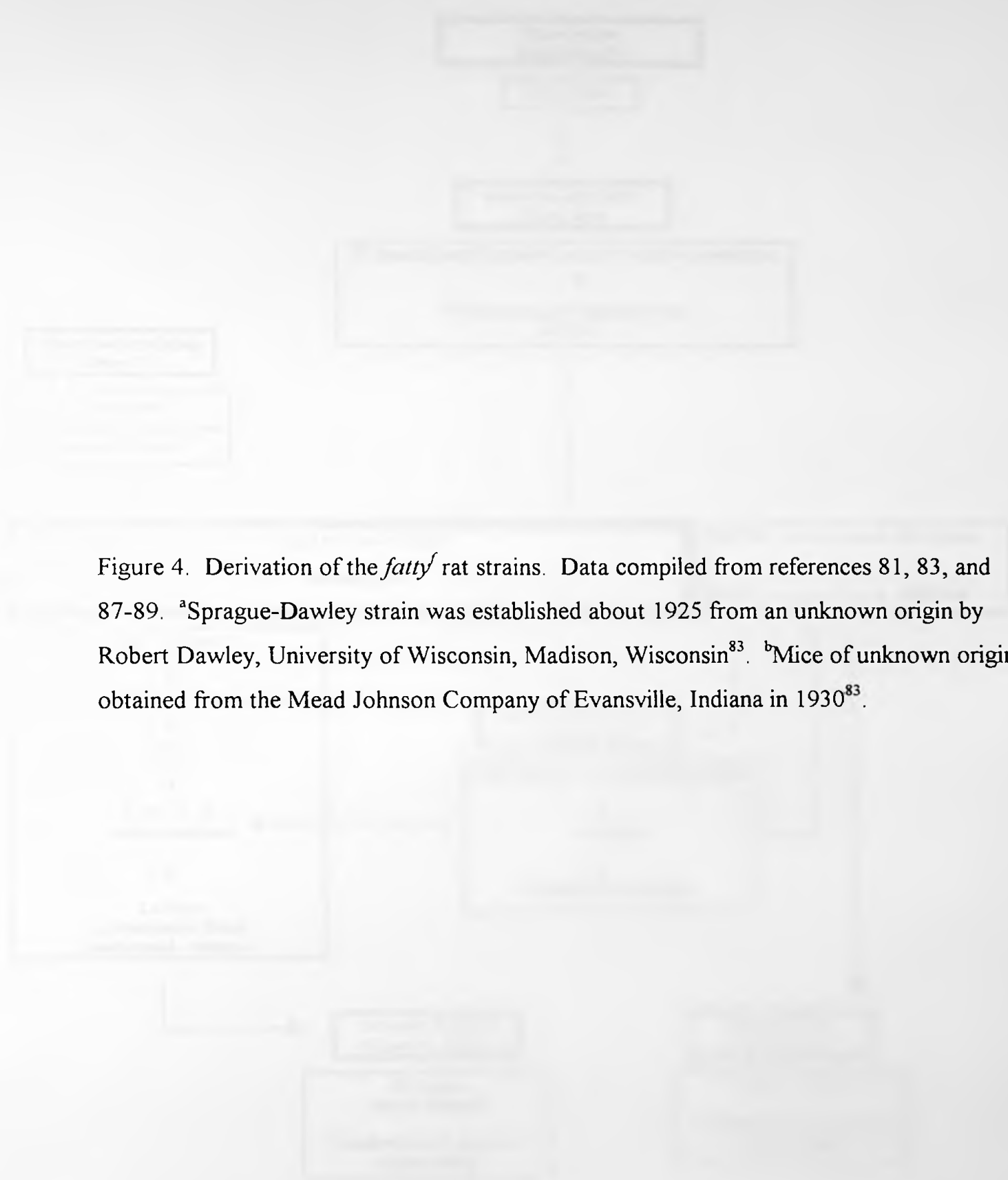awley, University of Wisconsin, Madison, Wisconsin[83]. [b]Mice of unknown origin obtained from the Mead Johnson Company of Evansville, Indiana in 1930[83].

Wistar Institute
Philadelphia, PA

WI (outbred)

Kyoto University SOM
Kyoto, Japan

WI (spontaneously hypertensive) male x female w/ elevated b.p.

SHR (spontaneously hypertensive rat)
(inbred)

Albany Medical College
Albany, NY

"Albany strain"[b]
(high incidence of spontaneous
mammary tumors)

National Institutes of Health

Bethesda, MD

WKY/N ⟶ WKY/N-cp

SHR/N ⟶ SHR/N-cp

ALB/N (inbred)

ALB/N x hooded stock of unknown origin

LA/N

LA/N x cp
outcross-testcross)

LA/N-cp
(normotensive, obese)
(backcrossed - congenic)

Case Western Res. Univ.
Cleveland, OH

SHR/N female x normotensive SD male[a]

inbreeding

spontaneous cp mutation

University of Alberta
Edmonton, Alberta

Jcr: LA-cp
(closed, outbred)

Transferred at N5, not a true
congenic strain

Ohio State Univ.
Columbus, OH

SHHF / Mcc-cp

Transferred at N7, not a true
congenic strain

Table 2. Segregation analysis of Zucker, Koletsky, and Zucker X Koletsky hybrid rats.
(A) Original breeding data for Zucker and Zucker (1961) of 13M Zucker-*fatty* rats[77].
(B) Breeding data for Yen et al (1977)[3]. Results include frequencies for Koletsky and
Zucker parental strains as well as Koletsky-Zucker hybrid animals.

STRAIN	CROSS	# OF LITTERS	TOTAL RATS	OBESE PROGENY	FREQ. OF OBESE	χ^2	P
Zucker & Zucker (1963)[77]							
Zucker							
	fa/+ x *fa*/+	49	475	94	0.199	6.602	<0.10
	fa/+ x *fa/fa*	6	55	19	0.345	2.187	>0.10
Yen et al (1977)[3]							
Zucker							
	fa/+ x *fa*/+	131	1037	244	0.235	1.196	>0.10
	fa/+ x *fa/fa*	15	90	42	0.467	0.400	>0.10
Koletsky							
	cp/+ x *cp*/+	5	54	11	0.204	0.617	>0.10
Hybrid							
	cp/+ x *fa*/+	12	136	31	0.228	0.245	>0.10

MATERIALS AND METHODS

I. ANIMALS

Animal Care Protocol. Rat strains were maintained in the Marshall University Biology Animal Facilities with routine husbandry guidelines recommended by the Marshall University Animal Care Committee. All rats were housed in plastic cages with the standard wood chip bedding. Animals were maintained in a light (on-off: 0600-1800 hours) and temperature (22°C) controlled environment. All rats were permitted rat chow (PMI Feeds, St. Louis, Missouri); proximate analysis: 58% carbohydrate from cane molasses and grains, 23.5% protein, 4.5% fat, 6% fiber, and 8% ash plus vitamins and minerals) *ad libitum* and free access to tap water from plastic and glass water bottles with rubber stoppers and stainless steel sipper tubes.

Progenitor Breeding Stock. (1) LA/N-*corpulent* (*cp*). Progenitor animals were provided by Carl T. Hansen of the Small Animal Breeding Section of The National Institutes of Health (Bethesda, Maryland) in 1991. Homozygous (*cp/cp*) obese males and females exhibit extremely reduced fertility and possible sterility necessitating the use of heterozygote (*cp/+*) lean males and females as breeding stock. Heterozygosity was determined either tediously by random male-female matings, or whenever possible, by mating of an unknown animal with a known heterozygote of the opposite sex. An animal's heterozygosity is confirmed by its parentage of an obese litter (i.e. a litter containing at least one obese member). Obese litters statistically yielded 25% homozygous lean, 50% heterozygous lean and 25% homozygous obese animals (Figure 5).

(2) 13M/Vc Zucker-*fatty* (*fa*). Progenitor animals were provided by Ruth Kava of Vassar College (Poughkeepsie, New York) in September 1992. Propagation of the 13M/Vc strain was also accomplished via the breeding of heterozygotes (*fa/+*) (Figure 6).

Breeding was alternatively accomplished by replacing heterozygote males with homozygote (*fa/fa*) obese males; selective obese Zucker males are fertile[109]. Obese litters sired by a homozygous *fa/fa* statistically contain 50% heterozygous lean and 50% homozygous obese progeny (Figure 7).

(3) Brown Norway. Progenitor animals were provided by Charles River Breeding Laboratories (Wilmington, Massachusetts) in September 1992. BN was a non-obese strain propagated through the breeding of random fertile males and females disregarding phenotype and genotype.

Interstrain Crosses. (1) LA/N-*cp* x 13M/Vc Zucker-*fa*. Heterozygote LA/N-*cp* rats were crossed with heterozygote / homozygote 13M/Vc Zucker-*fa* rats to confirm the homology of the *cp* and *fa* genes (Figures 8,9).

(2) LA/N-*cp* x Brown Norway. A two-step breeding protocol was constructed using the outcross-intercross methodology[123]. First, LA/N-*cp* heterozygotes ($fa/+$) were bred with the non-obese wild-type Brown Norway ($+/+$) (outcross). Half of the F1 progeny were predicted to be carriers of the *cp* gene. The resultant F_1 heterozygotes ($cp^{cp}/+^{BN}$) were crossed to produce an F_2 generation with the expected population of 25% obese mutants (intercross) (Figure 10).

Litter Care Protocol. A heterozygote male was placed in a cage with 1-5 fertile heterozygote females. Beginning at approximately 14 days post-introduction, females were observed for overt signs of pregnancy. The pregnant dam was removed from the sire prior to parturition and placed in a clean cage. Upon birth, record was made of the date, number of pups, and the dam and sire of the litter. The cage was visually inspected on a daily basis to observe the growth of the pups and document potential abnormalities. Initial attempts were made at 21 days of age to discern expression of the obese phenotype. Visual inspection of the entire litter with specific comparison of animals according to sex allowed a preliminary designation of phenotype.

Rats were weaned at 31 days of age, which involved removal of the dam, separation of male and female pups, ear-tagging for identification, and documentation of each animal in a record book. Documentation included the animal's ear tag number, sex, and phenotype and geneotype at the obese locus (where applicable).

If no obese animals were discernible by 31 days, the animals were maintained as a litter for an additional 7 -10 days to recheck for obesity. Lack of obesity by age 42 resulted in designation as a nonobese litter.

II. PHENOTYPIC ANALYSIS

Tissue Collection. Rats, following a 15 to 20 hour overnight (beginning at 1800-2000 hour) fasting period, were weighed (nearest 0.1 gram) and then sacrificed by intra-peritoneal injection of sodium pentobarbitol (Nembutol) (50 mg / 1 kg rat BWT). A midventral incision through the skin and muscles into the body cavity was made beginning at the inguinal region continuing anteriorly to the diaphragm. Two flaps of the body wall were pulled laterally to expose the thoracic cavity. Beginning at the diaphragm, a cut was made along the midventral line through the sternum to a point midway between the forelegs opening the peritoneal cavity and exposing the heart. Whole blood (1-2 milliliters) was withdrawn by cardiac puncture using a 3 cc syringe, immediately transferred to a labeled 1.5 mL polypropylene microcentrifuge tube with sodium heparin (1000 Units/ mL) in isotonic saline as an anticoagulant, and stored on ice until processed.

The liver was removed by cutting the hepatic veins from the postcava and the hepatic artery from the descending artery; the spleen was removed by cutting the splenic artery and vein and associated messentery. The excised tissues were cleaned of extraneous tissue and blood, weighed (nearest milligram), wrapped individually in aluminum foil, and quick frozen in liquid nitrogen. (A new, autoclaved forceps was used for each animal to prevent foreign contamination). Expediency was necessary in handling the liver and spleen to prevent degradation of DNA by nuclease activity. Tissues were stored at - 70°C for future analysis.

Fat pads were removed based on sex. In males, the testes were pushed anteriorly into the body cavity from the scrotal sac and the right inguinal pad was cut from the entire length of the right vas deferens. In females, the right ovarian fat pad was removed from the right uterine horn beginning anteriorly at the level of the ovary. Fat pads were removed and weighed (nearest milligram).

Plasma Preparation. Whole blood was centrifuged in a microcentrifuge at 14000 rpm for 5 minutes. The plasma was decanted, placed in a clean and labeled 1.5 mL tube, and stored at -70°C for subsequent analysis of insulin, glucose, and corticosterone. Analysis of plasma was conducted by Dr. Sam J. Bhathena at the Carbohydrate Nutrition Laboratory, Beltsville Human Nutrition Research Center, Agriculture Research Service, United States Department of Agriculture (Beltsville, Maryland). Plasma insulin was measured by a radioimmunoassay kit (Immunonuclear Corp., Stillwater, Minnesota) with rat insulin standards (gift of Eli Lilly, Indianapolis, Indianapolis). Plasma corticosterone was measured via an RIA kit (ICN Biochemicals, Inc., Costa Mesa, California). Plasma glucose was analyzed enzymatically (hexokinase) using a Centrifichem Automated System (Trace-America, Miami, Florida).

Genotype at the _corpulent_ locus. F_2 animals were scored for genotype at the _cp_ locus with regard to three criterion: appearance at sacrifice, body weight at sacrifice, and adiposity index of body fatness[110]. At the time of sacrifice each rat was designated lean or obese based on its appearance and weighed (nearest 0.1 gram). The adiposity index was calculated from the weight of the right inguinal (male) or ovarian (female) adipose tissue weight as a percentage of total body weight.

III. GENOTYPIC ANALYSIS

DNA Extraction. High molecular weight DNA was extracted from liver[111]. A liver sample was removed from storage at -70°C and a small section (approximately 200 milligrams) expeditiously cut. (A new, autoclaved scalpel and forceps was used for each

sample). The section was immediately minced and placed in a sterile 15 mL polypropylene Falcon tube containing 3.0 mL of freshly prepared digestion buffer [100 mM NaCl (Fisher Scientific, Pittsburgh, Pennsylvania), 10 mM Tris-HCl, pH 8 (Fisher), 25 mM EDTA, pH 8 (Fisher), 0.5% sodium dodecyl sulfate (Fisher) and 0.1 mg/mL proteinase K (Fisher)]. The tube was wrapped with parafilm and incubated in a 55°C shaking water bath for 18 - 20 hours. Approximately 3-4 times during the course of incubation, each tube was removed, vortexed gently to mix components, and "spiked" with 50 µl of 10 mg/mL proteinase K to enhance protein degradation. Samples were removed from the water bath and centrifuged (10 minutes at 1200 x g) to pellet undigested tissue and debris. The supernatant was carefully poured into a new 15 mL tube and heated to 95°C for 10 minutes to "kill" the proteinase K.

Each tube received an equal volume of phenol:chloroform [10:9 (v/v)], was inverted several times to mix phases, and centrifuged (10 min at 1700 x g). The aqueous (top) layer was transferred to a new tube and organic extraction performed a second time to enhance the DNA collected. The second aqueous layer received 0.5 volumes of 7.5 M ammonium acetate (Fisher) and 2 volumes of 100% ethanol (Fisher). The DNA was recovered in a white pellet by centrifugation (5 min. at 1700 x g). The supernatant was decanted, pellet washed with 70% ethanol to remove residual salt and phenol, and pellet allowed to air dry. The DNA pellet was resuspended in TE (10 mM Tris / 1 mM EDTA), pH 8 by gentle shaking or incubation in a 65°C water bath for 10 - 15 minutes. DNA concentration and purity was determined by the measurement of optical density (OD) at 260 and 280 nanometers using a spectrophotometer. DNA was stored at –20°C until further use.

