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Vaspin and amylin are expressed in human and rat placenta and regulated by nutritional status

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Summary. Amylin (islet amyloid polypeptide) and vaspin (visceral adipose tissue specific serpin) are gut and adipocyte hormones involved in the regulation of body weight homeostasis. The aim of this study was to examine whether amylin and vaspin are expressed in human and rat placenta, as well as their regulation by nutritional status. Our results demonstrate that amylin and vaspin are localized in both human and rat placenta. In the rat term placenta vaspin was demonstrated in the trophoblast of the fetal villi, the labyrinth. Vaspin immunostaining in human placenta was localized in cytotrophoblast and syncytiotrophoblast in the first trimester placentas while in the third trimester vaspin was localized in the syncytiotrophoblast. Regarding amylin, rat placenta of 16 days of gestational age showed an intense immunostaining, mainly localized in the labyrinth. On the other hand, in the human third trimester placenta amylin immunoreactivity was intense in the syncytiotrophoblast of the chorionic villi and in decidual cells. Furthermore, placental amylin and vaspin showed an opposite pattern of expression during pregnancy, with vaspin showing the highest expression level at the end and amylin at the beginning of pregnancy. Finally, food restriction also has contrary effects on their expression, increasing vaspin but decreasing amylin placental mRNA and protein levels. Taken together, our results demonstrate that vaspin and amylin are modulated by energy status in the placenta, which suggests that these proteins may be involved in the regulation of placental metabolic functions.

Key words: Varspin, Amylin, Placenta, Food restriction

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Introduction

The regulation of body weight involves modulation of feeding, metabolism and energy expenditure. This interplay is carried out by a complex inter organ circuit between the central nervous system (CNS) and the periphery, involving neural and hormonal signals (Hoggard et al., 1998; Pasquali et al., 2006; Chaudhri et al., 2007; López et al., 2007; Wiedmer et al., 2007).

During recent decades, placenta and its circulating factors have been widely studied (Myatt, 2006). Fetal development is critically determined by the availability and flux of nutrient and oxygen across the placenta from mother to fetus during pregnancy. The placenta also appears to influence fetal programming by controlling perturbations in maternal environment, such as malnutrition. This current view has generated a new placenta status that might affect irreversibly the fetus, making it more susceptible to develop diseases in adult life (Klaus, 2004; Jansson and Powell, 2007).

Amylin (islet amyloid polypeptide) is a pancreatic hormone, localized within the secretory granules of beta-cells and has also been found in low quantities in the gut and other tissues (Mulder et al., 1997). Amylin regulates important gastrointestinal functions (Mulder et al., 1997), providing feedback to the CNS on availability of nutrients, and then regulating food intake and energy balance (Riediger et al., 2004). Chronic administration

of amylin acts as a satiety and adiposity signal in rats, triggering hypoinsulinemia and hypoleptinemia, as well as a decrease in body weight (Isaksson et al., 2005). Interestingly, a recent report has also provided both non-clinical and clinical evidence that amylin agonist restored leptin responsiveness in diet-induced obesity (Roth et al., 2008).

In addition to the gastrointestinal tract, adipose tissue also controls energy homeostasis by producing several endocrine and paracrine mediators, including adiponectin, leptin, resistin and tumor necrosis factor-alpha (TNFα), which are involved in short and long-term regulation of food intake (Klaus, 2004a). Vaspin (visceral adipose tissue specific serpin), an adipocytokine with potential antiprotease properties, is a member of the largest and most widely distributed family of serine-protease inhibitor family (Hida et al., 2005) and was recently isolated from visceral white adipose tissue (WAT) of Otsuka Long Evans Tokushima Fatty (OLETF) rats, an animal model of type 2 diabetes, which is characterized by abdominal obesity, dyslipidemia, insulin resistance, and hypertension (Hida et al., 2005).

