Pair feeding-mediated changes in metabolism: stress response and pathophysiology in insulin-resistant, atherosclerosis-prone JCR:LA-cp rats

James C. Russell,1 Spencer D. Proctor,1 Sandra E. Kelly,1 and David N. Brindley2
1Metabolic and Cardiovascular Diseases Laboratory, Alberta Institute for Human Nutrition; and 2Department of Biochemistry (Signal Transduction Research Group), University of Alberta, Edmonton, Alberta, Canada

Submitted 27 February 2008; accepted in final form 3 April 2008

Russell JC, Proctor SD, Kelly SE, Brindley DN. Pair feeding-mediated changes in metabolism: stress response and pathophysiology in insulin-resistant, atherosclerosis-prone JCR:LA-cp rats. Am J Physiol Endocrinol Metab 294: E1078–E1087, 2008. First published April 15, 2008; doi:10.1152/ajpendo.90257.2008.—Rats of the JCR:LA-cp strain, which are homozygous for the cp gene (cplcp), are obese, insulin-resistant, and hyperinsulinemic. They exhibit associated micro- and macrovascular disease and end-stage ischemic myocardial lesions and are highly stress sensitive. We subjected male pair feeding (providing the rats each day with the amount of food eaten by matched freely fed animals), a procedure that alters the diurnal feeding pattern, leading to a state of intermittent caloric restriction. Effects on insulin, glucose, and lipid metabolism, response to restraint stress, aortic contractile/relaxant response, and myocardial lesion frequency were investigated. Pair-fed young (12-wk-old) cp/cp rats had lower insulin and glucose levels (basal and following restraint), consistent with increased insulin sensitivity, but a greater increase in plasma nonesterified fatty acids in response to restraint. These effects were unrelated to lipolytic rates in adipose tissue but may be related to reduced fatty acid oxidation in skeletal muscle. Older (24-wk-old) pair-fed cp/cp rats had significantly reduced plasma triglyceride levels, improved micro- and macrovascular function, and reduced severity of ischemic myocardial lesions. These changes indicate a significant amelioration of end-stage disease processes in this animal model and the complexity of metabolic/physiological responses in studies involving alterations in food intake. The effects illustrate the sensitivity of the JCR:LA-cp rat, an animal model for the metabolic syndrome and associated cardiovascular disease, to the environmental and experimental milieu. Similar stress-related mechanisms may play a role in metabolically induced cardiovascular disease in susceptible human beings.
intermittent fasting or intermittent metabolic deprivation through inhibition of glycolysis. We have also reported that metabolic deprivation, through administration of the glucose analog 2-deoxy-D-glucose (2-DG), improves cardiovascular function and reduces end-stage myocardial lesions in an animal model of the metabolic syndrome (49).

The JCR:LA-cp rat is a unique strain that has been used in the study of the metabolic syndrome and putative pharmacological interventions (32). If homozygous for the autosomal recessive cp gene (cp/cp), the rats are obese and spontaneously develop important elements of the pathophysiological status associated with the metabolic syndrome in humans (27, 43), including advanced intimal (atherosclerotic) lesions, myocardial ischemic lesions, and renal microvascular dysfunction and glomerular sclerosis (34, 48). The atherosclerosis is associated with a vasculopathy that includes increased vascular contractility and reduced vascular relaxation. Heterozygous (cp/+ or homozygous normal (+/+)) rats are lean and metabolically normal.

The cp mutation results in the presence of a stop codon in the extracellular domain of the leptin receptor (ObR) (56), leading to the absence of membrane-bound ObR, of any of the isoforms in cp/cp animals. In the absence of the ObR, the animals develop a marked hyperleptinemia and hyperphagia. A profound insulin resistance develops between the ages of 4 and 7 wk with accompanying VLDL hypertriglyceridemia (40, 51). The insulin-resistant state is accompanied by an exaggerated sensitivity to brief immobilization stress that has both metabolic and neural components (21, 26). The cp/cp rats maintain eu glycaemia at the expense of extremely high circulating insulin concentrations (47). The hyperphagic cp/cp rats must divert glucose derived from a high-carbohydrate diet to the liver, with disposal by conversion to triglyceride (TG) and export to the plasma as VLDL (17, 46), leading to VLDL hyperlipidemia. The hypertriglyceridemia is thus not caused by an impaired clearance of lipoproteins from the circulation but by enhanced hepatic secretion and subsequent modification of the lipoproteins (40, 51). The findings suggest that the insulin resistance of the cp/cp rats, and possibly also of humans, is related to abnormal lipid metabolism, associated with decreased fatty acid (FA) oxidation, increased TG levels in tissues (particularly in muscle) (3, 4, 42), and stress-induced release of FA from adipose tissue (26). High insulin levels appear, in themselves, to have pathological effects and may be a major contributor to the atherosclerosis and vascular dysfunction (1, 27, 36). Circulating FA levels of the cp/cp rats in response to immobilization-induced stress (26), which reached those normally associated with severe diabetes, could also contribute to the vascular damage and dysfunction as well as aggravate the insulin resistance. Our earlier findings also suggest that stress may play a significant exacerbating role in the abnormal metabolism and pathophysiology of this animal model (21).