Genotype at *Glut1* and *Pgm1*. (1) Chromosomal analysis at the Glut1 locus[2]. F_2 progeny were scored for LA/N-*cp* and BN/Crl alleles at the *Glut1* locus through the detection of PCR-amplified simple sequence length polymorphisms (SSLP). Oligonucleotide primers (Marshall University DNA Core Facility) were constructed to flank a CA repeat within intron H of *Glut1*[2] (Figure 11). DNA samples were thawed, vortexed, and diluted to the correct concentration. A master mix of reagents [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 0.2 mM dATP,

dCTP, dGTP, and dTTP, 250 ng of each primer and sterilized, deionized water](GeneAmp PCR Core Reagents, Perkin-Elmer, Norfalk, Connecticut) was prepared, vortexed, and distributed equally (50 µl each) among samples and controls. Each tube received 1 µg of DNA template, was overlaid with 50 µl of light mineral oil, and heated to 95°C for 1.5 minutes. Two units of Ampli*Taq* polymerase (Perkin-Elmer) in 5 µl of 1X PCR Buffer was added with a new pipet tip for each sample. The "Hot Start" modification was used to prevent mis-priming and optimize results[112]. Thirty-two cycles were performed at 94°C to denature, 60°C to anneal, and 72°C to extend; each cycle lasted 30 seconds. An additional 10 minute extension at 72°C was added to allow completion of amplification. Tubes were removed from the thermal cycler and stored at 4°C until evaluation. The PCR-amplified polymorphisms yielded strain-specific products: LA/N [195 base pairs (bp)] and BN (185 bp). Each PCR assay contained control DNA from LA/N-*cp* and BN rats.

(2) Chromosomal analysis at *Pgm1*[113]. F_2 progeny were scored for LA/N-*cp* and BN alleles at a $(GT)_{18}$ repeat microsatellite locus by an amplified sequence polymorphism. Oligonucleotide primers (Marshall University DNA Core Facility) were constructed for the *D5Rhm1* locus approximately 1 cM from *fatty*[113](Figure 11). The PCR master mix was prepared including [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 0.2 mM dATP, dCTP, dGTP, and dTTP, 100 ng of each primer and water]. A 20 µl reaction mixture was used containing 100 ng of template DNA. The "Hot start" modification was used with a three minute preincubation at 95°C and then the addition of 2 Units of Ampli*Taq* polymerase in 5 µL of 1X PCR Buffer II to each tube. The thermal cycler was programmed for the "touchdown protocol"[114]: 94°C to denature, 65°C to 55°C decreasing 0.5 degrees per cycle to anneal, and 72°C to extend at 30 seconds per segment for 20 cycles. This was followed for an additional 34 cycles at the final setting (94°C / 55°C / 72°C). Amplification was completed in a final 10 minute extension period at 72°C. Upon completion, tubes were stored at 4°C until gel electrophoresis. The PCR-amplified polymorphisms yielded strain-specific products: LA/N (178 bp) and BN (190 bp). Each PCR assay contained control DNA from LA/N-*cp* and BN/Crl rats.

Agarose Gel Electrophoresis of Amplification Products. PCR amplified products were observed on a 2% agarose [1.5 g NuSieveTM: 0.5 g SeakemTM GTG (FMC Products)] gel in 1X TBE [Trizma base, boric acid, EDTA (Sigma, St. Louis, Missouri)]. Fifteen μL of amplified DNA product plus 5 μL of loading buffer (bromphenol blue / xylene cyanol / glycerol / EDTA) was loaded in each well. The HaeIII digest of pBR322 (8bp - 587 bp) was run in the middle lane as a molecular weight standard. The gel was run at 60 milliamps for approximately 2 hours with 1X TBE as the running buffer. Upon completion, the gel was stained for 30 minutes with ethidium bromide (5μg / mL) and visualized and photographed in the dark under UV illumination.
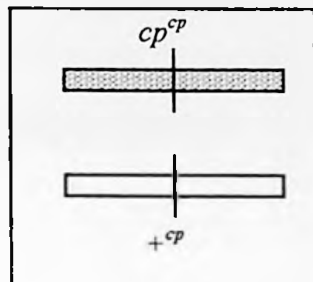
Statistical Analysis. Standard statistical analyses were performed using STATGRAPHICS for Windows, Version 2.0 (Statistical Graphics Corp.) computer software. A chi square analysis was performed on litter gene segregation data using the "Yates Correction for Continuity"[115] which corrects for small sample sizes. Degrees of freedom (df) was calculated as the number of classes of phenotype minus one. Acceptance of the null hypothesis (the difference between observed and expected observations attributed to chance alone) occurred below the critical probability value of 0.05 or 5%. Independent Student's t-tests were used to analyze physiological characteristics. A level of $p < 0.05$ was established for significance for all tests.

Figure 5. LA/N-*cp* x LA/N-*cp* cross. Cross used to propagate progenitor strain, LA/N-*cp*. The *cp* superscript denotes an allele derived from the LA/N-*cp* strain.
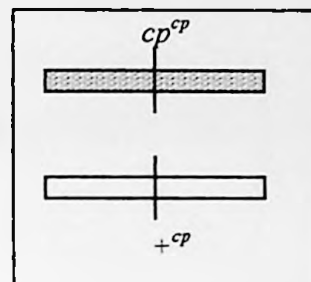
$$\boxed{P}$$

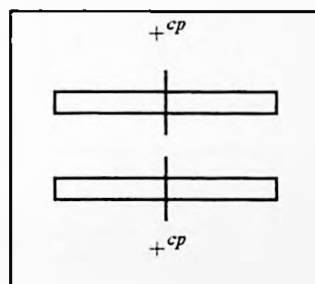$$cp^{cp}$$

$$+^{cp}$$

$$\mathbf{X}$$

$$cp^{cp}$$

$$+^{cp}$$

LA/N-*cp* (*cp*/+)

LA/N-*cp* (*cp*/+)

$$\boxed{F_1}$$

$$+^{cp}$$

$$+^{cp}$$
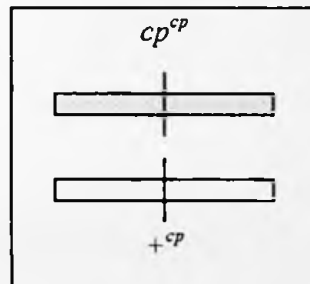
$$cp^{cp}$$

$$+^{cp}$$

$$cp^{cp}$$

$$cp^{cp}$$

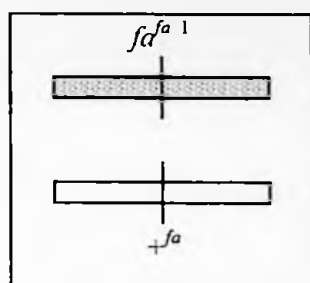| LA/N-*cp* (+/+) 1 (Lean)	LA/N-*cp* (*cp*/+) 2 (Lean)	LA/N-*cp* (*cp*/*cp*) 1 (Obese)
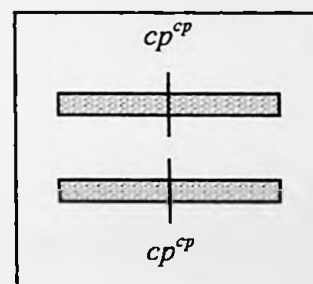
Figure 6. 13M/Vc Zucker-*fatty* x 13M/Vc Zucker-*fatty* cross (*fa*/+ sire). Cross used to propagate progenitor strain, 13M/Vc Zucker-*fa* using a heterozygote sire. The *fa* superscript denotes an allele derived from the 13M/Vc Zucker-*fa* strain.

P

$fa^{fa\ 1}$

fa^{fa}

X

$+^{fa}$

$+^{fa}$

13M/Vc Zuc-*fa* (*fa*/+)

13M/Vc Zuc-*fa* (*fa*/+)

F$_1$

$+^{fa}$

fa^{fa}

fa^{fa}

$+^{fa}$

$+^{fa}$

fa^{fa}

13M/Vc Zuc-*fa* (+/+)

13M/Vc Zuc-*fa* (*fa*/+)
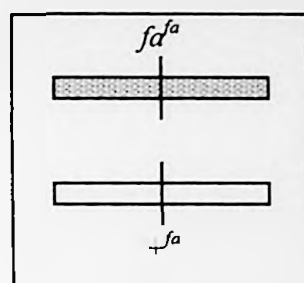
13M/Vc Zuc-*fa* (*fa*/*fa*)

1 (Lean)

2 (Lean)

1 (Obese)

Figure 7. 13M/Vc Zucker-*fatty* x 13M/Vc Zucker-*fatty* cross (*fa/fa* sire). Cross used to propagate progenitor strain, 13M/Vc Zucker-*fa*, using obese male sire. The *fa* superscript denotes an allele derived from the 13M/Vc Zucker-*fa* strain.
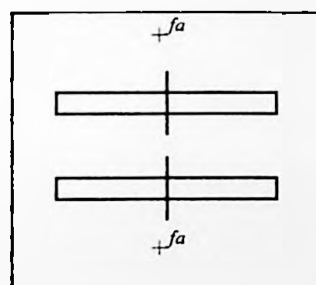
P

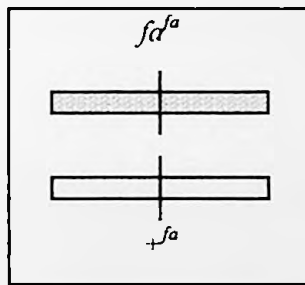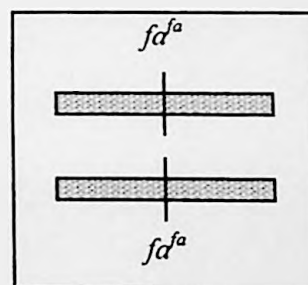13M/Vc Zuc-*fa* (*fa/fa*)   X   13M/Vc Zuc-*fa* (*fa/+*)

F₁

13M/Vc Zuc-*fa* (*fa/+*)   13M/Vc Zuc-*fa* (*fa/fa*)

1 (Lean)   1 (Obese)

Figure 8. LA/N-*corpulent* x 13M/Vc Zucker-*fatty* cross (*fa*/+ sire). Cross constructed to test the proposed homology of the *cp* and *fa* genes, using LA/N-*cp* heterozygotes and 13M/Vc Zucker-*fa* heterozygotes. The *cp* and *fa* superscripts denote alleles derived from the LA/N-*cp* and 13M/Vc Zucker-*fa* strains, respectively.

$$P$$

cp^{cp} $+^{cp}$	fd^{fa} $+^{fa}$
LA/N-cp ($cp/+$)	13M/Vc Zuc-fa ($fa/+$)