Pregnancy is a hypermetabolic state associated with insulin resistance, and gestational diabetes mellitus has long been recognized as a risk factor for a number of adverse outcomes during pregnancy (Ecker and Greene, 2008). For instance, in patients with gestational diabetes mellitus, the levels of amylin, as well as the secretion of insulin and C-peptide remain elevated when compared to women with normal glucose tolerance (Kinalski et al., 2004). Pregnancy is also characterized by a series of metabolic changes that promote adipose tissue accretion in early gestation to meet increased metabolic demands; thereby the expression and secretion of many adipocytokines are profoundly altered during gestation (Mitchell et al., 2005). To date it is well-established that placenta tissue is an active endocrine organ (Hoggard et al., 1997; Señarís et al., 1997; Bado et al., 1998; Xiao et al., 1998; Garcia et al., 2000; Casabiell et al., 2001; Gualillo et al., 2001; Caminos et al., 2005a,b, 2008) and an intriguing number of factors have been identified as essential during intrauterine maturity for developing placenta and extraembryonic structures in mammals. However, the underlying mechanisms remain to be established. The aim of this study was to examine the expression of amylin and vaspin in rat and human placenta, as well as their regulation by nutritional status. We demonstrate for the first time that vaspin and amylin are strongly expressed in both rat and human placenta. Furthermore, we show robust evidence that nutritional status has a significant impact on placental vaspin and amylin gene regulation.

Materials and methods

Human samples

First trimester human placentas (n=8) were obtained from the Department of Morphological Science of the University of Santiago de Compostela (USC). Third trimester human placentas (n=7; 33±1.64 weeks of pregnancy, 240±10.0 g of weight) were obtained from the service of Obstethrics and Ginecology of Complejo Hospitalario Universitario de Santiago (CHUS). All the women were provided with written information and gave written consent to enter and all of them were healthy and did not show metabolic alterations.

Animals

Sprague Dawley female rats (Animalario General, USC), were housed in standard 12 hours lighting and were given water and fed with standard rat chow *ad libitum* or food-restricted. Food consumption by control animals was measured daily and used to calculate the food amount given to the food-restricted group (Gualillo et al., 2002). All experiments and procedures involved in this study were reviewed and approved by the Ethics Committee of the USC, in accordance with EU normative for the use of experimental animals.

Chronic food restriction

Pregnant rats were randomly assigned on day 1 to one of two dietary groups as previously described (Gualillo et al., 2002): pregnant rats fed ad libitum and a restricted group of pregnant rats fed 30% of the amount of food ad libitum rats ate the previous day. Pregnant rats at different stages of gestation were sacrificed between 16:00 and 17:00 hours and rat placenta samples were collected at 12, 16, 19, and 21 days of gestation (term is 21–22 days). Tissues were collected and frozen at -80°C until processing. We detected about 5% of miscarriages; those animals were excluded from the study. We used 8-10 placentas per experimental group; each placenta was dissected from each dam.

Acute food restriction

On day 16 of pregnancy three groups of animals were deprived of food for 24, 48 or 72 hours, whereas a fourth group was fed *ad libitum*. All animals had free access to tap water. Animals were sacrificed between 16:00 and 17:00 hours and the placenta was extracted and frozen at -80°C until processing. We used 8-10 placentas per experimental group; each placenta was dissected from each dam. We detected 10% of miscarriages in the groups under 48 and 72 hours of fasting and those were excluded form the study.

RNA isolation and real time RT-PCR

Total RNA was isolated from rat tissues using TRIzol® (Invitrogen, Life Technologies, Carlsbad, CA, USA), according to the manufacturer's recommendations. First-strand cDNA was synthesized from 2 µg of total RNA by random priming RT. The

resulting cDNA was subjected to PCR amplification (Nogueiras et al., 2004; López et al., 2006, 2008; Chakravarthy et al., 2007; Vázquez et al., 2008) using sense and antisense primers specific for the rat amylin and vaspin mRNAs (Table 1). To verify the identity of amplified cDNAs, PCR products were electrophoresed on a 1.5% agarose gel; yielded DNA fragments of the expected length for all specific genes mRNAs and were confirmed by sequencing. Primers spanned an intron, providing a control for potential amplification of genomic DNA. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) for rat was used as a control housekeeping gene. Measurement of placental rat expression for amylin and vaspin, was assessed by real time RT-PCR using the LightCycler 2 (Roche Diagnostics, Germany), as described elsewhere (Heid et al., 1996; Lage et al., 2006, 2007; Caminos et al., 2007). The PCR protocol consisted of initial denaturation at 96°C for 5 min, followed by 35 cycles of denaturation (96°C, 2s), annealing (62°C, 15s) and elongation (72°C, 15s). This was followed by melting curve analysis consisting of 1 cycle at 95°C 30 s, 60°C 15 s and a temperature rise to 85°C. Amylin and vaspin mRNAs were normalized to HPRT RNA expression levels. Changes in gene expression level were calculated as previously described by using the $2^{\Delta\Delta ct}$ method (Heid et al., 1996; Livak and Schmittgen, 2001; Lage et al., 2006, 2007; Caminos et al., 2007).