Our hypothesis is that stress, as suggested by Brindley and Rolland (10) and supported by Björntorp (6), plays a significant role in both the metabolic syndrome and associated pathophysiology. However, “stress responses” arise in a variety of ways, are not a single entity, and have proven difficult to study experimentally. We suggest that some variants of psychological stress, especially in sensitive individuals and those with the metabolic syndrome, are deleterious and exacerbate the pathophysiological processes (19). In contrast, there is evidence that intermittent food deprivation, or inhibition of oxidative metabolism and accompanying physiological changes, can have beneficial effects on metabolism and pathological sequelae (25, 49, 54). The metabolic and physiological abnormalities of the cp/cp rat develop and change as the animals develop from juveniles to mature adults, and our experimental study was designed to address effects on critical elements of the pathophysiological process at appropriate ages. Thus, we subjected juvenile/adolescent animals of the JCR:LA-cp strain to pair feeding and then assessed both basal metabolism and response to restraint stress when they were young adults. The metabolic end points were changes in insulin and glucose metabolism, synthesis and oxidation of FA, and rates of lipolysis by adipose tissue. In a second study, we used fully adult rats subjected to pair feeding, without reduction of total food intake, to assess effects on metabolism and end-stage pathophysiology represented by micro- and macrovascular dysfunction and ischemic myocardial lesion frequency.

METHODS

Experimental Protocols

Experiment 1: metabolic effects of pair feeding and immobilization stress in adolescent cp/cp rats. Pair-fed cp/cp animals were subjected to the procedure from 8 wk of age (fully insulin resistant) to 12 wk of age (young adults). In this study, the rats were pair fed to match the food intake of freely fed cp/cp animals and compared with control (freely fed) cp/cp and +/+ rats. At 12 wk of age, a restraint stress test was performed with measurement of indexes of lipid and glucose metabolism followed by the rats being killed and blood and tissue sampling for measurement of biochemical parameters.

Experiment 2: metabolic and physiological effects of pair feeding of adult cp/cp rats. Adult rats were placed in the feeding protocol at 12 wk of age and with food intake paired to freely eating cp/cp control rats. At 24 wk of age a meal tolerance test was performed, with the rats killed 1 wk later and blood and urine sampling taken for biochemical parameters. Aortae were removed for vascular function studies and hearts and kidneys for histology.

Experimental Procedures

Animals. Male JCR:LA-cp rats, cp/cp (obese) and +/+ (lean; 2:1 cp/+ and +/+, were bred and maintained in our established rat colony (43). The rats were housed individually in polycarbonate cages and placed on a reversed light cycle (lights on at 1800 and off at 0600) 1 wk prior to the start of the pair-feeding protocol to facilitate metabolic studies during the active (dark) phase of their diurnal cycle. All food was Lab Diet 5001 (PMI Nutrition International, Brentwood, MO). Rats were weighed and food intake determined twice/wk throughout the experimental period. All care and treatment of the rats was in accordance with the guidelines of the Canadian Council on Animal Care and was reviewed and approved in advance by the Health Sciences Animal Policy and Welfare Committee of the University of Alberta.

Blood samples were obtained from the tip of the tail of conscious, unrestrained rats (45) during the dark phase between 0800 and 1200. At termination (either 13 or 25 wk of age), all rats were killed under anesthesia with isoflurane in oxygen during the same time period. Terminal blood samples were obtained by cardiac puncture and urine collected from the bladder. Tissues were removed for in vitro study and used immediately or flash-frozen in liquid nitrogen and stored at −80°C for later assay.

Feeding protocol. Control rats were provided with a significant excess of food in the cage top hopper at all times such that the remaining food was equivalent to 3 days consumption. Pair-fed cp/cp
rats were placed on a protocol that provided, in the hopper at a fixed
time of day (6 h into the dark period), the amount of food that control
animals ate on the corresponding experimental day. In all other
respects, the rats in the different groups were treated identically.
Under these circumstances, pair-fed rats ate the food provided within
12 h and then remained without food for the remainder of each 24-h
period. Occasionally (<1% of feeding periods), individual rats had
small amounts of food (~10%) remaining after 24 h.

Restrained stress. Rats were subjected to a single stress response test
with a 15-min restraint period, but without chronic vessel cannulation
as in our previous work (26). The animals were studied in the fed
state, postabsorption phase, during the first 4 h of the dark (active)
period of their diurnal cycle. An initial sample of blood was taken
from the tip of the tail, following which the rat was placed in a
polyethylene cone restraining device (Decapi-cone; Braintree Sci-
tific, Braintree, MA) for 15 min (21). A second blood sample was
taken while the animal was in the cone, after which it was released
and returned to its cage. A final blood sample was taken at 60 min from
the start of restraint.