$$X$$

$$F_1$$

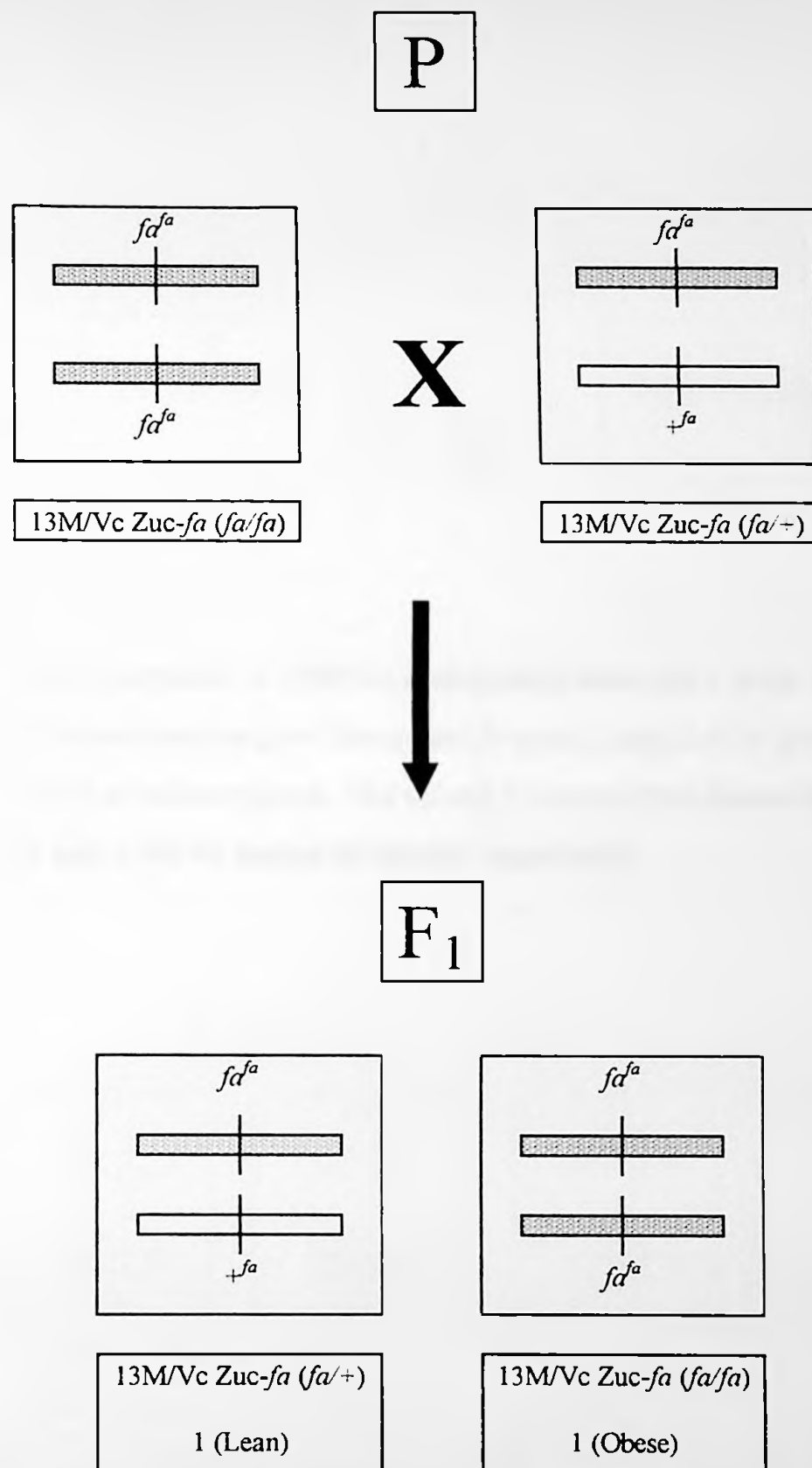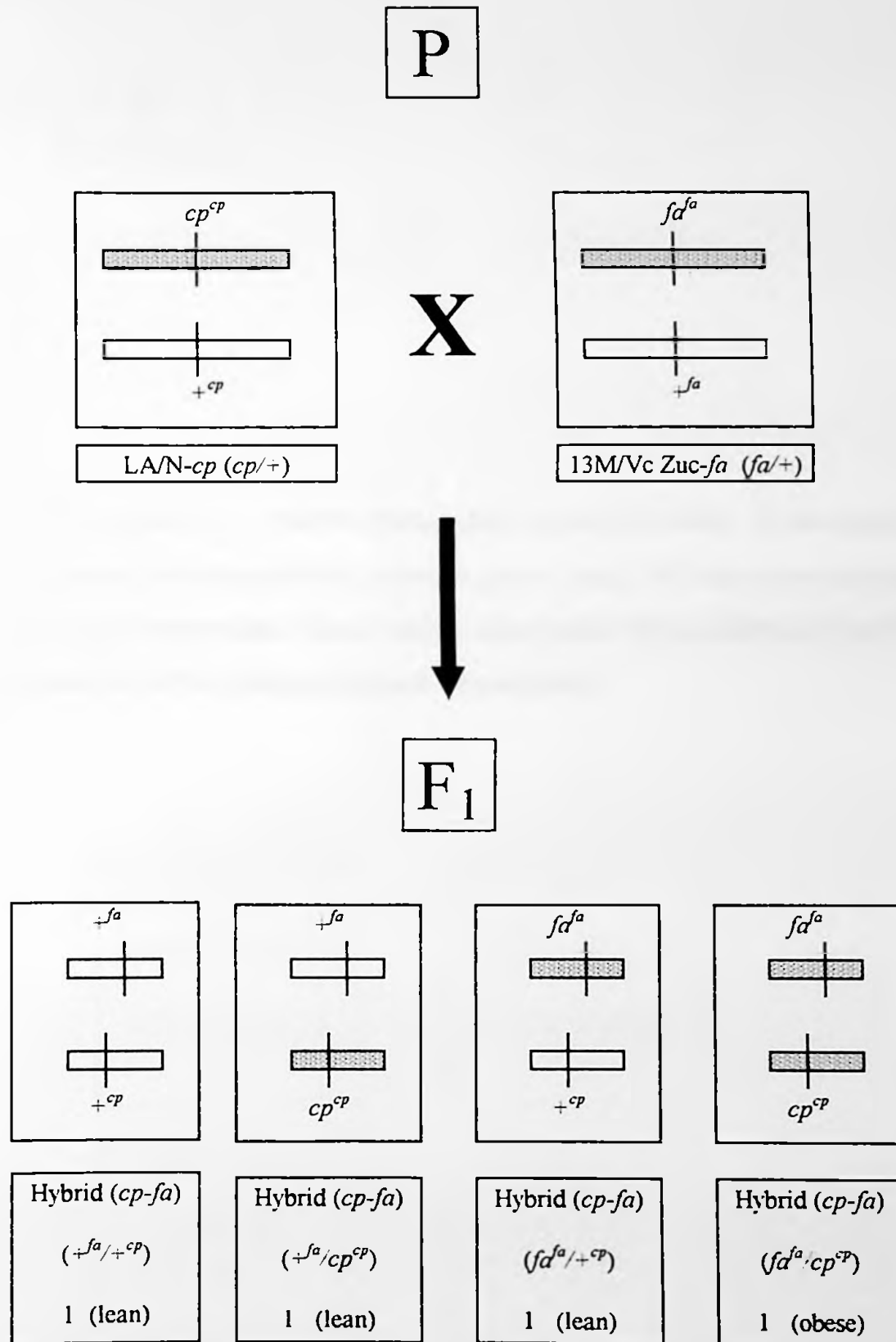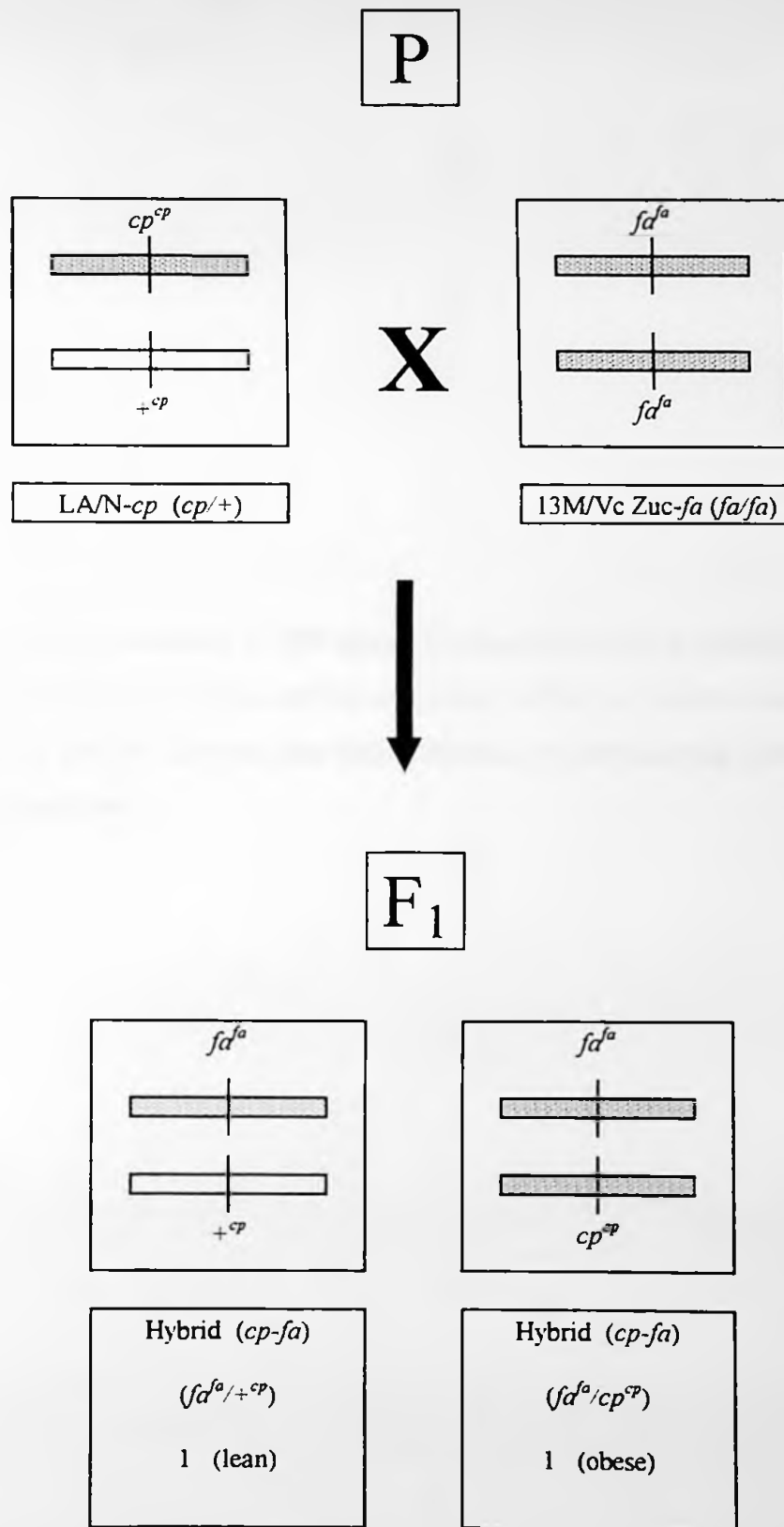|			
$+^{fa}$ $+^{cp}$	$+^{fa}$ cp^{cp}	fd^{fa} $+^{cp}$	fd^{fa} cp^{cp}
Hybrid (cp-fa) ($+^{fa}/+^{cp}$) 1 (lean)	Hybrid (cp-fa) ($+^{fa}/cp^{cp}$) 1 (lean)	Hybrid (cp-fa) ($fd^{fa}/+^{cp}$) 1 (lean)	Hybrid (cp-fa) (fd^{fa}/cp^{cp}) 1 (obese)

Figure 9. LA/N-*corpulent* x 13M/Vc Zucker-*fatty* cross (*fa/fa* sire). Cross constructed to test the proposed homology of the *cp* and *fa* genes, using LA/N-*cp* heterozygotes and 13M/Vc Zucker-*fa* obese males. The *cp* and *fa* superscripts denote alleles derived from the LA/N-*cp* and 13M/Vc Zucker-*fa* strains, respectively.

P

cp^{cp}

$+^{cp}$

LA/N-*cp* (*cp*/+)

X

fa^{fa}

fa^{fa}

13M/Vc Zuc-*fa* (*fa/fa*)

F_1

fa^{fa}

$+^{cp}$

Hybrid (*cp-fa*)

($fa^{fa}/+^{cp}$)

1 (lean)

fa^{fa}

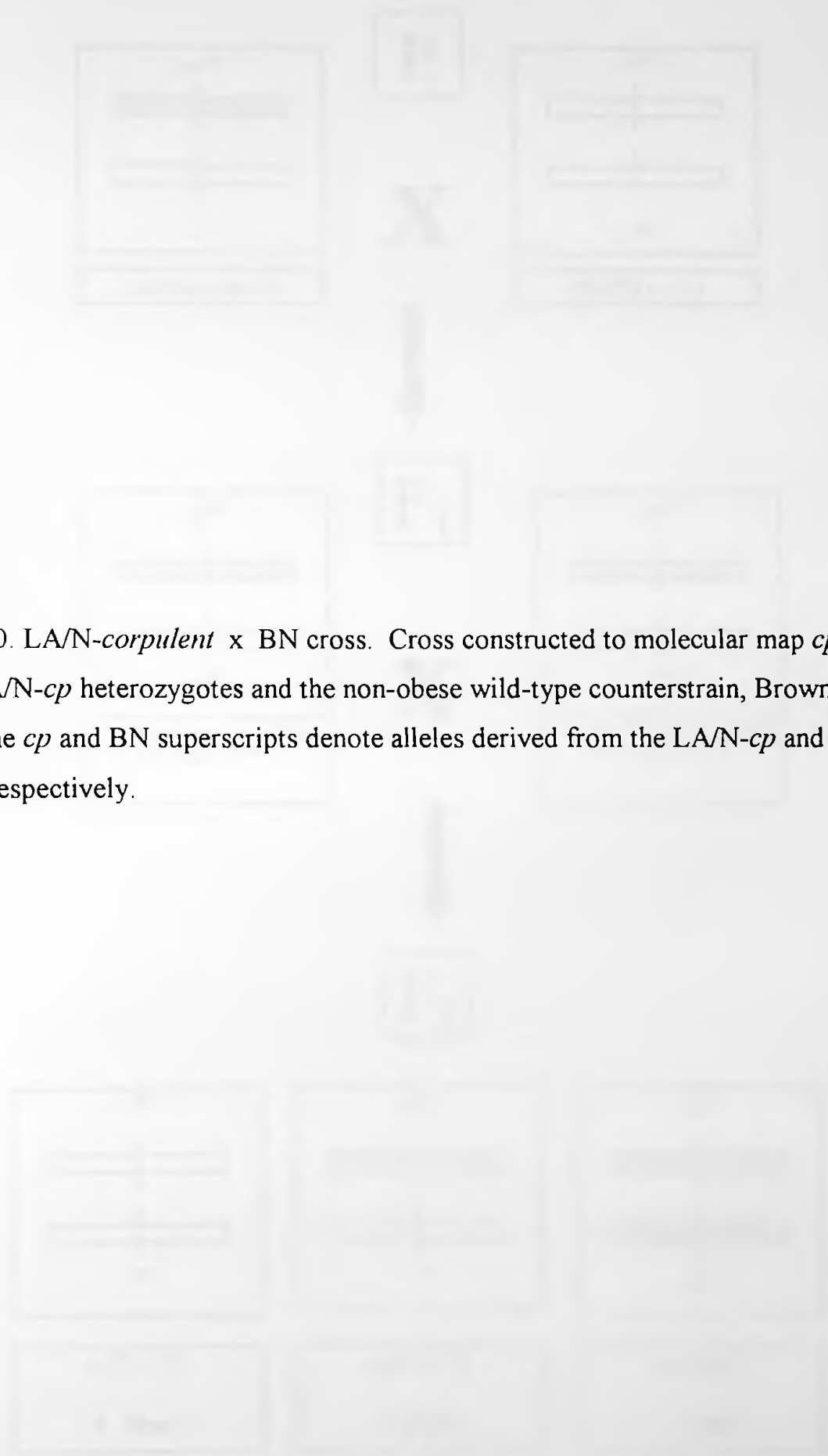cp^{cp}

Hybrid (*cp-fa*)

(fa^{fa}/cp^{cp})

1 (obese)

Figure 10. LA/N-*corpulent* x BN cross. Cross constructed to molecular map *cp* gene, using LA/N-*cp* heterozygotes and the non-obese wild-type counterstrain, Brown Norway (BN). The *cp* and BN superscripts denote alleles derived from the LA/N-*cp* and BN strains, respectively.
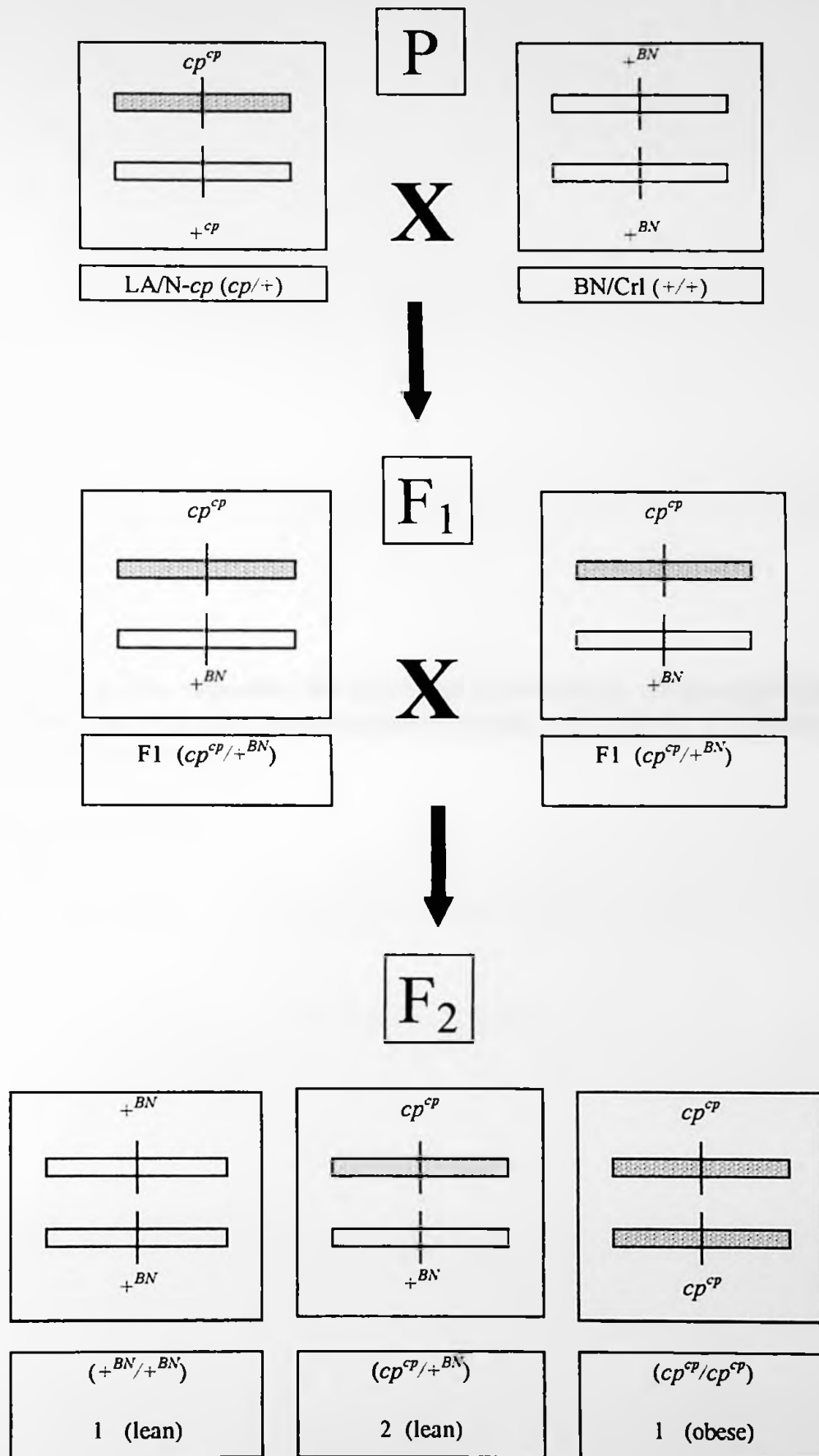
Figure 11. PCR primer sequences for *Glut1* and *Pgm1* assays. Oligonucleotide sequences used to amplify strain-specific simple sequence repeats in (LA/N-*cp* x BN) interstrain rats. (A) *Glut1*. (B) *Pgm1*.

