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# Differential expression of hypothalamic CART mRNA in response to body weight change following different dietary interventions

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ABSTRACT

Cocaine- and amphetamine-regulated transcript (CART) peptide is widely expressed in the hypothalamus and is involved in the central regulation of energy balance. Using *in situ* hybridization, this study examined the roles of CART peptide in the hypothalamus of diet-induced obese (DIO) or diet-resistant (DR) mice under different dietary interventions including high-fat (HF), low-fat (LF) and pair-feeding (PF) diet for 6 weeks. Pair feeding the energy intake of the DIO and DR mice was used to determine whether there is an inherent difference in baseline CART expression that may cause the DIO and DR phenotypes. The results demonstrated that CART mRNA expression in the hypothalamus of the DIO mice responded differently on the high-fat diet compared to DR mice. The arcuate nucleus and paraventricular nucleus showed a significant reduction in CART mRNA expression in DIO mice compared to DR mice on the HF diet (−19.6%, $p = 0.019$; −26.1%, $p = 0.003$); whilst a profound increase in CART mRNA expression was observed in the dorsomedial nucleus and lateral hypothalamic area (+44.5%, $p = 0.007$; +37.4%, $p = 0.033$). Our study suggests that the decrease of CART mRNA expression in Arc and PVN regions of DIO mice may contribute to the development of high-fat diet-induced obesity. In addition, CART in the dorsomedial nucleus (DM) of hypothalamus and lateral hypothalamus (LH) may be involved in the activation of an orexigenic effect. Since pair feeding of the high-fat diet eliminated both the body weight and CART mRNA differences between the DIO and DR mice, it is likely that their alterations in gene expression were a consequence of their dissimilar body weight levels.



## 1. Introduction

The hypothalamus plays a key role in the regulation of energy homeostasis and consequently, body weight (Badman and Flier, 2005; Arora and Anubhuti, 2006). Hypothalamic cocaine- and amphetamine-regulated transcript (CART) peptide is believed to be an important neurotransmitter that mediates food intake and body weight in both rodents and humans (Kristensen et al., 1998; Menyhert et al., 2007).

CART mRNA and peptides are widely expressed in the hypothalamus, with dense expression in the arcuate nucleus (Arc), paraventricular nucleus (PVN), dorsomedial nucleus (DM) of hypothalamus and the lateral hypothalamus (LH) (Koylu et al., 1998; Elias et al., 2001; Kramer et al., 2007). All these areas are involved in energy balance regulation and weight control (Kalra et al., 1999). In these areas, CART co-expresses with both anorexigenic and orexigenic neuropeptides in a regionally specific manner. First, CART co-localizes with the neurons containing proopiomelanocortin (POMC), a precursor for alpha-melanocyte-stimulating hormone (α-MSH), and leptin receptor in the Arc (Elias et al., 1998). Secondly, CART is co-expressed with melanin-concentrating hormone (MCH) in the DM and LH (Broberger, 1999; Vrang, 2006). Functionally, leptin can significantly elevate CART mRNA expression (Kristensen et al., 1998). Conversely, disrupted leptin signalling in obese Zucker rats (fa/fa rats) and obese (ob/ob) mice results in a decreased CART mRNA expression in the hypothalamus (Kristensen et al., 1998).

It is traditionally believed that CART promotes negative energy balance. However, pharmacological studies have shown different results regarding CART and its involvement in energy balance. Injection of $CART_{55–102}$ into the cerebral ventricles reduces food intake in normal rats, either fasted or non-fasted, and obese Zucker fa/fa rats (Kristensen et al., 1998; Lambert et al., 1998; Vrang et al., 1999; Larsen et al., 2000; Aja et al., 2001). While intrahypothalamic injection of CART has been found to increase food intake (Abbott et al., 2001). In addition, several lines of evidence have shown that in various genetic backgrounds and nutritional conditions, CART is expressed differently in specific brain regions that mediate energy balance (Kristensen et al., 1998; Abbott et al., 2001; Sergeyev et al., 2001; Robson et al., 2002). It has been shown that CART mRNA is