Meal tolerance test. A meal tolerance test was performed at 24 wk
of age, following a standardized protocol (47). Animals were previ-
sely subjected to a mock procedure 1 wk before the meal tolerance
test. The rats were deprived of food over the light (inactive) period,
and the test was conducted in the first 3 h of the dark period.
Conscious, unrestrained rats were subjected to three blood samplings
during each session. Initially, animals were placed on a heated table
to ensure vasodilation of the tail, and 0.5 ml of blood was taken from
the tip of the tail (time 0). Rats were then replaced in their cages and
given a 5-g food pellet (the test meal). Timing began when 50% of the
test meal had been consumed, and samples of blood were taken at 30
and 60 min for the analysis of glucose and insulin. All rats ate the full test
meal within 15 min of presentation.

Analytical methods. Plasma glucose was determined using a glu-
cose oxidase assay procedure (Diagnostic Chemicals, Charlottetown,
PEI, Canada). Insulin was assayed by rat ELISA assay (Mercodia,
Uppsala, Sweden). Plasma triglyceride (L-type TG;H), total chole-
sterol (Cholesterol E), and low-density lipoprotein (LDL) cholesterol
(L-type LDL-C) assays were obtained from Wako Pure Chemicals
USA (Richmond, VA). High-density lipoprotein (HDL) cholesterol
was assayed using direct HDL assay (Diagnostic Chemicals). Unes-
terified FA in the serum was measured using an assay kit (NEFAC;
Wako Pure Chemicals USA) and a 96-well plate reader (8). Lipids
were extracted from samples of liver, aorta, and skeletal muscle (7);
the chloroform phase was dried and then dissolved in 100 μl of
propan-1-ol. Triglyceride concentrations were then measured using 20
μl of propanol solution and 200 μl of TG assay reagent. Urine
albumin measurements were performed on a Beckman Coulter LX20i
analyzer using an immunoturbidimetric method.

Rate of lipolysis. The rate of lipolysis was measured using adipose
tissue (~100 mg) in preference to isolated adipocytes, since the latter
are highly fragile when isolated from obese rats (8). Fresh adipose

tissue was preincubated at 37°C for 30 min in 900 μl of medium, as
described previously (24). A 100-μl sample of medium was collected
before 100 μl of new medium that either did or did not contain
norepinephrine (final concentration: 1.1 μmol/l) was added. Samples
were then incubated for a further 3 h at 37°C, after which the clear
medium was collected. Medium collected following the 30-min and
3-h incubation periods was assayed for glycerol content, and the
resulting values were used to calculate the amount of glycerol released
during the 3-h incubation period.

FA oxidation. The rate of FA oxidation in resting intact soleus
muscle and in slices of liver was determined using [14C]palmitic acid
(18). Briefly, fresh soleus muscle (~100 mg) was tied lightly to a
tungsten wire with suture material so as to maintain its normal resting
length. A sample of liver (~100 mg) was cut into small pieces. The
muscle and liver samples were crimp-sealed in 25-ml serum vials with
2 ml of Krebs-Henseleit buffer containing 0.2 mmol/l [1-14C]palmit-
tate (0.083 Ci/mol) adsorbed onto BSA (0.2% wt/vol) and 11 mmol/l
glucose. The vials were gassed with 95% O2–5% CO2 via a stainless
steel needle inserted through the seal, and effluent gas was collected
through a second needle and bubbled through 1.5 ml of hyamine
hydroxide (ICN Pharmaceuticals Canada, Montreal, QC, Canada).
Vials were incubated for 2 h at 37°C, after which the reaction was
stopped by injecting 1 ml of 8% perchlorylic acid through the seal.
After a further 30 min of gassing and collection of the effluent, 10 ml of
ACS scintillation cocktail (Amersham Canada, Oakville, ON, Can-
da) was added to the hyamine solution and radioactivity was deter-
mined by scintillation counting.

Vascular function studies. The vascular function of aortic rings,
with intact endothelium, was assessed using established methods (41).
Briefly, rats were anesthetized using isoflurane in oxygen. The chest

cavity was exposed and the thoracic aorta excised, trimmed of
adhering fat and connective tissue, and cut into 3-mm-long transverse
rings. Aortic rings were mounted on stainless steel hooks under 1.5-g
resting tension in 10-ml organ baths and bathed at 37°C in Krebs
solution containing in mmol/l: 116 NaCl, 5.4 KCl, 1.2 CaCl2, 2
MgCl2, 1.2 Na2PO4, 10 glucose, and 19 NaHCO3) and gassed with
95% O2 and 5% CO2. Tension was recorded isometrically with Grass
FT03C transducers (Grass Medical Instruments, Quincy, MA) and
displayed on a Digi-Med tissue force analyzer (Model 210; Micro-
Med, Louisville, KY) linked to an IBM-compatible computer that
acquired data digitally using DMSI 210/4 (Micro-Med) software.