Immunohistochemical analyses

We used rat and human stomach, rat placenta from 16 days of gestational age, first and third trimester human placentas (n=7-8 each) and rat brown adipose tissue (BAT) (interescapular). Protocols were performed as previously described (López et al., 2006, 2008; Chakravarthy et al., 2007; Vázquez et al., 2008). Briefly, samples were fixed by immersion in 10% buffered formalin for 24 h, dehydrated and embedded in paraffin by a standard procedure. Sections 5 µm thick were mounted on slides, dewaxed and rehydrated. Slides were incubated with rabbit vaspin primary antibody for human and rat (anti-vaspin (386-414) IgG; G-002-66; 1:1000; Phoenix Pharmaceuticals, Inc., Belmont, CA, USA), rabbit amylin primary antibody for human and rat (anti-amylin serum Phoenix Pharmaceuticals, H-017-11, Inc.; Belmont, CA, USA; 1:100) at a dilution of 1:1000, and rabbit cytokeratin 7 (CK7) (Dakopatts; Glostrup, Denmark) at a dilution of 1:50, overnight at room temperature. Diaminobenzidine staining (Dakocytomation; CA, USA) was prepared according the manufacturer instructions. Controls for the immunohistochemistry were performed by 1) applying the primary antibody to a known positive tissue (rat and human stomach), resulting in a positive control and 2) substituting the primary antibody by non immune rabbit serum in the same tissues, then resulting in a negative reaction.

Western blot analysis

Western blot analysis of human and rat amylin and vaspin was performed as previously described (López et al., 2006, 2008; Caminos et al., 2007; Chakravarthy et al., 2007; Vázquez et al., 2008). Briefly, 250 µg protein extracts from WAT, stomach mucosa and placenta tissue were resolved by SDS-PAGE and transferred to a PVDF membrane (Hybond C-Super, Amersham Biosciences, Arlington Heights, IL,USA). The membrane was then incubated with rabbit vaspin primary antibody for human and rat (anti-vaspin (386-414) IgG; G-002-66; 1:1000; Phoenix Pharmaceuticals, Inc., Belmont, CA, USA), rabbit amylin primary antibody for human and rat

Table 1. Primer sequences for vaspin, amylin and HPRT.

mRNA	Primer Sequence (5´-3´)	Product size (bp)	Accession Number
Vaspin			
Forward	AGTCGGAAAACCCACAACAG	163	AF245398
Reverse	CGGTCTTGCCTTCCTCTATG		
Amylin			
Forward	CTGCCAGCTGTTCTCCTCAT	151	J04544
Reverse	TGGAGCGAACCAAGAAGTTT		
HPRT			
Forward	CAGTCCCAGCGTCGTGATTA	137	NM_012583
Reverse	AGCAAGTCTTTCAGTCCTGTC		