(A) *Glut1*

     r*Glut1-1*   5' GAA TGA AGC TAA GAA TTG ACC TTA GGT 3'

     r*Glut1-2*   5' GTC CAT GCC TGT CCT TTA GTG CTC TTG 3'

(B) *Pgm1*

     forward   5' GCG TGA TGG CTG AGA GAG A   3'

     reverse   5' CTG GCC ACC CCC TCC CT   3'
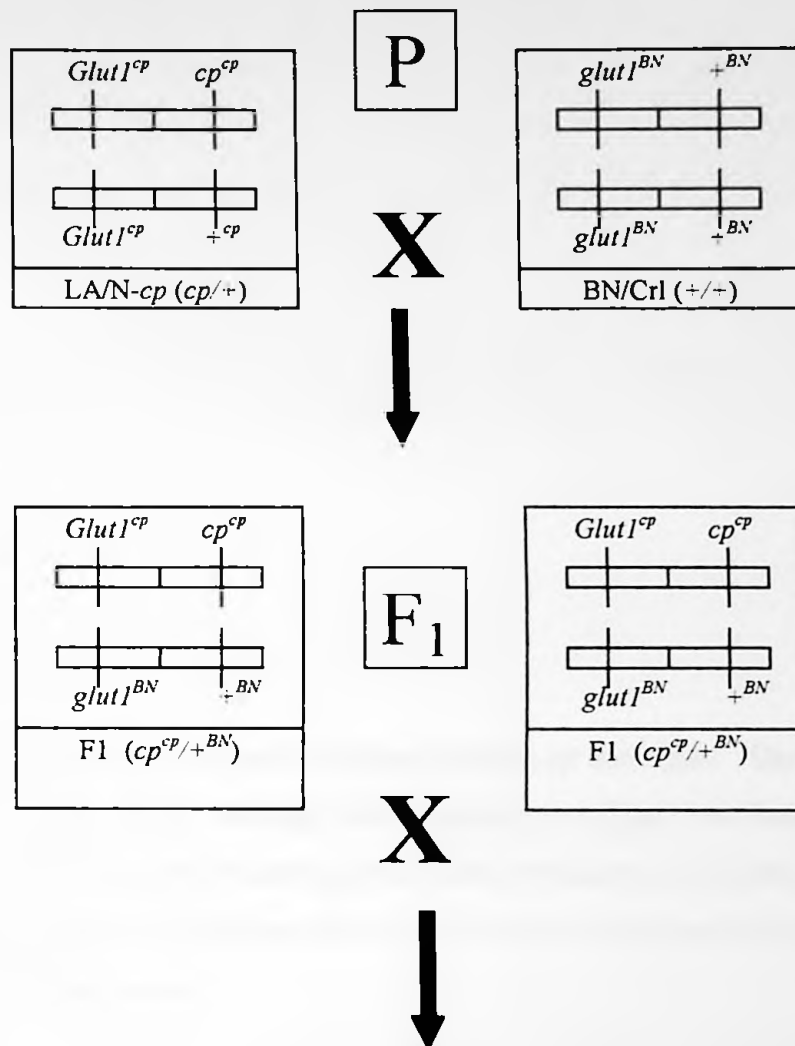
Figure 12. Representation of genetic linkage between *cp* and *Glut1*. Illustration of the crosses used to follow genetic linkage between the *cp* and *Glut1* loci throughout two generations. Only F_2 progeny resulting in an obese phenotype are depicted. Recombinants indicate the existence of a crossover event as shown by the presence of a BN allele at the *Glut1* locus.

45
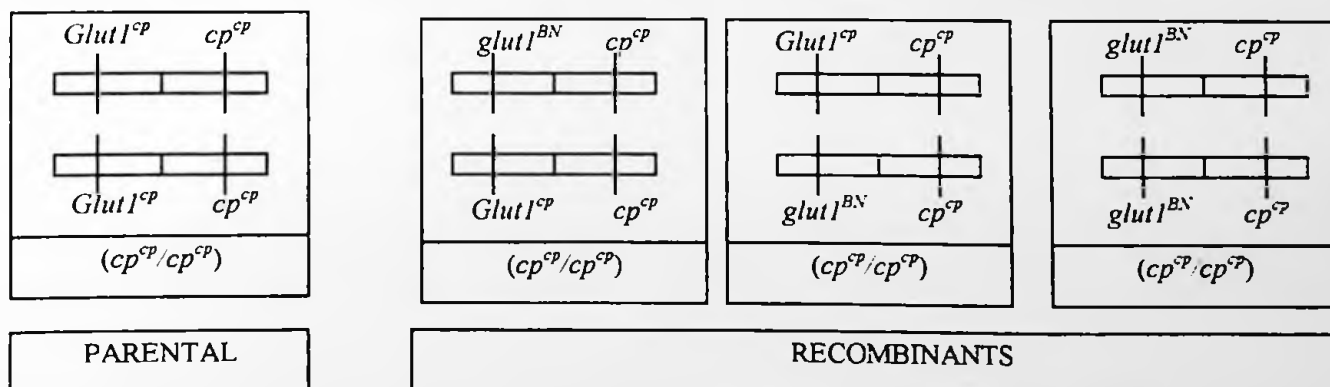
Figure 13. Representation of genetic linkage between *cp* and *Pgm1*. Illustration of the crosses used to follow genetic linkage between the *cp* and *Pgm1* loci throughout two generations. Only F_2 progeny resulting in an obese phenotype are depicted. Recombinants indicate the existence of a crossover event as shown by the presence of a BN allele at the *Pgm1* locus.

P

LA/N-cp (cp/+)   X   BN/Crl (−/+)

F₁

F1 (cp^{cp}/+^{BN})   F1 (cp^{cp}/+^{BN})

X

OBESE F₂ PROGENY

(cp^{cp}/cp^{cp})   (cp^{cp}/cp^{cp})   (cp^{cp}/cp^{cp})   (cp^{cp}/cp^{cp})

PARENTAL   RECOMBINANTS
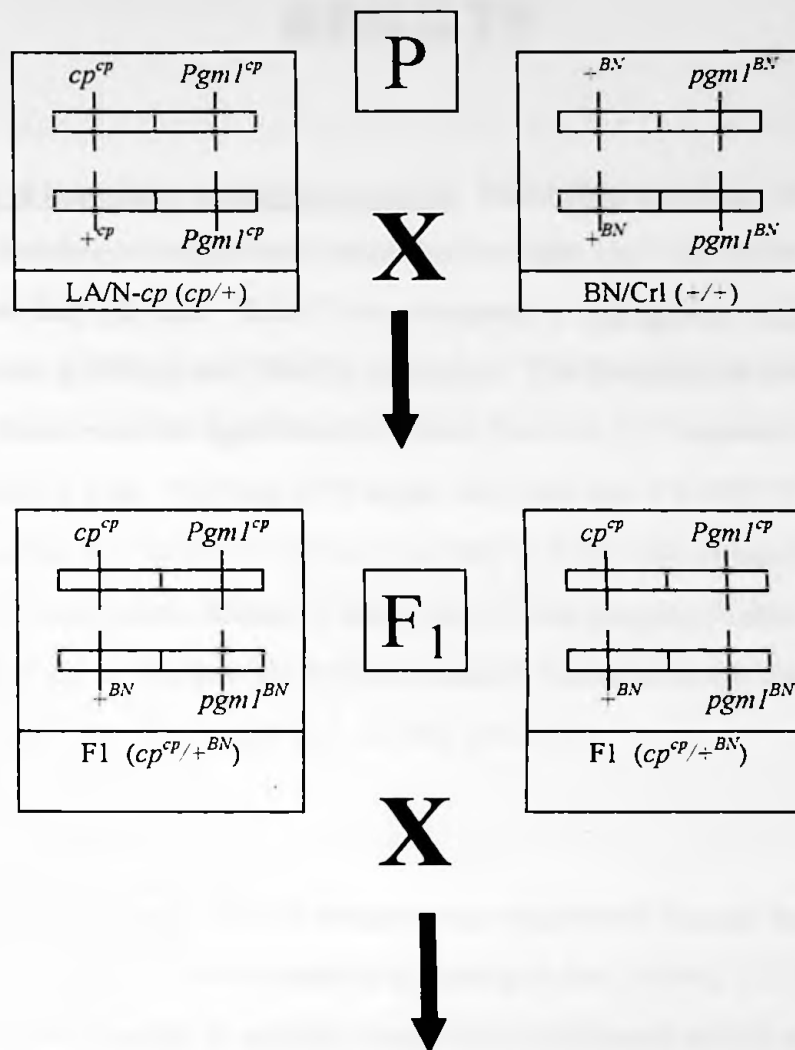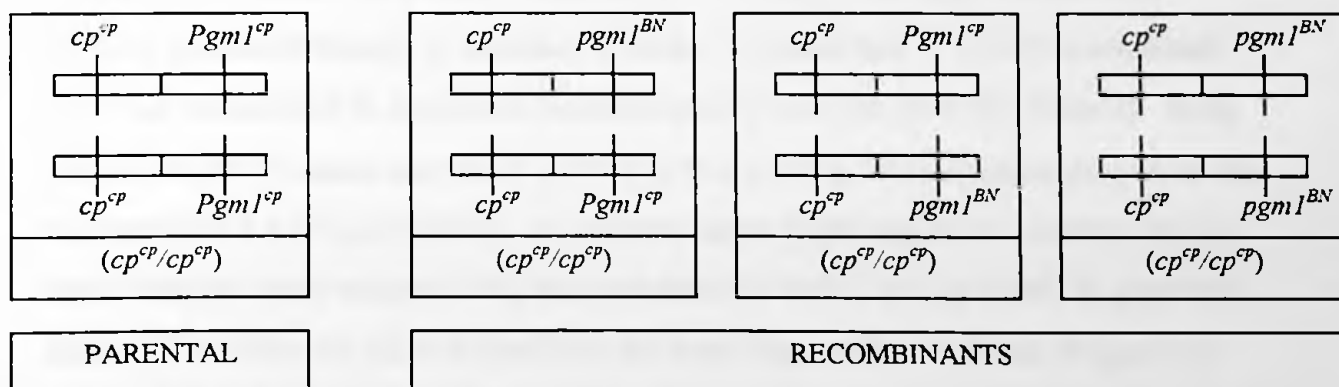
RESULTS

Confirmation of homology between _cp_ and _fa_. To confirm homology of the _corpulent_ and _fatty_ loci, interstrain crosses were established between LA/N-_corpulent_ (_cp_) and 13M/Vc Zucker-_fatty_ (_fa_) rats. Table 3 lists comparative segregation analysis of the two progenitor strains, LA/N-_cp_ and 13M/Vc Zucker-_fa_. The frequency of obese progeny in both parental strains was not significantly different from the 0.25 expected of an autosomal recessive trait: LA/N-_cp_ (104 obese /434 total rats = 0.240) (χ^2 = 0.197, p>0.10) and Zucker-_fa_ (10/43 = 0.232) (χ^2 = 0.008, p>0.10). Six crosses using heterozygotes of each strain yielded 10 obese and 32 lean progeny (Table 3). The interstrain (LA/N-_cp_ x Zucker-_fa_) hybrid evaluation was numerically similar to that of both parent strains (10 / 42 =0.238) (χ^2 = 0.030, p>0.10).

Identification of _cp/cp_ rats. The _cp_ mutation was segregated through interstrain crosses with BN. Obese _cp/cp_ rats were classified according to two criteria: (1) visible appearance and body weight at sacrifice compared to littermates and (2) adiposity index calculated from the weight of the inguinal (male) or ovarian (female) fat pad weight as a percentage of total body weight. Visual inspection established the presence of 30 obese (10 male / 20 female) _cp/cp_ and 81 lean (-/+ or +/+) rats. The frequency of obese progeny produced through F_2 crosses (31 obese / 112 total rats = 0.277) is consistent with that expected of an autosomal recessive trait (χ^2 = 0.230, p>0.10) (Table 4). Body weights of the 30 obese rats (mean ± SEM) (215.2 ± 9.3 g) were significantly greater than the lean (172.3 ± 4.2 g) (Table 6). An adiposity index [right inguinal or ovarian adipose tissue weight / body weight x 100] was calculated for each LA/N-_cp_ x BN) F_2 generation animal. The adiposity index divided both the male (Figure 14) and female (Figure 15) groups into 2 distinct and unambiguous populations within sex. The adiposity values (mean ± SEM) 0.86 ± 0.05 for the obese rats and 0.27 ± 0.02 for lean animals, were highly significant (p <0.0001).

Physiological analysis of (LA/N-*cp* x BN) F₂ rats. Characterization of the obesity phenotype in the newly created (LA/N-*cp* x BN) interstrain was established through body weight, tissue weights, and plasma analysis. Table 6 summarizes the physical characteristics of the rats used. At sacrifice, obese rats weighed more, possessed heavier livers and fat pad weights than their lean counterparts. These differences were also present when males and females were analyzed separately. Also, significant differences were observed (male > female) when lean males and lean females were compared. The same pattern existed for the obese rats, with the exception of adiposity indices. While the mean values for obese males were greater (0.90 ± 0.06) vs. (0.84 ± 0.07), they were not statistically significant. Further analysis showed obese females to have a greater percentage increase compared to lean females in body weight (143%) and adiposity index (646%) than was observed in male animals: liver (118%) and adiposity index (225%). Increased liver weights for both obese sexes compared with their lean counterparts were similar 136% (male) and 132% (female).

The characteristics of glucose homeostasis in the F₂ progeny are provided in Table 7. Fasting plasma insulin concentrations were highly elevated in both sexes of the *cp/cp* rats above their lean counterparts. Both sexes of obese animals exhibited plasma glucose values compared with their lean littermates. Corticosterone levels are not significantly different between obese males and females and lean rats. Values are slightly elevated in obese males (412 ± 90 µg / L) vs. lean males (370 ± 25 µg / L); in females, lean rats have higher corticosterone levels (543 ± 50 µg / L) compared to obese *cp/cp* mutants.