The contractile response of endothelium-intact rings of aortae to
phenylephrine (PE) was assessed through concentration-response
curves for PE (1 nmol/l to 300 mmol/l). The basal nitric oxide
(NO)-mediated relaxation of aortic rings (precontracted with PE to
80% of maximal contraction) was assessed by determining the con-
centration response to the endothelial NO-releasing agent acetylcho-
line (ACh) and the NO donor sodium nitroprusside. Direct assessment
of NO-mediated effects was also determined through addition of
N5-nitro-L-arginine methyl ester, at 10−4 mol/l, to inhibit NO syn-
thesis activity (33).

Myocardial lesions. Hearts were cut transversely into four blocks,
fixed in formalin, subjected to conventional processing, embedded in
a single paraffin block, and sectioned followed by hematoxylin and
eosin staining. Heart sections were examined blind by an experienced
observer and the number of ischemic lesions identified in each of the
sections summed for each heart. The lesions were categorized by four
stages, as described previously (34, 36).

Statistical analysis. Results are expressed as means ± SE and were
analyzed with SigmaStat (Jandel Scientific, San Rafael, CA) and
plotted using SigmaPlot (Systat Software, San Jose, CA) and Prism
(Graphpad, San Diego, CA). Results were compared using one- and
two-way analysis of variance followed by multiple comparison tests.
Concentration-response curves were analyzed using the ALLFIT
program (15), which fits the complete data set to the logistic equation
and permits independent testing of differences between individual
parameters. A value of <0.05 was taken as being statistically
significant.

RESULTS

Experiment 1: Metabolic Effects of Pair Feeding
in Adolescent cpl/cpl Rats

Food intake and body weight. Figure 1 (left) shows food
intake and body weight of cpl/cpl rats that were either freely fed
or pair fed over the period from 8 to 12 wk of age. Data from
freely fed +/− control rats is also shown for reference. Despite
having the same daily food intake over each 24-h period as rats
fed ad libitum, the pair fed cpl/cpl rats showed an 8% lower
body weight (P < 0.05) from 9 wk of age and beyond.

Restrained stress. Figure 2 depicts normal fed-state insulin
and glucose levels (baseline values) and values following
PAIR FEEDING OF INSULIN-RESISTANT JCR:LA-cp RATS

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The restraint stress, with a small decrease in the recovery period. Pair-fed cp/cp rats also had lower baseline plasma glucose concentrations that were not altered by the restraint stress (Fig. 2).

The release of FA into the circulation is a major component of the response to stress. Compared with animals in the fed state (baseline values), fasted rats +/? and cp/cp at 12 wk of age showed significantly elevated plasma FA levels (P < 0.001; Fig. 3), with the cp/cp rats having higher levels than the +/? animals (P < 0.0005). Although baseline (fed state) FA concentrations were not different between freely fed +/? and cp/cp rats, pair-fed cp/cp rats had ~75% higher levels than freely fed cp/cp rats (P < 0.01). FA levels of freely fed cp/cp rats increased nonsignificantly during the period of restraint, with a further increase during recovery (P < 0.01). The significantly higher FA levels of pair-fed cp/cp rats at time 0 were further exacerbated both during and following restraint (P < 0.001).

Fat pads and lipolysis. Retroperitoneal fat pads of the cp/cp rats are larger than those of the +/? rats, reflecting their obese status (Table 1). Basal lipolytic activity, expressed per gram of tissue, did not differ with genotype. However, when stimulated with norepinephrine, adipose tissue from the +/? rats showed significantly higher rates of lipolysis, on a per gram basis, than did that from the cp/cp rats. When expressed on a per fat pad basis, the basal and norepinephrine-stimulated release of glycerol from adipose tissue of the cp/cp rats remained about fourfold higher than that from the +/? rats due to higher fat pad weights. Rate of lipolysis by adipose tissue of cp/cp rats was not significantly affected by pair feeding under the experimental conditions.

FA oxidation. The rate of FA oxidation by soleus muscle showed no significant difference between freely fed +/? and cp/cp rats (Fig. 4) but was significantly reduced in the pair-fed cp/cp animals (P < 0.05). Livers of 12-wk-old cp/cp rats had a much lower rate of FA oxidation than did those of +/? rats, with pair feeding having no significant effect.

Tissue triglyceride content. Soleus muscle TG concentrations were significantly higher in cp/cp than in +/? rats and

Fig. 1. Food intake and body weights of +/? male rats freely fed and cp/cp rats freely fed and pair fed. Experiment 1 was performed using young (adolescent) rats, and experiment 2 was performed using young adult rats (see METHODS for details). Values are means ± SE, 10 rats/group. Pair-fed rats in experiment 1 had significantly lower body weights from 9 wk of age (P < 0.05).

Fig. 2. Plasma insulin (A) and glucose (B) concentrations of JCR:LA-cp rats in experiment 1 in the fed state before and during 15 min of immobilization and at 60 min following a recovery period. Values are means ± SE, 10 rats at 13 wk of age in each group. †P < 0.05; ††P < 0.01 vs. time 0; **P < 0.01; ***P < 0.001 vs. freely fed cp/cp rats.