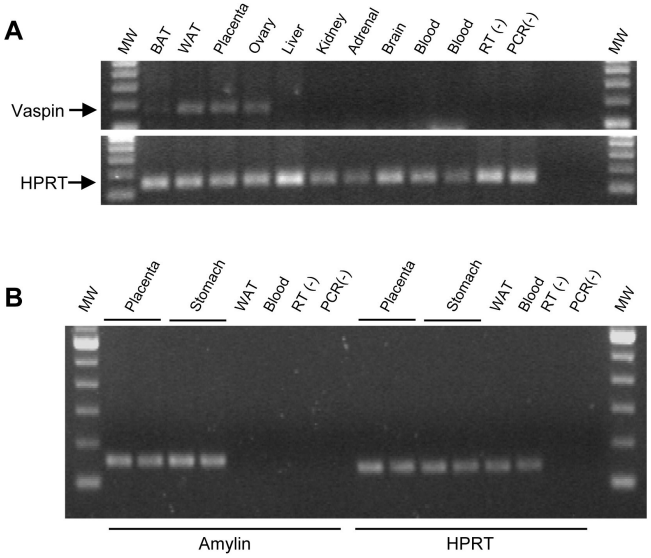
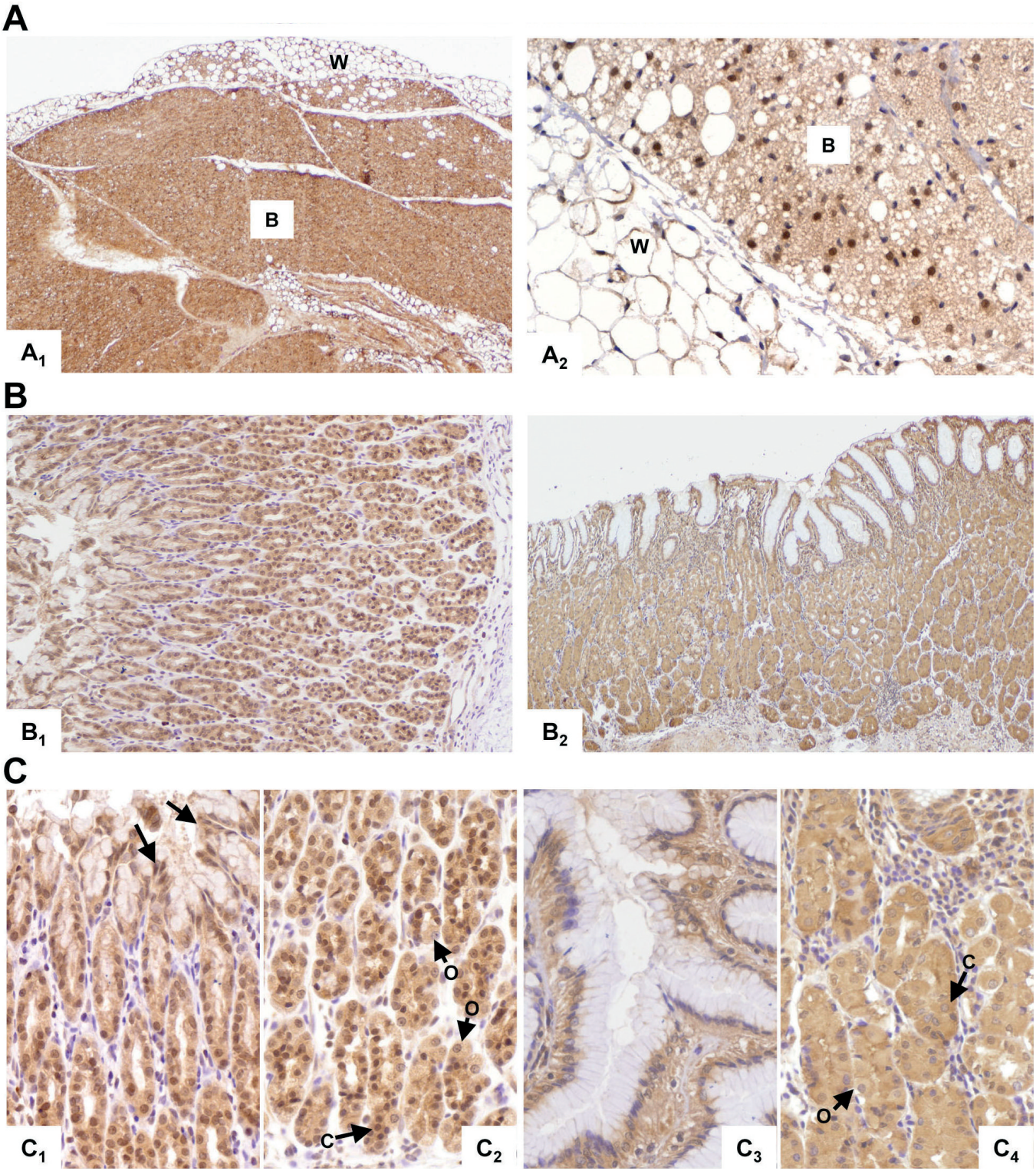


Fig. 1. Representative RT-PCR analysis of vaspin and amylin. **A.** Vaspin transcripts in different rat tissues; in addition, positive (WAT) and negative (blood, RT(-), PCR(-)) controls are shown. **B.** Amylin transcripts in rat placenta; in addition, positive (stomach) and negative (WAT, blood, RT(-), PCR(-)) controls are shown. Integrity of RNA was confirmed by amplification of HPRT. A 100-bp molecular weight marker (MW) was used.