Genotypes at *Glut1* and *Pgm1*. The 30 *cp/cp* F₂ rats were scored for LA/N-*cp* and BN alleles at the *Glut1* and *Pgm1* loci using the SSLP assays described in the Materials and Methods section. Pedigree analysis of the specific DNA haplotypes manifested by each rat was established (Figure 16). Four BN alleles were detected at the *Glut1* locus through

amplified sequence polymorphisms; only 1 BN allele was detected at the *Pgm1* locus (Figures 12, 13). No rat inherited more than a single BN allele at either locus.

Linkage analysis and genetic distances. Linkage and map distances were derived from the recombination frequencies between *Glut1*, *Pgm1*, and *cp* (Table 8). The 30 obese rats are homozygous for LA/N-*cp* alleles at the *cp* locus; therefore, recombinant chromosomes are represented by BN alleles at the *Glut1* and *Pgm1* loci. The recombination fraction between *cp* and *Glut1* (4 recombinations in 60 meioses) does not approach 50 recombinations per 100 meioses (χ^2=43.35), suggesting that *cp* and *Glut1* are linked. Similarly, *cp* and *Pgm1* are linked (1 recombination in 60 meioses) (χ^2=54.15). Assuming that one centiMorgan represents 1 recombination / 100 meioses, the gene order with intervening calculated map distances is: *Pgm1* --- 1.67 cM --- *cp* --- 6.67 cM --- *Glut1* (Figure 17).

Table 3. Comparative segregation analysis of progenitor strains, LA/N-*corpulent* and 13M/Vc Zucker-*fatty*, and hybrid interstrain (LA/N-*cp* x 13M/Vc Zucker-*fa*).

STRAIN	CROSS	# OF LITTERS	TOTAL RATS	OBESE PROGENY	FREQ. OF OBESE	χ^2	P

PROGENITOR

LA/N-*cp*

| | *cp*/+ x *cp*/+ | 61 | 434 | 104 | 0.240 | 0.197 | >0.10[a] |

Zucker 13M/Vc Zucker-*fa*

| | *fa*/+ x *fa*/+ | 5 | 43 | 10 | 0.232 | 0.008 | >0.10[a,b] |
| | *fa/fa* x *fa*/+ [c] | 3 | 27 | 9 | 0.333 | 8.026 | <0.10 |

INTERSTRAIN HYBRID

LA/N-*cp* x 13M/Vc Zucker-*fa*

| | *fa*/+ x *cp*/+ | 6 | 42 | 10 | 0.238 | 0.030 | >0.10[a,b] |
| | *fa/fa* x *cp*/+ [c] | 3 | 26 | 8 | 0.308 | 8.500 | <0.10 |

D_F equals 1 for all crosses.

[a] Acceptance of null hypothesis (P>0.10) as ratio conforms to that expected of an autosomal recessive trait.

[b] Highly significant at P>0.90.

[c] Use of *fa/fa* obese male as the sire.

Table 4. Segregation analysis of the (LA/N- *cp* x Brown Norway) interstrain cross for molecular mapping techniques.

STRAIN	CROSS	# OF LITTERS	TOTAL RATS	OBESE PROGENY	FREQ. OF OBESE	χ^2	P
LA/N-*cp* x BN							
	F$_1$ *cp/+* x F$_1$ *cp/+*	14	112	31	0.277	0.230	>0.10[a]

D$_F$ equals 1.

[a]Acceptance of null hypothesis (P>0.10) as ratio conforms to that expected of an autosomal recessive trait.

Table 5. Body weight at 60 days of age in obese, lean, and combined populations of compound mutant (LA/N-*corpulent* x 13M/Vc Zucker-*fatty*) rats.

Sex	Phenotype	n	Body Weight (g)
Male	Obese	5	$230.4 \pm 6.4^{a,b}$
	Lean	8	207.5 ± 6.7^{b}
Female	Obese	5	209.8 ± 10.4^{a}
	Lean	9	147.9 ± 3.6
Combined	Obese	10	220.1 ± 6.5^{a}
	Lean	17	175.9 ± 8.4

All values are stated as means $\pm$ SEM.

[a]Phenotypic (obese vs. lean) variance - statistically significant at $p<0.05$ by an Independent t-test.

[b]Genotypic (obese vs. obese, lean vs. lean) variance - statistically significant at $p<0.05$ by an Independent t-test.

Table 6. Body weight at sacrifice, liver weight, and adiposity indices in obese, lean, and combined populations of (LA/N-*corpulent* x BN/Crl) hybrid rats.

Sex	Phenotype	n	Body Weight (g)	Liver Weight (g)	Adiposity Index[a]
Male	Obese	10	235.5 ± 15.3^b	$8.672 \pm 0.499^{b,c}$	0.90 ± 0.06^b
	Lean	41	199.7 ± 4.5^c	6.339 ± 0.157^c	0.40 ± 0.02^c
Female	Obese	20	205.1 ± 11.3^b	6.832 ± 0.350^b	0.84 ± 0.07^b
	Lean	39	143.6 ± 3.4	5.152 ± 0.149	0.13 ± 0.01
Combined	Obese	30	215.2 ± 9.3^b	7.446 ± 0.325^b	0.86 ± 0.05^b
	Lean	80	172.3 ± 4.2	5.760 ± 0.127	0.27 ± 0.02

All values are stated as means $\pm$ SEM.

[a] Adiposity index = [(weight of the right inguinal (male) or ovarian (female) adipose tissue / body weight) x 100].

[b] Phenotypic (obese vs. lean) variance - statistically significant at $p<0.05$ by an independent t-test.

[c] Genotypic (obese vs. obese, lean vs. lean) variance - statistically significant at $p<0.05$ by an independent t-test.

Table 7. Fasting glucose, insulin, and corticosterone concentrations in obese, lean, and combined populations of (LA/N-*cp* x BN/Crl) hybrid rats.

Sex	Phenotype	n	Glucose (mg /dL)	Insulin (pmol/l)	Corticosterone (μg/l)
Male	Obese	10	144.1 ± 17.3^{a}	2175 ± 815^{a}	412 ± 90
	Lean	40	111.1 ± 5.1	490 ± 86	370 ± 25^{b}
Female	Obese	20	120.8 ± 5.4^{a}	2731 ± 509^{a}	515 ± 68
	Lean	39	108.2 ± 4.5	256 ± 46	543 ± 50
Combined	Obese	30	128.8 ± 7.1^{a}	2545 ± 429^{a}	480 ± 54
	Lean	79	109.7 ± 3.4	374 ± 51	455 ± 29

All values are stated as means $\pm$ SEM.

[a]Phenotypic (obese vs. lean) variance - statistically significant at $p<0.05$ by an Independent t-test.

[b]Genotypic (obese vs. obese, lean vs. lean) variance - statistically significant at $p<0.05$ by an Independent t-test.

Figure 14. Male adiposity indices of (LA/N-*cp* x BN) F_2 rats segregating *cp* plotted in comparison to littermates within litter. The plot results in two clusters: upper cluster (>~0.6%) as obese (*cp/cp*) and lower cluster (<~0.6%) designated as lean (*cp/+*) or (+/+).

Adiposity Indices (Males)
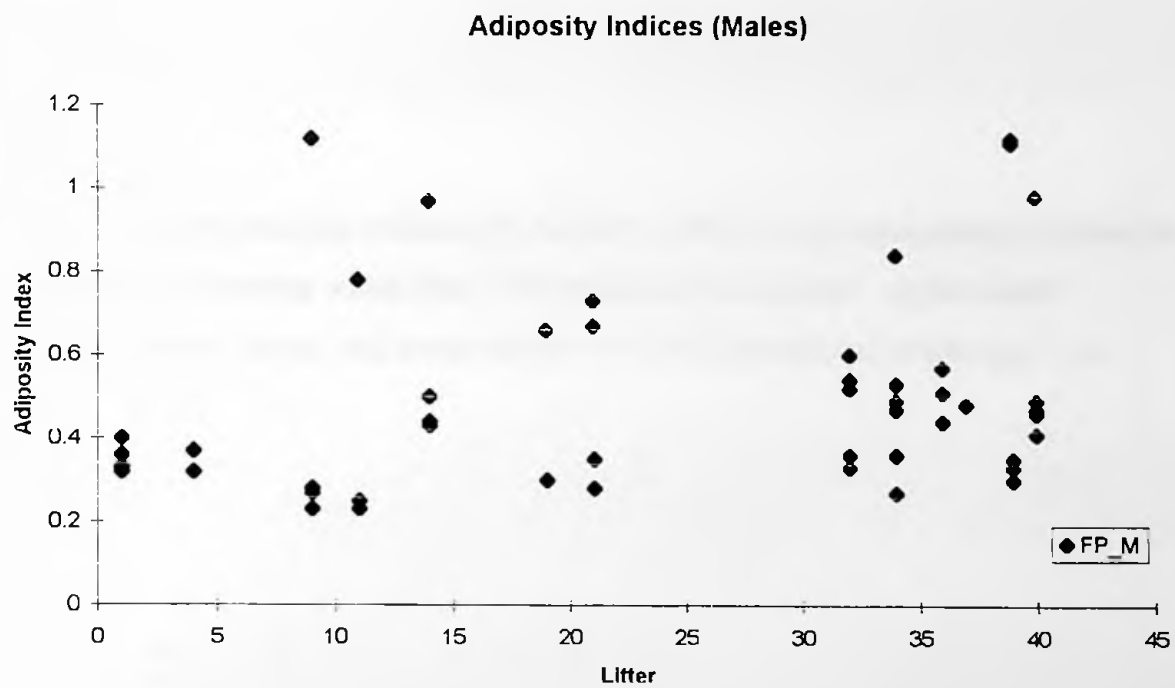
Adiposity index = [right inguinal adipose tissue weight / body weight] x 100.
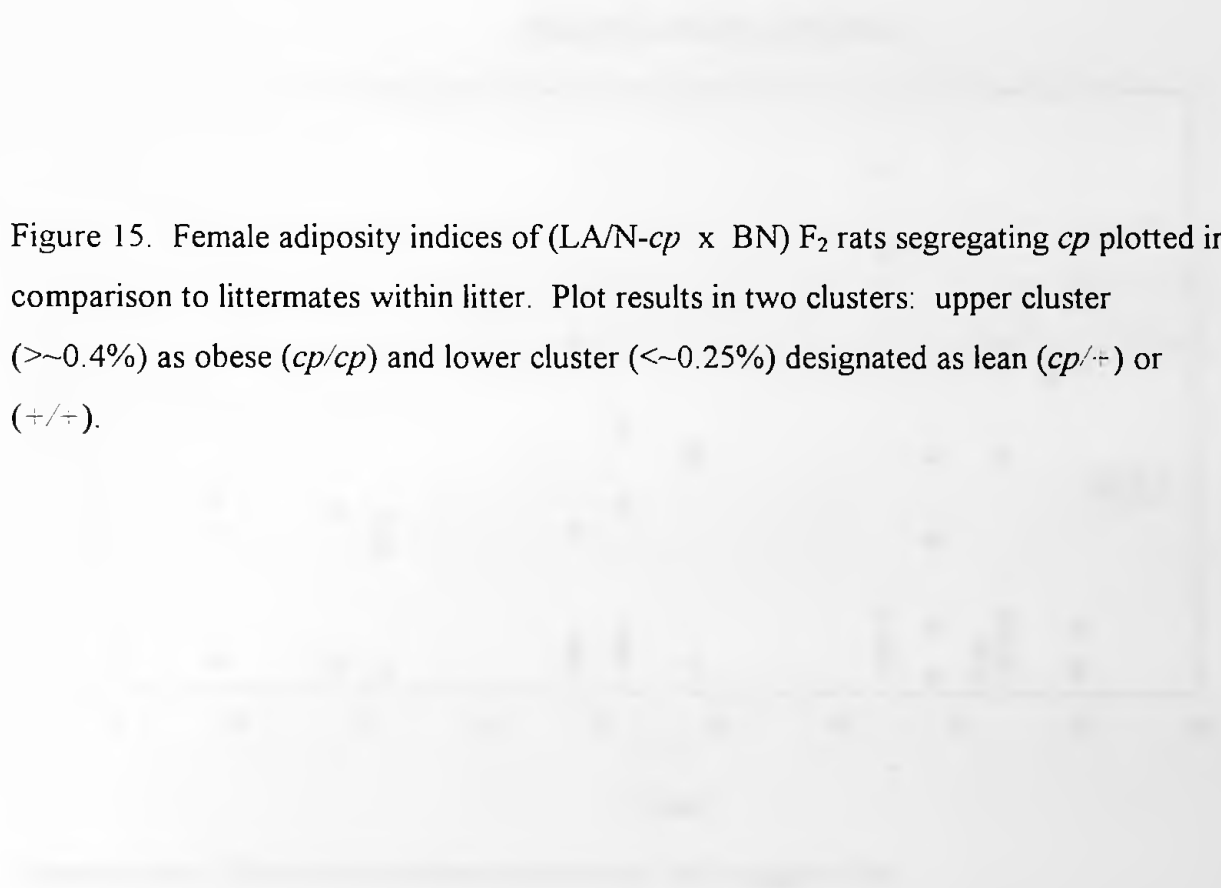
Figure 15. Female adiposity indices of (LA/N-*cp* x BN) F$_2$ rats segregating *cp* plotted in comparison to littermates within litter. Plot results in two clusters: upper cluster (>~0.4%) as obese (*cp/cp*) and lower cluster (<~0.25%) designated as lean (*cp/+*) or (*+/+*).