Fig. 3. Plasma free fatty acid (FFA) concentrations of JCR:LA-cp rats in experiment 1, fasted and in the fed state, before and during 15 min of immobilization and at 60 min following a recovery period. Values are means ± SE, 10 rats in each group at 13 wk of age. For the fasted values: **P < 0.001 vs. time 0 (fed state); ††P < 0.001 vs. cp/cp for baseline and restraint values; **P < 0.01; ***P < 0.001 vs. freely fed rats; †P < 0.05; ††P < 0.01; †††P < 0.001 vs. time 0.
unaffected by pair feeding (Fig. 4). TG content of the liver was also significantly greater in the cp/cp than in the +/? rats, and this was reduced by pair feeding (P < 0.05).

Experiment 2: Metabolic and Physiological Effects of Pair Feeding in Adult cp/cp Rats

Food intake and body weight. Figure 1 (right) shows food intake and body weights over the period of 12 to 24 wk of age. In these mature rats, there was no difference in body weights between the freely fed and pair-fed animals.

Meal tolerance test. Meal tolerance tests performed on rats at 24 wk of age (Fig. 5) showed much higher fasting and postprandial insulin levels in freely fed control cp/cp rats than in +/? rats. Also, reflecting the insulin-resistant state of these animals, plasma glucose concentrations, fasting and following the test meal, were elevated in the cp/cp rats. Fasting plasma insulin levels of the pair-fed cp/cp rats were not significantly elevated above those of the freely fed cp/cp rats but were significantly elevated 30 min after the meal challenge (P < 0.05). Plasma glucose concentrations of the pair-fed rats were consistently lower than those of the freely fed animals, with this difference being highly significant 60 min after the meal challenge (P < 0.001).

Plasma lipid concentrations. Pair feeding caused a significant decrease in plasma TG concentrations of the cp/cp rat (P < 0.01; Fig. 6). There was no change in the plasma concentrations of total, LDL, or HDL cholesterol in the pair-fed animals (data not shown).

Vascular dysfunction. As shown in Fig. 7, aortic vascular function is significantly impaired in the cp/cp rat, compared with the +/? rat, with elevated contractile response to the noradrenergic agonist PE and impaired ACh-mediated relaxation. Figure 7 also shows a reduced contractile response to PE by the aortic rings from pair-fed cp/cp rats. The calculated parameters in Table 2 show that the reduction in the hypercontractile response to PE in the pair-fed rats was significant (P < 0.001), but there was no significant improvement in the ACh-mediated relaxation.

Microalbuminuria. Glomerular dysfunction, and resultant albuminuria, is an element of the microvascular disease associated with the cp/cp rat.

### Table 1. Retroperitoneal fat pad weight and rates of lipolysis in male JCR:LA-cp rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Fat Pad Weight, g</th>
<th>Basal (Unstimulated), μmol·h⁻¹·g⁻¹</th>
<th>NE (Stimulated), μmol·h⁻¹·g⁻¹</th>
<th>Basal/Fat Pad, μmol/h</th>
<th>NE (Stimulated)/Fat Pad, μmol/h</th>
<th>Ratio (Stimulated/Unstimulated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/? Freely fed</td>
<td>0.95±0.06*</td>
<td>0.53±0.04</td>
<td>4.13±0.12*</td>
<td>0.51±0.05*</td>
<td>3.93±0.27*</td>
<td>8.19±0.80</td>
</tr>
<tr>
<td>cp/cp Freely fed</td>
<td>4.67±0.38</td>
<td>0.46±0.06</td>
<td>2.93±0.17</td>
<td>2.03±0.23</td>
<td>3.93±0.86</td>
<td>7.20±0.74</td>
</tr>
<tr>
<td>cp/cp Pair fed</td>
<td>4.28±0.15</td>
<td>0.41±0.03</td>
<td>2.69±0.14</td>
<td>1.77±0.14</td>
<td>11.46±0.64</td>
<td>6.70±0.51</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE, 10 rats/group, 12 wk of age. NE, norepinephrine. *P < 0.001 vs. values for cp/cp control rats.

Fig. 4. Rate of fatty acid (FA) oxidation (A) and tissue triglyceride content of soleus muscle and liver (B) of male JCR:LA-cp rats. *P < 0.05; **P < 0.005 vs. values for cp/cp freely fed rats.

Fig. 5. Plasma insulin (A) and glucose (B) concentrations of 24-wk-old JCR:LA-cp rats in experiment 2 during a meal tolerance test. Values are means ± SE, 10 rats/group. *P < 0.05; ***P < 0.001 vs. cp/cp freely fed rats.
associated with the metabolic syndrome. Figure 8 shows that pair feeding causes a significant reduction in the elevated urinary albumin loss of the cplcp rat.