(anti-amylin serum Phoenix Pharmaceuticals, H-017-11, Inc.; Belmont, CA, USA; 1:100). Detection was performed using a chemiluminescent system (Tropix, Bedford, MA, USA) (Caminos et al., 2007). To confirm protein loading, the same blot was stripped and incubated with monoclonal ß-actin (Sigma-Aldrich



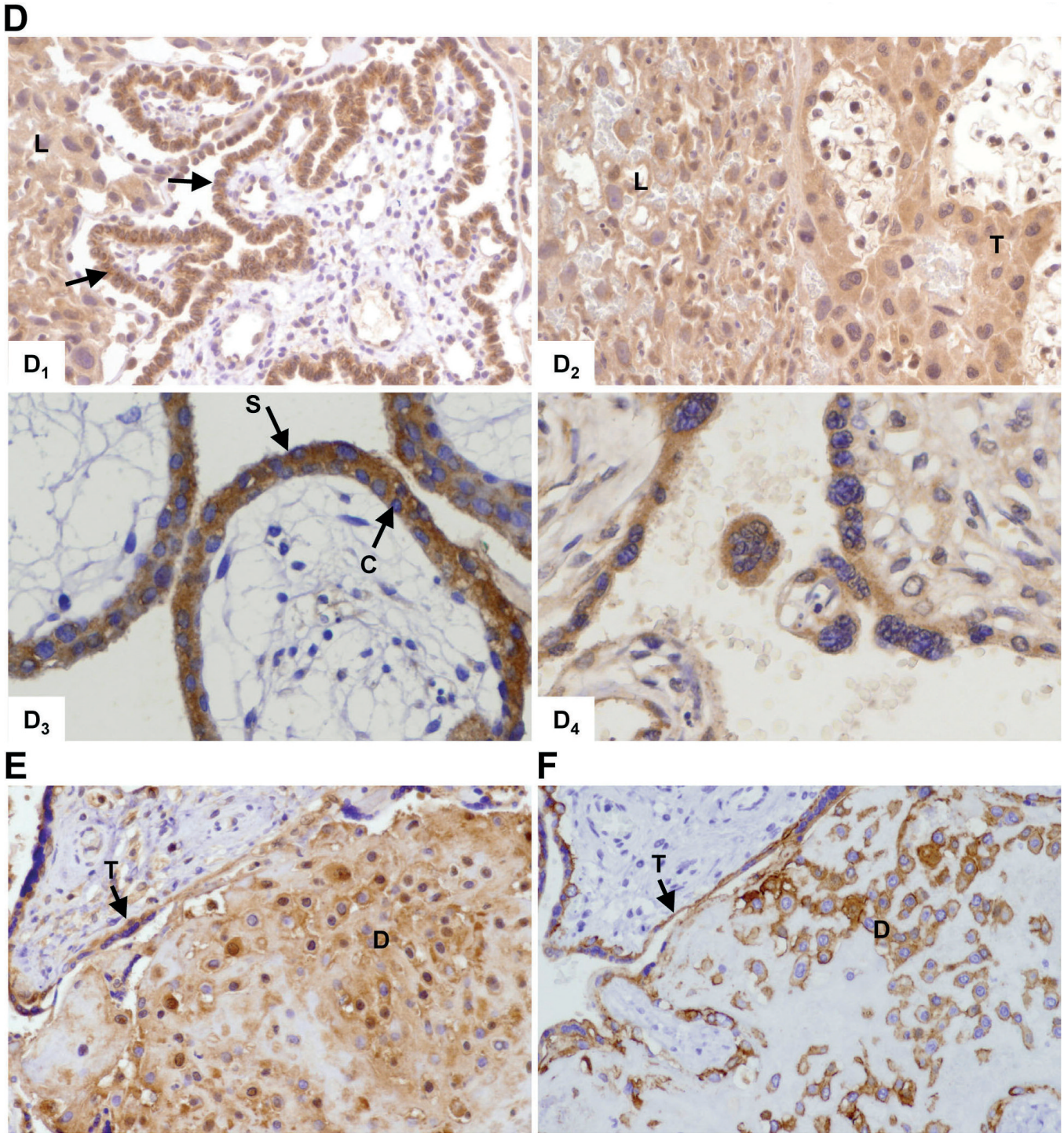


Fig. 2. Vaspin immunoreactivity in human and rat placenta. **A.** Positive controls of adipose tissue immunostained for vaspin. **A1)** Low magnification micrograph (4x) showing immunoreactivity for vaspin in the brown (B) and white (W) adipose lobes. **A2)** At higher magnification (20x) immunostaining was localized in the cytoplasm of adipocyte. **B.** B1 Rat (10x) and B2 human (4x) fundic mucosae showing intense vaspin immunoreactivity localized in the gastric glands. **C.** Cellular localization of vaspin immunoreactivity in stomach. **C1 and C2)** rat fundic mucosa (10x). In the rat gastric glands vaspin was immunoreactive in the epithelia of surface (C1, arrows) and gastric pits. Both the oxyntic (O) and chief (C) cells of the fundic glands were immunoreactive for vaspin **(C2)**. **C3 and C4)** human stomach surface epithelium (10x) expressed vaspin at the base of epithelial cells while apical cytoplasm was negative. As in the rat gastric glands vaspin immunostaining was demonstrated in the oxyntic (O) and chief (C) cells, the latter being more intensely stained. **D)** Human and rat placenta immunostained for vaspin. **D1 and D2)** Micrographs (10x) show the intense rat vaspin immunoreactivity of the trophoblast (arrows), while mesenchymal cells were not stained. Micrograph **D1** corresponds to fetal villi within the labyrinth (L) which was also positive for vaspin (10x). Micrograph D2 shows vaspin immunostaining of labyrinth (L) and trophospongium (T). Micrograph D3 (20x) belongs to human fetal placenta of the first trimester which shows vaspin immunoreaction in the cytotrophoblast (C) and syncytiotrophoblast (S). In the third trimester (20x; D4) human vaspin was positive in the syncytiotrophoblast. Semiserial sections immunostained for vaspin (E) and for cytokeratin 7 (CK7)(F); Trophoblast (T) and decidual cells (D) were positively stained with both antibodies. The analysis was performed on 8 human placentas and 10 rat placentas dissected from 10 different dams.

Corp., St. Louis, MO, USA; 1:1000).

Statistical analysis

Data are reported as mean±SEM and analyzed by using two-way ANOVA, followed by *post hoc Bonferroni* test. $P< 0.05$ was considered significant.

Results

RT-PCR Analysis

Vaspin and amylin transcripts were detected in rat placenta (Fig. 1A,B). Amplification products of the expected size for vaspin (163 bp) and amylin (151 bp) were obtained.

Immunohistochemical analyses

Intense vaspin positive signal was detected in the positive controls: WAT and BAT (Fig. 2A1, 2A2) and rat and human fundic mucosal glands (Fig. 2B1, 2B2). On the other hand, no immunostaining was observed as a result of the substitution of the anti-vaspin antibody with non immune rabbit serum (data not shown). Within the gastric glands the cellular distribution was similar in rat