Adiposity Indices (Females)
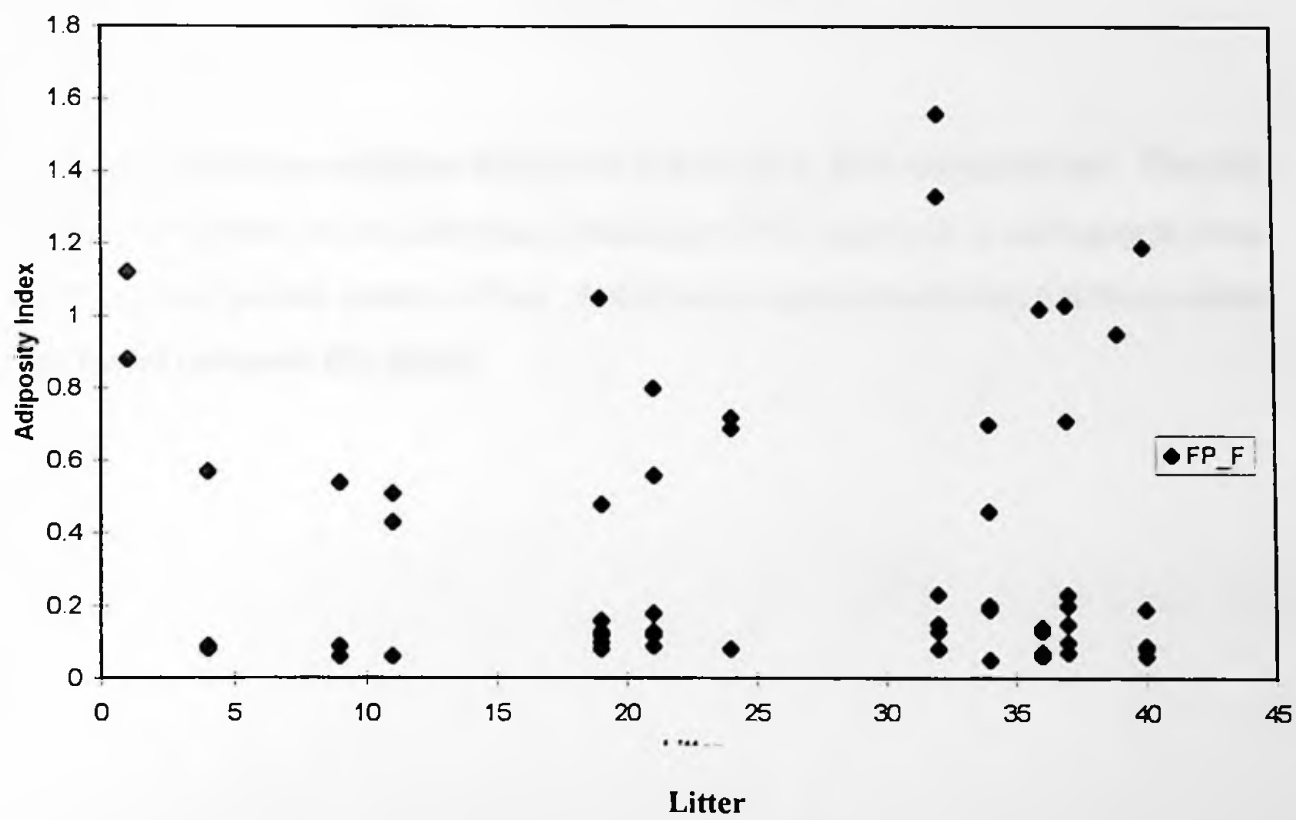
Adiposity index = [right ovarian adipose tissue weight / body weight] x 100.

Figure 16. Haplotype pedigree analysis of (LA/N-*cp* x BN) interstrain rats. The plot includes the number of rats inheriting a particular DNA haplotype at each genetic locus and the overall genetic pattern of loci. Solid boxes represent inherited LA/N-*cp* alleles; open boxes represent BN alleles.

Locus
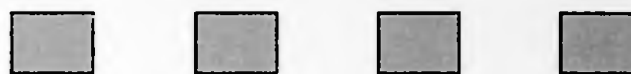
Pgm1	■	■	□	□
corpulent	■	■	■	■
Glut1	■	□	■	□
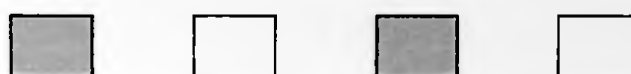
# rats	25	4	1	0

■ LA/N-*cp* allele

□ BN allele

Table 8. Linkage data with calculated map distances for the *cp*, *Glut1*, and *Pgm1* loci mapped in 30 obese F₂ (LA/N-*cp* x BN) interstrain progeny.

PAIRED LOCI	RECOMBINATION FRACTION PER TOTAL MEIOSES SCORED	χ^2	MAP DISTANCE cM ($\pm$ SEM)
Pgm1 - *cp*	1 / 60	54.15	1.67 $\pm$ 1.65
cp - *Glut1*	4 / 60	43.35	6.67 $\pm$ 3.22

DISCUSSION

The relationship between *corpulent* and *fatty* was established over 15 years ago by Yen et al (1977)[3]. His breeding of Koletsky and Zucker rats yielding obese hybrid progeny supported a hypothesis that the mutations emanated from genetic defects at the same locus. In 1977, limitations in molecular genetic techniques prevented further analysis. Our work is the first to establish the molecular genetic locus of the *fa* (formerly *corpulent*) gene. This was accomplished through complementation breedings between *cp* and *fa* and segregation of *cp* on a non-obese counterstrain with subsequent mapping using microsatellite markers for two genes flanking *fa*.

Preliminary complementation studies to establish genetic relationship.

Obese syndromes produced by mutations in the *cp* and *fa* genes are similar, but not identical[92]. Difficulty in analyzing complex phenotypic characteristics is due to the evolution of the mutations on different strain backgrounds with varied lineages and impacting epistatic loci[116, 117]. Discriminating the primary genetic defect from resultant secondary effects further obscures the dissection of a distinct phenotype[118].

We revisited the work of Yen using a descendent of the Koletsky rat, the LA/N-*cp*. The congenic LA/N-*cp* strain was created by Hansen at NIH using the Koletsky rat as the contributor of the *cp* gene[1]. With the placement of *cp* on the LA/N background through more than ten backcross generations and the intervening fifteen years, the potential for additional mutations at this locus exists. This genetic locus has been shown to be highly mutable with five mutations documented in the mouse[119], two in rats (including *cp*)[77, 87], and one in humans[120]. Therefore, the possibility of a different obese expression in our hybrid rats existed.

Our interstrain crosses between LA/N-*cp* and 13M/Vc Zucker-*fa* rats produced F_1 generation obese *cp* /*fa* compound mutants displaying a 3 (lean):1 (obese) ratio. By comparing breeding results and segregation analysis between the progenitor strains (LA/N-*cp* and 13M/Vc Zucker-*fa*) and the hybrid (LA/N-*cp* x 13M/Vc Zucker-*fa*) animal, we see no differences and our results are those typically expected for a fully penetrant

autosomal recessive trait. Through these crosses we were able to replicate the previously suggested homology of *cp* and *fa*, and provide the impetus for the use of molecular markers defining the exact chromosomal location (mapping) in the rat.

We did not attempt to extensively analyze the phenotype of the hybrid *cp/fa* mutant rats. The recent cloning of both *fa*[121] and *fa*[122] genes, which represent the leptin receptor (*lepr*), has shown that they are the result of two different mutations at the same genetic locus. The analysis of the hybrid *cp/fa* mutant would provide insight into the potentially differing molecular pathways involved in the manifestation of two different alleles.

Our results confirm the work of Yen and his co-workers more than 20 years ago. The *cp* and *fa* mutations occur within the same gene. The use of a derivative of the original Koletsky rat crossed with the 13M/Vc Zucker-*fa* produced rats that were 25% heavier than their lean counterparts. However, these crosses could not permit definitive analysis of chromosomal gene location. The breeding analysis or resultant phenotypes could not conclusively establish if *cp* and *fa* are allelic or the same mutation. Therefore, we attempted to map the *cp* gene mutation utilizing established molecular markers tightly linked to *fa*.

Segregation of the *cp* gene on a non-obese counterstrain, Brown Norway.

Through interstrain crosses using the Brown Norway background, we created a new obese rat strain, (LA/N-*cp* x BN). Our crosses successfully established the *cp* mutation on BN as shown by obese F_2 progeny. (LA/N-*cp* x BN) F_2 rats exhibited obesity in the expected 3 (lean): 1 (obese) ratio. Obese rats were heavier with liver hypertrophy and significantly increased adiposity. These progeny were hyperinsulinemic and although not statistically different, they showed trends of glucose homeostasis characteristics different from the parental LA/N-*cp* strain. Interstrain animals are more hyperglycemic than the LA/N-*cp* and differ from the expression of *fa* on the Zucker background in several characteristics[92, 124].

The variability of phenotypic expression is most likely due to the effect of the background strain and epistatic influences[117]. Interstrain crosses inherently create

admixtures of alleles at individual genetic loci. After the F_2 cross, heterozygosity (BN and LA/N-*cp* alleles) would theoretically be expected at greater than 87% of loci; only 12.5% of all loci would be expected to be "fixed" for one parental allele[123]. Therefore, unique genetic combinations exist producing undetermined phenotypes.

Background-dependent phenotypic expression is conclusively established in rodent obesity models. When maintained on the same background strain, the *db* and *ob* mouse mutations manifest identical obesity syndromes. On the C57BL/6 background there is severe obesity with mild glucose intolerance and transient hyperglycemia, while *ob/ob* or *db/db* expression on the C57BL/KsJ strain leads to a rapidly developing and life-shortening diabetes[125, 126, 127, 128]. This phenomenom is also present in rat models where both *fa* and *cp* have been placed on different backgrounds to compare phenotype. In addition to LA/N-*cp*, 5 other strains incorporate the *corpulent* mutation: SHR/N-*cp*[1], WKY/N-*cp*[129], Jcr: LA-*cp*[88], SHHF/Mcc-*cp*[89], and DSS/N-*cp*[130]. Expression of the *cp* phenotype on the SHR/N and WKY/N backgrounds demonstrates impaired glucose tolerance advancing toward non-insulin dependent diabetes mellitus (NIDDM), while LA/N-*cp* rats remain nondiabetic throughout their entire lives[131]. Partially backcrossed (prior to complete congenic status) substrains derived from Hansen's breeding, the Jcr: LA-*cp* (separated at N5) and SHHF/Mcc-*cp* (N7) exhibit phenotypic variability from the completely congenic (> 10 backcrosses) LA/N-*cp* and SHR/N-*cp*, respectively. Jcr: LA-*cp* obese rats suffer atherosclerosis and myocardial lesions[88]; the SHHF/Mcc-*cp* strain manifests obesity with congestive heart failure[89]. The Dahl salt-sensitive/NIH-*cp* rat is a newly created congenic strain derived from SHR/N-*cp* and DSS/N rats which manifests organ vasculopathy with vascular lesions in the kidneys, intestines, pancreas, and testes[130].

We segregated the *cp* mutation on a non-obese counterstrain, BN. The Brown Norway has been used previously in mapping studies of loci flanking *fa*[2]. In microsatellite loci that have been PCR-amplified, BN alleles exhibit differential sized polymorphisms[113] with respect to both *cp* and *fa*. By expressing both LA/N-*cp* and BN alleles at various loci, the newly created strain will allow us to "track" and map strain-specific polymorphisms.

Molecular mapping of *corpulent* using polymorphic microsatellite markers flanking *fatty*.

To map the *cp* gene in the (LA/N-*cp* x BN) F_2 rats, we relied on classical genetics and meiotic mechanisms to create a physical molecular map. Mendel's 2nd law or the law of independent assortment, states that the segregation of a gene at a particular locus will have no influence on the segregation of a gene from any other locus. This law fails when the genes are located on the same chromosome (linked). Linked genes will segregate together during meiosis unless a crossover event has occurred. Crossovers occur during the first prophase period of meiotic division with the exchange of chromosomal segments between nonsister chromatids. By counting the number of crossover events between two genetic loci, a map distance in centiMorgans (1 cM equals 1% recombination) can be calculated[115].

To quantify crossovers occurring between *cp* and the flanking *Pgm1* or *Glut1*, we used single sequence length polymorphism (SSLP) assays for microsatellites at each locus.[2, 113] Microsatellites, or simple sequence repeats (SSR), are short (1 - 4 base pair) sequences repeated multiple times in a tandem array interspersed throughout mammalian genomes. They are highly polymorphic among different rat strains. Variability in length of the repeated sequences can be measured through PCR amplification and product length determination via electrophoresis[132].

Complementation crosses between LA/N-*cp* and Zucker-*fa* provided the preliminary information to place *cp* at the *fa* locus. Because of our breeding results and segregation analysis, we were confident that *cp* and *fa* were homologous mutations on rat chromosome 5. This information allowed us to map *cp* using molecular markers linked to *fa* and did not require us to scan the entire genome. The *cp* gene was segregated by interstrain crosses between LA/N-*cp* and a genetically distant counterstrain, Brown Norway (BN). This breeding protocol allowed the use of molecular mapping techniques[2]. Scoring by visual appearance, body weight, and adiposity indices divided the (LA/N-*cp* x BN) F_2 population into two unambiguous non-overlapping populations. Analysis of DNA haplotypes for 30 obese F_2 rats (60 informative meioses) yielded 4 BN alleles at the *Glut1*

locus and 1 BN allele at *Pgm1*. A resultant BN allele at either the *Glut1* or *Pgm1* locus represented a crossover event.

Our results place *cp* on rat chromosome 5, flanked by *Glut1* and *Pgm1*: *Pgm1* -- 1.67cM -- *cp* -- 6.67cM -- *Glut1*. Thus, we have mapped *cp* to the same genetic interval as *fa*. Gene order and map distances are similar to research previously reported for *fa*: *Pgm1* – 1.0cM -- *fa* -- 8.6cM -- *Glut1*[2]. The symbol *fatty^f* (*fa^f*) is now used to represent *corpulent* (*cp*) and Koletsky (*f*) and reflects the fact that both *fa* and *fa^f* occur at the same genetic locus. Mapping *fa^f* to this locus on chromosome 5 solidifies the synteny between rat chromosome 5, mouse chromosome 4 and the short arm of the first human chromosome (Figure 17)[108, 133, 134]. The *db* obesity mutation on mouse chromosome 4, which is homologous to the *fa* mutation in rats, is found within a well-conserved syntenic group shared by mice and humans[135].

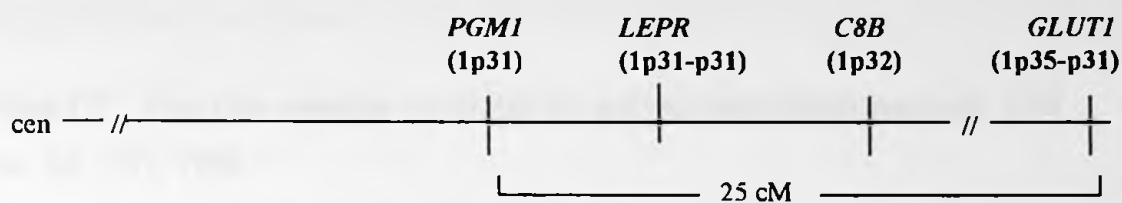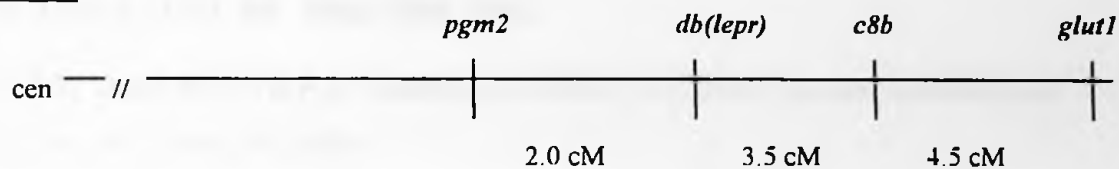## The leptin receptor and skeletal muscle in the LA-N-*fa^f* model.

Following the discovery of the *ob* gene product (leptin),[72] Tartaglia et al (1995) reported the expression cloning of its corresponding receptor, *lepr*, as the putative cause of obesity in the *db* mouse model[75]. The leptin receptor is an alternatively-spliced, single-membrane spanning receptor related to the class I cytokine receptor family[136]. Synteny homology between mouse chromosome 4 (*db* mutation) and rat chromosome 5 (*fa* mutation) permitted the establishment of the rat *fatty* mutation as a defect in *lepr*. *Fatty* is the result of a base transversion at nucleotide 880 converting amino acid 269 from glutamine to a proline residue[121]. Our placement of *cp* within the same chromosomal region as *fatty* was supported by Wu-Peng et al (1997) with the cloning of the Koletsky gene (*f*). The nonsense mutation (a thymine to adenine base transversion at nucleotide 2349 within the intracellular domain) converts a tyrosine residue to a stop codon yielding a truncated receptor[122].