Ischemic myocardial lesions. There were relatively few stage 1 myocardial lesions (areas of early ischemia or necrosis) in the hearts (Fig. 9). The frequency of stage 1 lesions was lower, but not significantly, in the hearts of pair-fed cplcp rats. Similarly, there were relatively few stage 2 lesions (foci of chronic inflamed inflammatory cells without cell lysis) seen, and there were no significant differences between groups. Stage 3 lesions (areas with strong inflammatory cell infiltration and cell lysis) were infrequent in hearts of the +/− rats but present at a significant frequency in the hearts of freely fed cplcp rats. Hearts of pair-fed cplcp rats had significantly fewer stage 3 lesions (>50%, P < 0.05) than the freely fed cplcp animals. Stage 4 lesions were infrequent in these 24-wk-old hearts, reflecting the cumulative nature of these old scarred lesions and the relatively young age of the rats, with no significant differences between groups.

DISCUSSION

Although a hormonally driven hyperphagia (55) with resultant obesity and insulin resistance is a prominent and critical element of the metabolic syndrome, abnormal stress responses are also clearly important (21, 22, 35). The response to stress may be related to the vascular dysfunction, which leads to end-stage cardiovascular disease (27, 32) but may also involve other metabolic elements (19, 35). This is consistent with our earlier work showing exaggerated metabolic response by the

![Fig. 6. Plasma triglyceride concentrations of 24-wk-old JCR:LA-cp rats. Values are means ± SE, 10 rats/group. **P < 0.01 vs. cplcp freely fed rats.](image)

![Fig. 7. Effect of pair feeding on phenylephrine (PE)-mediated contractile and acetylcholine (Ach)-mediated relaxant vascular function in cplcp rats. Calculated values of the logistic equation parameters are shown in Table 2 together with results of the statistical analysis of intergroup differences. Values are means ± SE, 10 rats/group.](image)

![Fig. 8. Effect of pair feeding on microalbuminuria in 24-wk-old cplcp rats. Values are means ± SE, 10 rats/group. *P < 0.05; ***P < 0.001 vs. cplcp freely fed rats.](image)

Table 2. Vascular function of JCR:LA-cp rats: effects of pair feeding on cplcp rats

<table>
<thead>
<tr>
<th></th>
<th>Freely Fed</th>
<th>cplcp</th>
<th>Pair-Fed cplcp</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE contractility</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum, g</td>
<td>1.82±0.07***</td>
<td>2.77±0.10</td>
<td>2.21±0.09***</td>
</tr>
<tr>
<td>EC50 (M × 10−8)</td>
<td>2.62±0.57**</td>
<td>6.02±1.35</td>
<td>3.36±0.78</td>
</tr>
<tr>
<td>PE contractility (L-NAME)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum, g</td>
<td>2.40±0.08</td>
<td>2.85±0.12</td>
<td>2.29±0.21</td>
</tr>
<tr>
<td>EC50 (M × 10−8)</td>
<td>2.71±0.06*</td>
<td>3.70±1.02</td>
<td>7.12±0.45</td>
</tr>
<tr>
<td>ACh-mediated relaxation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum, %</td>
<td>79.4±2.6</td>
<td>62.9±1.7</td>
<td>67.6±2.0</td>
</tr>
<tr>
<td>EC50 (M × 10−8)</td>
<td>2.61±0.52</td>
<td>3.58±0.62</td>
<td>5.43±0.81</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE, 10 rats/group, 24 wk of age. PE, phenylephrine; l-NAME, Nω-nitro-l-arginine methyl ester. *P < 0.05; ***P < 0.01; ***P < 0.001 vs. values for cplcp control rats.

cplcp rats to restraint and handling stress (21, 26). Overall, our results would indicate an integrated response to the ObR defect-mediated hyperphagia, obesity, and insulin resistance at central nervous system, metabolic, and cardiovascular levels.

Insulin Sensitivity

Pair feeding of rats, with the resultant enforced intermittent food intake, would appear to constitute a relatively minor environmental change. In contrast to overt food restriction employed in other studies (5, 15, 28, 50), there was no reduction in total caloric intake and therefore only a modest, albeit important, effect on body weight of adolescent animals, resulting in insulin resistance, indicated by reduced concentrations of insulin and glucose in the fed state. Unlike the freely fed rats, pair-fed cplcp rats responded to short restraint stress with a significant decrease during the recovery period, resembling the +/− freely fed rats. The fall in insulin concentration with a constant plasma glucose concentration implies an increased response to insulin during the stress and/or recovery periods and represents a significant improvement in the metabolic status of the animals. These effects are similar to those reported by others in Sprague-Dawley and Zucker fatty rats (11, 23) but are more extreme due to the metabolic dysfunction of the cplcp rat and were enhanced by
Interestingly, there is a contrast between the rise in FA seen in rats familiarized with this less aversive procedure in advance. Also handled in association with the tail-bleeding procedure but of stress to the animal (26). Animals in the present study were nonhandled rats, with handling constituting a significant form of stress exposure (11) and the absence of response by the +++? rats under the brief cone restraint of this study. As well, cplcp rats showed significant rises in FA during and following restraint, whereas no such increases were seen in fafa (obese) rats by Chaouloff et al. (11). The contrasting results are consistent with the different nature of the fa mutation (an amino acid substitution that leads to reduced affinity of the ObR receptor for leptin) and the cp mutation (a stop codon that leads to the absence of all isoforms of the ObR) (12). In this study, pair-fed cplcp rats had prestress FA levels that were almost double those of the freely fed rats, with an exaggerated response to the restraint stress that was comparable with that induced by the fasted state. These levels were comparable with those seen in our earlier study with rats that were handled (26) and indicate a major exacerbation of the stress response in pair-fed rats.