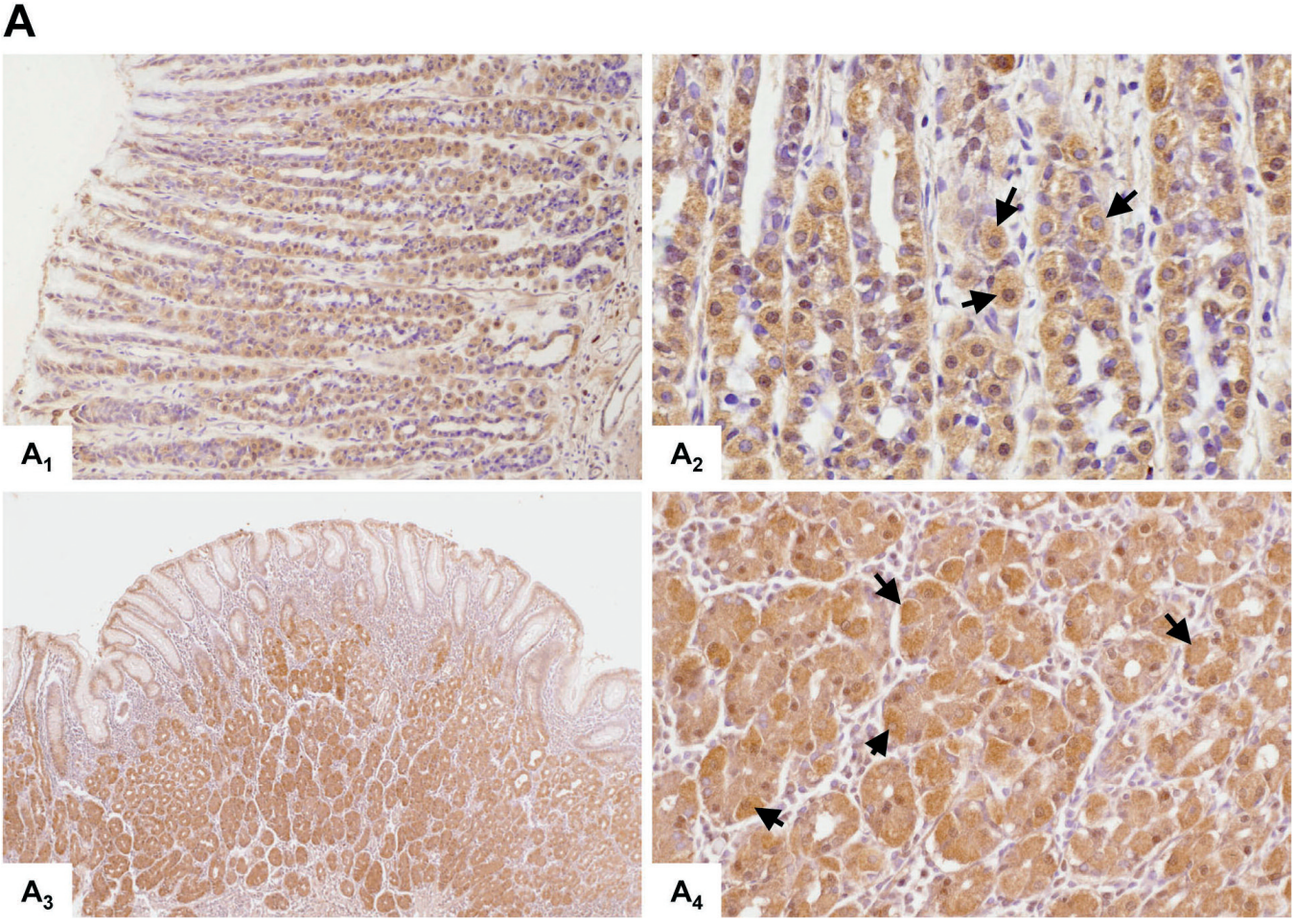