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virtually absent in the Arc but still relatively abundant in the DM in fa/fa Zucker rats and ob/ob mice (Kristensen et al., 1998). Also, intraperitoneal injection of leptin into ob/ob mice causes a 3.5-fold increase of CART mRNA in the Arc but only 0.5-fold increase in the DM (Kristensen et al., 1998). A study by Robson has revealed that 48 h fasting causes a 40% reduction of CART in the Arc with no change in the DM in Siberian hamsters (Robson et al., 2002). Furthermore, CART mRNA expression is significantly decreased in the Arc, but not the DM of rats that have been fasted for 24 h (Kristensen et al., 1998).

The alarming growth rate of obesity, a process of an interaction between genetic predisposition and environmental factors, makes more research into the area of obesity of great importance. We have previously shown a differential alteration of hypothalamic orexigenic and anorexigenic neurotransmitter systems in diet-induced obese (DIO) and diet-resistant (DR) mice including neuropeptide Y, POMC, and agouti-related protein (AgRP) (Huang et al., 2003a,b). Although some studies have shown that CART administration can affect food intake, there are large gaps in our knowledge about changes in CART expression in animal models of chronic high-fat diet-induced obesity. This study investigated the levels of hypothalamic CART mRNA in the hypothalamic nuclei of altered energy homeostasis models in high-fat diet-induced obese and diet-resistant mice. A pair-feeding method was also used in this study so that the DIO and DR mice were fed an identical amount of food. This technique was used to determine whether differences in CART expression remain once the body weights of the DIO and DR mice are normalised and therefore whether these CART differences are a cause or consequence of obesity development. In addition, body weight, energy intake, fat deposition and plasma leptin were also measured in this study under various nutritional conditions.

## 2. Materials and methods

### 2.1. Animals, diets and experimental procedures

Forty-five, 12-week-old, C57Bl/6J male mice were obtained from the Animal Resources Center (Perth, WA, Australia). The mice were housed in environmentally controlled conditions (temperature 22 °C, light cycle from 0600 to 1800 h and dark cycle from 1800 to 0600 h). They were fed standard laboratory chow for the first week to allow them to adapt to their new environment. They were then placed on a high-fat diet (3.8 kcal/g) containing 40% fat, 44% carbohydrate and 16% protein by calories (Table 1). Foods were made from semisynthetic materials according to the recommendation of AIN93 Diet for Laboratory Rodents as we have used previously (Reeves et al., 1993). After 8 weeks of high-fat diet, the 15 mice with the highest body weight gain were designated as the DIO mice and the 15 mice with the lowest body weight gain were designated as the DR mice, according to our and others' previous method (Levin and Keesey, 1998; Huang et al., 2005). The DIO or DR mice were divided into three groups each and then either continued on a high-fat diet (DIO-H, DR-H), switched to a low-fat diet (3.2 kcal/g, containing 10% fat, 74% carbohydrate and 16% protein; DIO-L, DR-L), or a pair-feeding diet for a further 6 weeks (DIO-P, DR-P) (Fig. 1). In the pair-feeding group, both the DIO and DR mice were supplied the same amount of high-fat diet which was equal to 85% average energy intake of the DR group in week 8 prior to dietary intervention. 85% of average DR group's energy intake was determined as it corresponds to the lower standard deviation of the DR group's energy intake. This ensured that all mice would consume all of their given food throughout the intervention study. All experimental procedures were approved by the Animal Ethics Committee, University of Wollongong, Australia, and complied with the Australian Code of practice for the care and use of animal for scientific purposes.