Under stimulation by norepinephrine, the rate of lipolysis, per gram of retroperitoneal fat, was higher in the +++? rats, as expected from the smaller size and greater number of adipocytes per gram tissue in these animals. However, when expressed on a per fat pad basis, +++? rats had lower rates of lipolysis, reflecting a smaller mass of adipose tissue (26). Thus, higher steady-state circulating FA concentrations in the fasted state seen in the cplcp rats compared with the +++? rats were due to the greater adiposity of the former and, therefore, potentially greater release of FA. The origin of the increased plasma FA response to the immobilization stress in the pair-fed rats (Fig. 3) does not appear to lie in enhanced catecholamine sensitivity of peripheral fat tissues, as Table 1 shows no effect of pair feeding on the rate of lipolysis. We speculate that the underlying mechanism is driven by either the central nervous system and/or a systemic hormonal response to the pair feeding through the sympathoadrenal axis (11, 13).

The rates of FA oxidation found in both the muscle and liver of JCR:LA-cp rats were qualitatively similar to those reported by Iso et al. (19) in the ZDF rat. Results in Fig. 4 are uniformly about 50% of those found in the ZDF rat (19), probably as a consequence of the differences between the fa and cp mutations and the different background genotypes of the two strains (12, 56). The greater FA increase in the plasma of the pair-fed rats may be related to the lower rate of oxidation in muscle tissue secondary to chronic stress (Fig. 4). The TG content of skeletal (soleus) muscle of the cplcp rat is fourfold higher than that of the +++? rat at 12 wk of age, and we have shown previously that this is directly related to the peripheral insulin resistance (42). The liver of the cplcp rat has a 10-fold higher TG content than that of the +++? rat, probably because of the greatly enhanced lipid synthesis and metabolism (46). In comparison, pair-fed cplcp rats had a threefold reduction in hepatic TG content that parallels the elevated plasma FA levels in the fed state, both basal and in response to immobilization stress (Fig. 3). The reduction in TG content does not involve enhanced FA oxidation in either liver or skeletal muscle, as Fig. 4 shows reduced FA oxidation in the pair-fed rats. Reduced hepatic FA oxidation is consistent with oversupply of glucose (46), which was not altered by pair feeding (food intake being unaltered). However, the reduced TG content of liver (but not soleus muscle) of pair-fed cplcp rats suggests diversion of FA to some other, at this point unidentified, tissue and presumably oxidation. Similarly, the reduced plasma TG concentration seen in pair-fed cplcp rats (Fig. 6) is consistent with diversion of FA to oxidation and away from TG synthesis and secretion as VLDL (37, 40, 51). These changes seen with pair feeding are consistent with the increase in insulin sensitivity and reduction in plasma insulin levels, with consequent increase in peripheral glucose uptake and oxidation and reduction in the plasma glucose seen in both fafa Zucker rats (10).

Mature, 24-wk-old, cplcp rats that were pair fed (experiment 2) showed no significant increase in fasting insulin levels compared with freely fed controls but exhibited a significant increase in insulin immediately postmeal challenge with no change in glucose concentration (30 min; Fig. 5). Thus, there is no evidence of an increase in insulin sensitivity and a possible small increase in insulin resistance, in contrast to the improvement in insulin sensitivity shown by young adult rats in experiment 1. These differences in response to pair feeding reflect metabolic changes as the obese cplcp rats age and mature.

FA Metabolism

Plasma FA concentrations are sensitive to both stress and insulin/glucose metabolism, illustrated by the significantly greater FA levels in fasted rats and the greater increase in response to stress of the pair-fed cplcp rats (Fig. 3). The FA response to stress in the present results was similar to, but lower than, that seen in earlier studies (26). Lower FA concentrations in this study may reflect differences in experimental protocol from our previous study (26) in which the animals were implanted with venous cannulae for blood sampling. The increase in FA seen during the recovery phase in the present study resembled that reported for handled rats but not for nonhandled rats, with handling constituting a significant form of stress to the animal (26). Animals in the present study were also handled in association with the tail-bleeding procedure but were familiarized with this less aversive procedure in advance. Interestingly, there is a contrast between the rise in FA seen in fafa (lean) Zucker rats studied under a more extreme restraint (11) and the absence of response by the +++? rats under the brief cone restraint of this study. As well, cplcp rats showed significant rises in FA during and following restraint, whereas no such increases were seen in fafa (obese) rats by Chaouloff et al. (11). The contrasting results are consistent with the different nature of the fa mutation (an
bias to FA metabolism characteristic of the prediabetic insulin-resistant state.