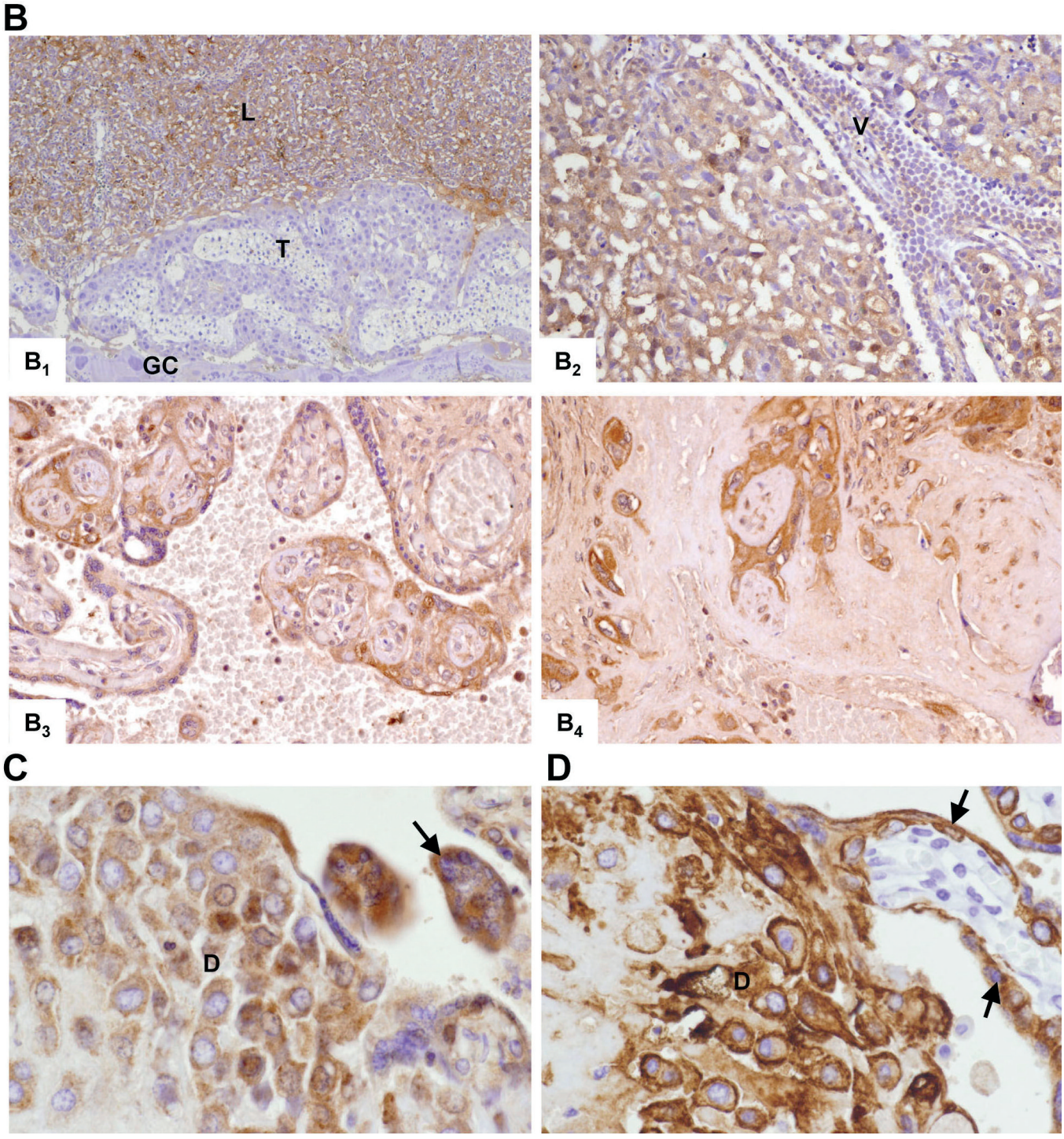