**Table 1**
Composition of the high-fat and low-fat diet

| | High-fat diet | Low-fat diet |
|---|---|---|
| Total energy (kcal/100 g) | | |
| Fat | 40 | 10 |
| Carbohydrate | 44 | 74 |
| Protein | 16 | 16 |
| Component (g/kg) | | |
| Beef tallow | 80 | 0 |
| Coconut oil | 80 | 0 |
| Sunflower oil | 41 | 41 |
| Cornstarch | 432 | 624 |
| Sucrose | 60 | 60 |
| Fatty acid composition, % fat | | |
| Saturated | 60 | 13 |
| Monounsaturated | 25 | 21 |
| $n-6$ PUFA[a] | 15 | 66 |
| Total energy (% kcal) | | |
| Saturated | 24 | 1 |
| Monounsaturated | 10 | 2 |
| $n-6$ PUFA | 6 | 7 |

Foods were made from semisynthetic materials according to the recommendation of "AIN93 Diet for Laboratory Rodents" (Reeves et al., 1993).
[a] Polyunsaturated fat.

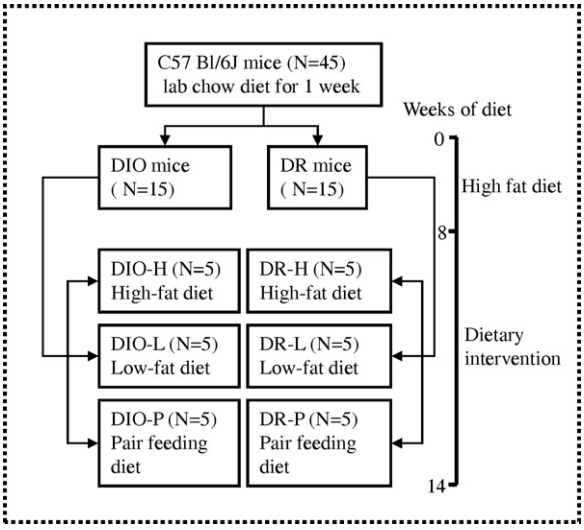


**Fig. 1.** A flowchart indicates the experiment design and assignment of mice. The diet-induced obese (DIO) and diet-resistant (DR) mice were induced by high-fat diet for 8 weeks, then either continued on the high-fat diet in groups DIO-H and DR-H, changed to low-fat diet in groups DIO-L and DR-L, or pair-feeding diet in groups DIO-P and DR-P for 6 weeks. In the pair-feeding group, both the DIO and DR mice were supplied the same amount of high-fat diet which was equal to 85% average energy intake of the DR group in week 8 prior to dietary intervention.

### 2.2. Food intake and body weight

Twenty-four hour energy intake was measured weekly during the dietary intervention. A weighted amount of fresh diet was given at the beginning of the dark cycle and both the remaining food in the cage plus spillage were collected and weighted after 24 h later. The body weight was determined every 2 weeks during the 14 weeks feeding study.

### 2.3. Tissue preparation

After a total of 14 weeks of experiment, the mice were sacrificed with an overdose of sodium pentobarbitone anesthesia (120 mg/kg, i.p.). All mice were killed between 0700 and 0900 h in order to minimize circadian variation in mRNA expression. Brains were immediately removed after death and frozen in liquid nitrogen. Coronal brain sections (14 μm) were cut at −18 °C with a cryostat, and thaw-mounted onto polysine slides. Hypothalamic sections were collected from Bregma 0.14 to −2.92 mm according to a standard mouse brain atlas (Paxinos and Franklin, 2002). White fat tissue from four regions (epididymal, perirenal, omental and inguinal) was collected and weighed.

### 2.4. Plasma leptin

Upon killing, blood was collected via right ventricle puncture with anticoagulant heparin in the container. The blood samples were centrifuged at 4000 rpm for 10 min. The plasma was then collected and stored at 4 °C. Using the mouse endocrine Lincoplex kit (Cat.# MENDO-75K, Linco Research, St. Louis, MO, USA), plasma leptin was determined.