**Vascular Function and Myocardial/Renal Disease**

Macrovascular dysfunction, evident in increased contractile response of aortic rings to PE and impaired endothelium-dependent relaxation, as seen in Fig. 7, is a major complication of the hyperinsulinemic prediabetic state (17). It is associated, in the cpl/cpl rat, with ischemic lesions in the heart (36) and is reduced by interventions that are cardioprotective in this model (38, 39, 42). The reduction in PE-mediated vascular contraction in the pair-fed 24-wk-old cpl/cpl rats was associated with a reduction in plasma TG concentrations but not with lower cholesterol, indicating that noncholesterol lipid elements were involved in the vasculopathy. The reduced vascular dysfunction is reflected in the lower incidence of stage 3 ischemic lesions in the heart (Fig. 9).

Microvascular dysfunction is the other major facet of the end-stage complications of the prediabetic state and type 2 diabetes. Microvascular damage is evident in microalbuminuria, as shown in Fig. 8, with progression to glomerular sclerosis and eventually renal failure (24, 30). Pair-fed cpl/cpl rats had significantly lower urinary albumin loss, consistent with improved microvascular function and with the improved macrovascular function. In these relatively young adult rats, there was no improvement in the severity of glomerular sclerosis (results not shown), although other interventions that cause significant reduction in albuminuria have been shown to be associated with reduced glomerular damage (30, 49).

Under the experimental conditions, rats eat the daily allocation of food rapidly (over ~12 h) and are food deprived for the ≥10-h balance of each diurnal cycle, inducing an intermittent metabolic deprivation. Although the length of time the rats are metabolically deprived is short in human terms, the passage of time and physiological processes are effectively faster in a short-lived species such as the rat (20). With a heart rate about six times that of humans, time and physiological processes effectively run equivalently rapidly. Thus, metabolic deprivation of a rat that normally eats numerous small meals throughout the diurnal cycle for 14 h each day is equivalent to recurring 3-day fasting by a human. The metabolic changes seen in the rats may be related to the stress experienced by animals that perceive that they have an intermittent or unreliable food supply. However, we have recent data (49) that show similar effects in cpl/cpl rats treated with 2-DG, a synthetic glucose analog that inhibits glucose oxidation and thus energy availability. Importantly, the effects of 2-DG are evident primarily with intermittent (alternating days), and not with continuous, treatment. We suggest that intermittent food deprivation, or metabolic inhibition, has similar effects. In support of this concept, Wan et al. (54) also used an intermittent-treatment 2-DG schedule as an analog to intermittent fasting, which they have shown to have beneficial effects on the cardiovascular system (25).

In previous studies, we have shown that reduction of insulin resistance and the resultant hyperinsulinemia, through a variety of interventions, leads to improved vascular function and sharply reduced myocardial lesion severity and glomerular sclerosis (8, 27, 31, 33, 35–38, 40–43). Intermittent caloric restriction, induced by the pair-feeding protocol, appears to have effects that are very similar to other insulin-sensitizing treatments. The mechanisms underlying the beneficial pathophysiological effects are probably related to reduction of insulin concentrations and thus of the cytokine activity of insulin at high levels (1, 2) and reduction of circulating triglyceride as VLDL and chylomicrons, both of which are atherogenic (31, 52).

**Summary**

The cpl/cpl rat exhibits exaggerated metabolic responses to changes in physical environment, including intermittent caloric restriction. The responses include lower insulin levels following restraint stress, consistent with an increase in insulin sensitivity, and a greater increase in circulating FA. Metabolism of the pair-fed cpl/cpl rat appears to be altered to favor FA release, and presumably metabolism, rather than glucose oxidation. Lower insulin levels are potentially beneficial in terms of the cardiovascular disease that accompanies obesity and insulin resistance in these animals and in humans (2, 17). On the other hand, the markedly higher stress-induced FA concentrations in young pair-fed rats fall within a range that could have adverse effects (9), leading to vascular damage and increased cardiovascular disease (2, 27). However, the older pair-fed cpl/cpl rats had significantly reduced plasma triglyceride levels, improved micro- and macrovascular function, and reduced severity of ischemic myocardial lesions. These constitute significant amelioration of the end-stage disease processes that accompany the metabolic syndrome and are analogous to the previously reported effects of food restriction on degenerative diseases (25, 54). It is possible that similar interactions exist in susceptible human beings.

**ACKNOWLEDGMENTS**

The technical assistance of K. MacNaughton, S. Sokolik, and X. Li is gratefully acknowledged.

**GRANTS**

This work was supported financially by Sanofi-Aventis, Rueil-Malmaison, France; Institut de Recherches Internationales Servier, Courbevoie, France; the NSERC Discovery Program; and the Heart and Stroke Foundation of Alberta and the Northwest Territories. D. N. Brindley is a Medical Scientist of the Alberta Heritage Foundation for Medical Research.

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