To date, the significance of the leptin receptor in the obesity syndrome is unclear and remains to be elucidated. The primary effect of leptin takes place via receptors in the central nervous system. However, leptin receptors have been identified in peripheral tissues, including skeletal muscle[74]. The role of skeletal muscle in overall organismal

lipogenesis and fatty acid synthesis has been documented[137]. Kahle's lab has established ultrastructural differences (increased lipid content) in skeletal muscle satellite cells in the LA/N-*fa* model[107]. Recent studies by Muoio et al (1997)[138] have shown the role of leptin in lipid partitioning and those of Wang et al (1998)[139] showed induction of leptin gene expression by the nutrient-sensing hexosamine biosynthetic pathway in skeletal muscle[139]. Further implication of the role of skeletal muscle metabolism in the LA/N-*fa* model with a defective leptin receptor provides the foundation for future analysis.

Figure 17. Syntenic relationship between human chromosome 1, mouse chromosome 4 and rat chromosome 5 at the *lepr* locus. Compiled from Truett et al (1991)[2], Kershaw et al (1996)[113] and current data.

HUMAN 1

| | PGM1 (1p31) | LEPR (1p31-p31) | C8B (1p32) | GLUT1 (1p35-p31) |

cen — // —————————————————————— // ————

PGM1 (1p31)   LEPR (1p31-p31)   C8B (1p32)   GLUT1 (1p35-p31)

L——————— 25 cM ———————

MOUSE 4

pgm2   db(lepr)   c8b   glut1

cen — // ————————————————————————————

2.0 cM   3.5 cM   4.5 cM

RAT 5 (13M Zucker-*fa* X BN) F$_1$ INTERCROSS

pgm1   fa (lepr)   c8b   glut1

cen — // ————————————————————————————

1.0 cM   0.4 cM   8.6 cM
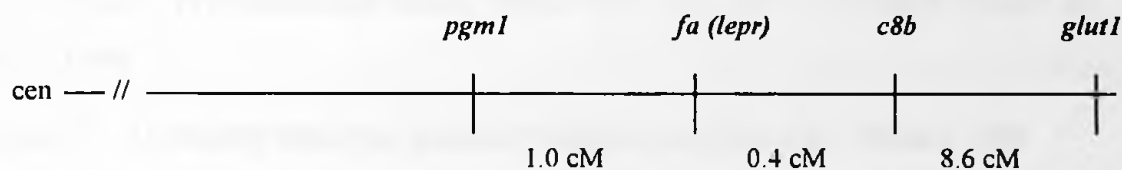
RAT 5 (LA/N-*cp* X BN) F$_1$ INTERCROSS

pgm1   cp = fa^d (lepr)   glut1

1.67 cM   6.67 cM
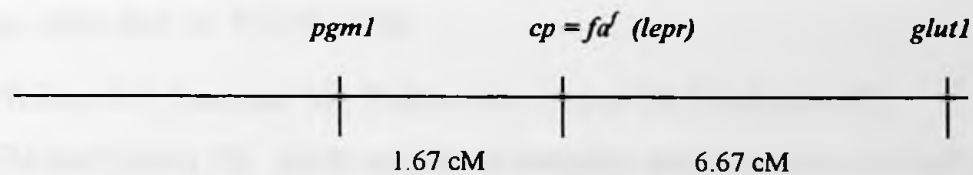
REFERENCES

1. Hansen CT. Two new congenic rat strains for nutrition and obesity research. *Fed Proc* 42: 537, 1983.

2. Truett GE, Bahary N, Friedman JM, Leibel RL. Rat obesity gene *fatty* (*fa*) maps to chromosome 5: Evidence for homology with the mouse gene *diabetes* (*db*). *Proc Natl Acad Sci USA* 88: 7806-7809, 1991.

3. Yen TT, Shaw WN, Yu P-L. Genetics of obesity in Zucker rats and Koletsky rats. *Heredity* 38: 373-377, 1977.

4. Kahle EB, Butz KG, Chua SC, Kershaw EE, Leibel RL, Fenger TW, Hansen CT, Michaelis OE. The rat *corpulent* (*cp*) mutation maps to the same interval on (*Pgm1 - Glut1*) rat chromosome 5 as the *fatty* (*fa*) mutation. *Obes Res* 5: 142-145, 1997.

5. Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL. Overweight and obesity in the United States: Prevalence and trends, 1960-1994. *Int J Obes Rel Metab Disord* 22: 39-47, 1998.

6. Taubes G. As obesity rates rise, experts struggle to explain why. *Science* 280: 1367-1368, 1998.

7. Kortt MA, Langley PC, Cox ER. A review of cost-of-illness studies on obesity. *Clin Ther* 20: 772-779, 1998.

8. Wolf AM, Colditz GA. Current estimates of the economic cost of obesity in the United States. *Obes Res* 6: 97-106, 1998.

9. Manson JE, Willett WC, Stampfer MJ, Colditz GA, Hunter DJ, Hankinson SE, Hennekens CH and Speizer FE. Body weight and mortality among women. *N Engl J Med* 333: 677-685, 1995.

10. Garfinkel L. Overweight and cancer. *Ann Int Med* 103: 1034 - 1036, 1985.

11. Van Itallie TB. Health implications of overweight and obesity in the United States. *Ann Int Med* 103: 983-988, 1985.

12. Jung RT. Obesity as a disease. *Br Med Bull* 53: 307-321, 1997.

13. Colditz GA, Willet WC, Rotnitzky A, Manson JE. Weight gain as a risk factor for clinical diabetes mellitus in women. *Ann Intern Med* 122: 481-486, 1995.

14. Chan JM, Stampfer MJ, Rimm EB, Willett WC, Colditz GA. Obesity, fat distribution, and weight gain as risk factors for clinical diabetes in men. *Diabetes Care* 17: 961-969, 1994.

15. Westlund K, Nicolayson R. A ten year mortality and morbidity study related to serum cholesterol. A follow-up of 3751 men aged 40-49. *Scand J Lab Invest* 30: 1-24, 1972.

16. Royal College of Physicians. Obesity. *J R Coll Physicians Lond* 17: 3-58, 1983.

17. Rabkin SW, Mathewsom FA, Hsu PH. Relation of body weight to development of ischemic heart disease in a cohort of young North American men after a 26 year observation period: The Mannitoba study. *Am J Cardiol* 39: 452-458, 1977.

18. Garrison RJ, Castelli WP. Weight and thirty-year mortality of men in the Framingham study. *Ann Intern Med* 103: 1006-1009, 1985.

19. Blumberg VC, Alexander J. Obesity and the heart. In: Bjorntorp P, Brodoff BN (eds.) *Obesity*. Philadelphia: Lippincott. 517-531, 1992.

20. Messerli FH. Cardiovascular effects of obesity and hypertension. *Lancet* 1: 1165-1168, 1982.

21. Alpert MA, Hashimi MW. Obesity and the heart. *Am J Med Sci* 306: 117-123, 1993.

22. Duflou J, Virmani R, Rabin I, Burk A, Farb A, Smialek J. Sudden death as a result of heart disease in morbid obesity. *Am Heart J* 130: 306-13, 1995.

23. Eckel RH. Obesity and heart disease; a statement for healthcare professionals from the Nutrition Committee, American Heart Association. *Circulation* 96: 3248-3250, 1997.

24. Bird CL, Frankl HD, Lee ER, Haile RW. Obesity, weight gain, large weight changes, and adenamatous polyps of the left colon and rectum. *Am J Epidemiol* 147: 670-680, 1998.

25. Russo A, Franceschi S, La Vecchia C, Dal Maso L, Montella M, Conti E, Giacosa A, Falcini F, Negri E. Body size and colorectal-cancer risk. *Int J Cancer* 78: 161-165, 1998.

26. Caan BJ, Coates AO, Slattery ML, Potter JD, Quesenberry Jr CP, Edwards SM. Body size and the risk of colon cancer in a large case-control study. *Int J Obes Rel Metab Disord* 22: 178-184, 1998.

27. Shoff SM, Newcomb PA. Diabetes, body size, and risk of endometrial cancer. *Am J Epidemiol* 148: 234-240, 1998.

28. Chu SY, Lee NC, Wingo PA, Senie RT, Greenberg RS, Peterson HB. The relationship between body mass and breast cancer among women enrolled in the cancer and steroid hormone study. *J Clin Epidemiol* 44: 1197-1206, 1991.

29. Franceschi S, Favero A, La Vecchia C, Baron AE, Negri E, Dal Maso L, Giacosa A, Montella M, Conti E, Amadori D. Body size indices and breast cancer risk before and after menopause. *Int J Cancer* 67: 181-187, 1996.

30. Lubin F, Ruder AM, Wax Y, Modan B. Overweight and changes in weight throughout adult life in breast cancer etiology. *Am J Epidemiol* 122: 579-588, 1985.

31. Ballard-Barbash R, Schatzkin A, Carter CL, Kannel WB, Kreger BE, D'Agostino RB, Splansky GL, Anderson KM, Helsel WE. Body fat distribution and breast cancer in the Framingham study. *J Natl Cancer Inst* 82: 286-290, 1990.

32. Cleary MP, Maihle NJ. The role of body mass index in the relative risk of developing premenopausal versus postmenopausal breast cancer. *Proc Soc Exp Biol Med* 216: 28-43, 1997.

33. Yuan J-M, Castelao JE, Gago-Dominguez M, Ross RK, Yu MC. Hypertension, obesity and their medications in relation to renal cell carcinoma. *Br J Cancer* 77: 1508-1513, 1998.

34. Keesey RE. Physiological regulation of body weight and the issue of obesity. *Med Clin N Am* 73: 15-27, 1989.

35. Harris RBS. Role of set-point theory in regulation of body weight. *FASEB J* 4: 3310-3318, 1990.

36. Friedman JM, Leibel RL. Tackling a weighty problem. *Cell* 69: 217-220, 1992.

37. Bray GA, York DA. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol Rev* 59: 719-809, 1979.

38. Bray GA, Inoue S, Nishizawa Y. Hypothalamic obesity: the autonomic hypothesis and the lateral hypothalamus. *Diabetologia* 20: 366-377, 1981.

39. Jeanrenaud B. An hypothesis on the aetiology of obesity: dysfunction of the central nervous system as a primary cause. *Diabetologia* 28: 502-513, 1985.

40. Bray GA. Obesity - a state of reduced sympathetic activity and normal or high adrenal activity (the autonomic and adrenal hypothesis revisited). *Int J Obes Rel Metab Disord* 14: 77-92, 1990.

41. Bray GA. Autonomic and endocrine factors in the regulation of energy balance. *Fed Proc* 45: 1404-1410, 1986.

42. Bray GA, York DA, Fisler JS. Experimental obesity: a homeostatic failure due to defective nutrient stimulation of the sympathetic nervous system. *Vit Horm* 45: 1-125, 1989.

43. Lardy H, Shrago E. Biochemical aspects of obesity. *Ann Rev Biochem* 59: 689-710, 1990.

44. Rosenbaum M, Leibel RL, Hirsch J. Obesity. *New Engl J Med* 337: 396-407, 1997.

45. Woods SC, Seeley RJ, Porter Jr. D, Schwartz MW. Signals that regulate food intake and energy homeostasis. *Science* 280: 1378-1383, 1997.

46. Bouchard C. Genetic factors in obesity. *Med Clin N Am* 73: 67-81, 1989.

47. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med* 337: 869-873, 1997.

48. Maffeis C, Talamini G, Tato L. Influence of diet, physical activity and parents' obesity on children's adiposity: a four-year longitudinal study. *Int J Obes Relat Metab Disord* 22: 758-764, 1998.

49. Stunkard AJ, Sorensen TI, Hanis C, Teasdale TW, Chakraborty R, Schull WJ, Schulsinger F. An adoption study of human obesity. *N Engl J Med* 314: 193-198, 1986.

50. Sorensen TI, Price RA, Stunkard AJ, Schulsinger F. Genetics of obesity in adult adoptees and their biological siblings. *BMJ* 298: 87-90, 1989.

51. Sorensen TI, Holst C, Stunkard AJ, Skovgaard LT. Correlations of body mass index of adult adoptees and their biological and adoptive relatives. *Int J Obes Relat Metab Disord* 16: 227-236, 1992.

52. Sorensen TI, Stunkard AJ. Does obesity run in families because of genes? An adoption study using silhouettes as a measure of obesity. *Acta Psychiatr Scand Suppl* 370: 67-72, 1993.

53. Sorensen TI, Holst C, Stunkard AJ. Adoption study of environmental modifications of the genetic influences on obesity. *Int J Obes Relat Metab Disord* 22: 73-81, 1998.

54. Turula M, Kaprio J, Rissanen A, Koskenvuo M. Body weight in the Finnish Twin Cohort. *Diabetes Res Clin Pract* 10: 533-536, 1990.

55. Carey DG, Nguyen TV, Campbell LV, Chisolm DJ, Kelly P. Genetic influence on central abdominal fat: a twin study. *Int J Obes Relat Metab Disord* 20: 722-726, 1996.

56. Austin MA, Friedlander Y, Newman B, Edwards K, Mayer-Davis EJ, King MC. Genetic influences on changes in body mass index: a longitudinal analysis of women twins. *Obes Res* 5: 326-331, 1997.

57. Comuzzie AG, Allison DB. The search for human obesity genes. *Science* 280: 1374-1377, 1998.

58. Zietkiewicz E, Labuda M, Sinnett D, Glorieux FH, Labuda D. Linkage mapping by simultaneous screening of multiple polymorphic loci using *Alu* oligonucleotide-directed PCR. *Proc Natl Acad Sci USA* 89: 8448-8451, 1992.

59. Paterson AH, Lander ES, Hewitt JD, Peterson S, Lincoln SE, Tanksley SD. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* 335: 721-726, 1988.

60. Chagnon YC, Perusse L, Bouchard C. The human obesity gene map: the 1997 update. *Obes Res* 6: 76-92, 1998.

61. Bouchard C. Genetics of human obesity: recent results from linkage studies. *J Nutr* 127: 1887S-1890S, 1997.

62. Bray, Bouchard C. Genetics of human obesity: research directions. *FASEB J* 11: 937-945, 1997.

63. Gauguier D, Frouel P, Parent V, Bernard C, Bihoreau MT, Portha B, James MR, Penicaud L, Lathrop M, Ktorza A. Chromosomal mapping of genetic loci associated with non-insulin dependent diabetes in the GK rat. *Nature Genet* 12: 38-43, 1996.

64. Galli J, Li LS, Glaser A, Ostenson CG, Jiao H, Fakhrairad H, Jacob HJ, Lander ES, Luthman H. Genetic analysis of non-insulin dependent diabetes mellitus in the GK rat. *Nature Genet* 12: 31-37, 1996.

65. Chung Wk, Zheng M, Chua M, Kershaw E, Power-Kehoe L, Tsuji M, Wu-Peng XS, Williams J, Chua Jr SC, Lebel RL. Genetic modifiers of *Leprfa* associated with variability in insulin production and susceptibility to NIDDM. *Genomics* 41: 332-344, 1997.

66. Leibel RL. And finally, genes for human obesity. *Nat Genet* 16: 218-220, 1997.

67. Ingalls AM, Dickie MM, Snell GD. *Obese*, a new mutation in the house mouse. *J Hered* 41: 317-318, 1950.

68. Coleman DL. Diabetes-obesity syndromes in mice. *Diabetes* 31: 1-6, 1982.

69. Coleman DL. *Obese* and *diabetes*: two mutant strains causing diabetes-obesity syndromes in mice. *Diabetologia* 14: 141-148, 1978.

70. Hummel KP, Dickie MM, Coleman DL. *Diabetes*, a new mutation in the mouse. *Science* 153: 1127-1128, 1966.

71. Coleman DL. Effects of parabiosis of *obese* with *diabetes* and normal mice. *Diabetologia* 9: 294-298, 1973.

72. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse *obese* gene and its human homologue. *Nature* 372: 425-432, 1994.

73. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the *obese* gene. *Science* 269: 543-546, 1995.

74. Houseknecht KL, Baile CA, Matteri RL, Spurlock ME. The biology of leptin: a review. *J Anim Sci* 76: 1405-1420, 1998.

75. Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 83: 1263-1271, 1995.

76. Chen H, Charlat LA, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP. Evidence that the *diabetes* gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in *db/db* mice. *Cell* 84: 491-495, 1996.

77. Zucker LM, Zucker TF. *Fatty*, a new mutation in the rat. *J Hered* 52: 275-278, 1961.

78. Zucker LM. Two-way selection for body size in rats, with observations on simultaneous changes in coat color pattern and hood size. *Genetics* 45: 467-483, 1960.

79. Zucker LM, Antoniades HN. Insulin and obesity in the Zucker genetically obese rat *"fatty."* *Endocrinology* 90: 1320-1330, 1972.

80. Ionescu E, Sauter JF, Jeanrenaud B. Abnormal glucose tolerance in genetically obese *(fa/fa)* rats. *Am J Physiol* 248: E500-E506, 1985.

81. Greenhouse DD, Hansen CT, Michaelis IV OE. Development of *fatty* and *corpulent* rat strains. *ILAR News* 32: 2-4, 1990.

82. Kava R, Greenwood MRC, Johnson PR. Zucker *(fa/fa)* rat. *ILAR News* 32: 4-8, 1990.

83. Lindsay JR. Historical Foundations. In: Baker HJ, Lindsey JR, Weisbroth SH, eds. The Laboratory Rat, Vol. 1. New York, NW: Academic, pp 1-36, 1979.

84. Kava R, Peterson RG, West DB. Wistar diabetic *fatty* rat. *ILAR News* 32: 9-13, 1990.

85. Peterson RG, Little LA, Neel M-A. WKY *fatty* rat as a model of obesity and NIDDM. *ILAR News* 32: 13-15, 1990.

86. Peterson RG, Shaw WN, Neel M-A, Little LA, Eichberg J. Zucker diabetic *fatty* rat as a model for NIDDM. *ILAR News* 32: 16-19, 1990.

87. Koletsky S. Obese spontaneously hypertensive rats - a model for study of atherosclerosis. *Exp Mol Path* 19: 53-60, 1973.

88. Russell JC, Koeslag DG. Jcr:LA-*corpulent* rat: a strain with spontaneous vascular and myocardial disease. *ILAR News* 32: 27-32, 1990.

89. McCune SA, Baker PB, Stills, Jr. HF. SHHF/Mcc-*cp* rat: model of obesity, non-insulin dependent diabetes, and congestive heart failure. *ILAR News* 32: 23-27, 1990.

90. Michaelis IV OE, Ellwood KC, Tulp OL, Greenwood MRC. Effect of feeding sucrose or starch diets on parameters of glucose tolerance in the LA/N-*corpulent* rat. *Nutr Res* 6: 95-99, 1986.

91. Ellwood KC, Michaelis IV OE, Emberland JJ, Bhathena SJ. Hormonal and lipogenic and gluconeogenic enzymatic responses in LA/N-*corpulent* rats. *Proc Soc Exp Biol Med* 179: 163-167, 1985.

92. Michaelis IV OE, Ellwood KC, Hallfrisch J, Hansen CT. Effect of dietary sucrose and genotype on metabolic parameters of a new strain of genetically obese rat: LA/N-*corpulent*. *Nutr Res* 3: 217-228, 1983.

93. Tulp OL. Characteristics of thermogenesis, obesity, and longevity in the LA/N-*cp* rat. *ILAR News* 32: 32-38, 1990.

94. Bhathena SJ, Revett K, Michaelis IV OE, Ellwood KC, Voyles NR, Recant L. Genetic obesity and dietary sucrose decrease hepatic glucagon and insulin receptors in LA/N-*corpulent* rats. *Proc Soc Exp Biol Med* 181: 71-77, 1986.

95. Ellwood KC, Michaelis IV OE, Bhathena SJ. Comparative hormonal profile of two newly developed obese congenic rat strains. *Comp Biochem Physiol* 89A: 371-375, 1988.

96. Timmers KI, Voyles NR, Recant L. Genetically obese rats with (SHR/N-*cp*) and without diabetes (LA/N-*cp*) share abnormal islet responses to glucose. *Metabolism* 41: 1125-1133, 1992.

97. Berdanier CD, Pan J-S, Hartle DK, Michaelis IV OE. Glucose turnover in lean and obese rats of the SHR/N-*cp* and LA/N-*cp* strains. *Comp Biochem Physiol* 106B: 87-94, 1993.

98. Yamini S, Staples RC, Hansen CT, Szepesi B. Effect of dietary carbohydrate on liver and kidney enzyme activities and plasma amino acids in the LA/N-*cp* rat. *Int J Obes Relat Metab Disord* 15: 189-203, 1991.

99. Tulp OL, McKee TD. Triiodothyronine (T3) neogenesis in lean and obese LA/N-*cp* rats. *Biochem Biophys Res Commun* 140: 134-142, 1986.

100. Tulp OL, Shields SJ. Thermogenesis in cafeteria-fed LA/N-*cp* (*corpulent*) rats. *Nutr Res* 4: 325-332, 1984.

101. Young NL, Tulp OL. The effects of norepinephrine and nutritional status on resting metabolic rates in the LA/N-*cp* rat. *Comp Biochem Physiol* 94A: 597-602, 1989.

102. Tulp OL, Hansen CT, Michaelis IV OE. Effects of dietary carbohydrate and phenotype on thyroid hormones and brown adipose tissue locularity in adult LA/N-*cp* rats. *Comp Biochem Physiol* 94A: 225-229, 1989.

103. Tulp OL, Jones CT. Effects of increased energy expenditure on weight gain and adiposity in the LA/N-*corpulent* rat. *Comp Biochem Physiol* 86A: 67-72, 1987.

104. Kahle, EB, Dadgari JM, Dudley GA, Hansen CT, Tulp OL, Michaelis OE. Adaptive response of enzymes of carbohydrate and lipid metabolism to exercise training. In: New Models of Genetically Obese Rats for Studies in Diabetes, Heart Disease, and Complications of Obesity. Bethesda, MD: National Institutes of Health, pp143-148, 1988.

105. Kahle B, Harapanahalli S, Cross M, Butz K, Music M, Wellman F, Guyer K. Triglycerides in the femoral artery and vein of exercised LA/N-*cp* rats. *FASEB J* 7: A608, 1993.

106. Kahle EB, Leibel RL, Domaschko DW, Raney SG, Mann KT. Obesity genes and insulin resistance syndrome. *Ann N Y Acad Sci* 827: 35-49, 1997.

107. Noah JA. Differences in lean and obese skeletal muscle myoblasts of adult and post-natal LA/N-*fa* rats: a confocal description of the skeletal muscle myoblast. M.S. Thesis. Huntington, WV: Marshall University, 1998.

108. Szpirer C, Riviere M, Szpirer J, Genet M, Dreze P, Islam MQ, Levan G. Assignment of 12 loci to rat chromosome 5: evidence that this chromosome is homologous to mouse chromosome 4 and to human chromosomes 9 and 1 (1p arm). *Genomics* 6: 679-684, 1990.

109. Chua Jr. SC, Hansen MH, Truett GE, Leibel RL. Utility of a *c-jun* microsatellite marker in determining gene dosage for *fatty* (*fa*). *Obes Res* 1: 475-480, 1993.

110. Lavau M, Bazin R. Inguinal fat pad weight plotted versus body weight as a method of genotype identification in 16-day-old Zucker rats. *J Lipid Res* 23: 941-943, 1982.

111. Gross-Bellard M, Oudet P, Chambon P. Isolation of high-molecular-weight DNA from mammalian cells. *Eur J Biochem* 36: 32-38, 1973.

112. Choe Q, Russel M, Birch DE, Raymond J, Bloch W. Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplification. *Nucleic Acids Res* 20: 1717-1723, 1992.

113. Kershaw EE, Chua SC, Williams JA, Murphy EM, Leibel RL. Molecular mapping of SSRs for *Pgm1* and *C8b* in the vicinity of the rat *fatty* locus. *Genomics* 27: 149-154, 1995.

114. Don RH, Cox PT, Wainright BJ, Baker K, Mattick JS. 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Res* 19: 4008, 1991.

115. Stansfield WD. Statistical Distributions. In: Stansfield WD. Schaum's Outline of Theory and Problems of Genetics, 3rd edition. New York, NY: McGraw-Hill, Inc., p163, 1991.

116. Smoller JW, Truett GE, Hirsch J, Leibel RL. A molecular genetic method for genotyping *fatty* (*fa/fa*) rats. *Am J Physiol* 264: R8-R11, 1993.

117. Leiter EH. The genetics of diabetes susceptibility in mice. *FASEB J* 3: 2231-2241, 1989.

118. Smoller JW, Truett GE, Hirsch J, Leibel RL. The Zucker *fatty* (*fa*) gene is not a mutation of corticotropin-releasing factor. *Am J Physiol* 264: R12-R15, 1993.

119. Bahary N, Leibel RL, Joseph L, Friedman JM. Molecular mapping of the mouse *db* mutation. *Proc Natl Acad Sci USA* 87: 8642-8646, 1990.

120. Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Grand B. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 392: 398-401, 1998.

121. Chua Jr. SC, White DW, Wu-Peng XS, Liu S-M, Okada N, Kershaw EE, Chung WK, Power-Kehoe L, Chua M, Tartaglia LA, Leibel RL. Phenotype of *fatty* due to Gln269Pro mutation in the leptin receptor (*lepr*). *Diabetes* 45: 1141-1143, 1996.

122. Wu-Peng XS, Chua, Jr. SC, Okada N, Liu S-M, Nicolson M, Leibel RL. Phenotype of the obese Koletsky (*f*) rat due to Tyr763Stop mutation in the extracellular domain of the leptin receptor (*lepr*): Evidence for deficient plasma-to-CSF transport of leptin in both the Zucker and Koletsky obese rat. *Diabetes* 46: 513-518, 1997.

123. Silver LM. Mouse Genetics: Concepts and Applications. New York, NY: Oxford University Press. 362 pages, 1995.

124. Kahle EB, Butz KG, Leibel RL, Hansen CT, Bhathena SJ, Michaelis OE. Glucose homeostasis in 3 interstrains (LA/N-BN/Crl *cp/cp*, Zucker 13M - BN/Crl *fa/fa*, and Zucker 13M - LA/N *fa^{cp}*) of genetically obese rats. In: E. Shafrir, ed. Lessons from Animal Diabetes, Vol. 6. Boston, MA: Birkhauser, pp411-418, 1996.

125. Hummel KP, Coleman DL, Lane PW. The influence of genetic background on expression of mutations at the *diabetes* locus in the mouse. I. C57BL/KsJ and C57BL/6J strains. *Biochem Genet* 7: 1-13, 1972.

126. Leiter EH. The influence of genetic background on expression of mutations at the *diabetes* locus in the mouse. IV. Male lethal syndrome in CBA/Lt mice. *Diabetes* 30: 1035-1044, 1981.

127. Leiter EH, Chapman HD, Coleman DL. The influence of genetic background on expression of mutations at the *diabetes* locus in the mouse. V. Interactions between the *db* gene and hepatic sex steroid sulfotransferase correlates with gender-dependent susceptibility to hyperglycemia. *Endocrinology* 124: 912-922, 1989.

128. Coleman DL. The influence of genetic background on expression of mutations at the *diabetes* locus in the mouse. VI. Hepatic malic enzyme activity is associated with diabetes severity. *Metabolism* 41: 1134-1136, 1992.

129. Kimmel PL, Velasquez MT, Michaelis IV OE. Diabetic nephropathy in non-insulin dependent diabetes mellitus: animal models with emphasis on recently developed *corpulent* rat strains. *ACTA Diabetologia* 29: 142-148, 1993.

130. Michaelis IV OE, Velasquez MT, Abraham AA, Servetnick DA, Scholfield DJ, Hansen CT. Development and characteristics of a new strain of obese hyperinsulinemic and hyperlipidemic Dahl Salt-Sensitive Rat: the Dahl Salt Sensitive/NIH-*corpulent* rat. *Am J Hypertens* 8: 467-473, 1995.

131. Tulp OL. Characteristics of thermogenesis, obesity, and longevity in the LA/N-*cp* rat. *ILAR News* 32: 32-38, 1990.

132. Serikawa T, Kuramoto T, Hilbert P, Mori M, Yamada J, Dubay CJ, Lindpainter K, Ganten D, Guenet J-L, Lathrop GM, Beckmann JS. Rat gene mapping using PCR-analyzed microsatellites. *Genetics* 131: 701-721, 1992.

133. Levan G, Szpirer J, Szpirer C, Klinga K, Hanson C, Islam MQ. The gene map of the Norway rat (*Rattus norvegicus*) and comparative mapping with mouse and man. *Genomics* 10: 699-718, 1991.

134. Dracopoli NC, O'Connell P, Elsner TI, Lalouel J-M, White RL, Buetow KH, Nishimura DY, Murray JC, Helms C, Mishra SK, Donis-Keller H, Hall JM, Lee MK, King M-C, Attwood J, Morton NE, Robson EB, Mahtani M, Willard HF, Royle NJ, Patel I, Jeffreys AJ, Verga V, Jenkins T, Weber JL, Mitchell AL, Bale AE. The CEPH consortium linkage map of human chromosome 1. *Genomics* 9: 686-700, 1991.

135. Nadeau JH. Maps of linkage and synteny homologies between mouse and man. *Trends Genet* 5: 82-86, 1989.

136. Tartaglia LA. The leptin receptor. *J of Biol Chem* 272: 6093-6096, 1997.

137. Hollands MA, Cawthorne MA. Important sites of lipogenesis in the mouse other than the liver and white adipose tissue. *Biochem J* 196: 645-647, 1981.

138. Muoio DM, Dohn GL, Fiedorek, Jr. FT, Tapscott EB, Coleman RA. Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* 46: 1360-1363, 1997.

139. Wang J, Liu, Hawkins M, Barzilai, Rossetti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. *Nature* 393: 684-688, 1998.

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