Figure 3. Amylin immunoreactivity in human and rat placenta. **A.** Rat (**A1 and A2**) and human (**A3 and A4**) fundic stomach showing intense amylin immunoreactivity in the mucosal gastric glands (4x; **A1 and A4**). At higher magnification (20x; **A2 and A4**) amylin expression was mainly localized in the parietal cells (arrows). The other cell types of the rat gastric glands, including chief cells, seem not to be stained (**A2**), while in the human fundic glands chief cells were weakly immunostained (**A4**). Rat (**B1 and B2**) placenta of 16 days of gestational age and human (**B3 and B4**) term placentas immunoreactive for amylin. In the rat placenta, amylin was localized in the labyrinth (L), the trophospongium (T) and giant cells (GC) not being stained (4x; **B1**). At high magnification (20x; **B2**) the micrograph shows a fetal villous ramification (V) covered by trophoblast that was negative; at left is the labyrinth immunoreactive for amylin. In the human placenta amylin was demonstrated in the syncytiotrophoblast of the chorionic villi (20x; **B3**) and decidual cells (20x; **B4**). Semiserial sections of human term placenta immunostained for amylin and (C) and cytokeratin 7 (CK7) **(F)**. Note that trophoblast (T) and decidua **(D)** were positive with both antibodies. The analysis was performed on 7-8 human placentas and 10 rat placentas dissected from 10 different dams.

(Fig. 2C1, 2C2) and human (Fig. 2C3, 2C4), e.g. mucous cells of the surface and gastric pits, parietal and chief cells were immunoreactive. Vaspin immunoreactivity was localized in rat and human placenta. In the rat term placenta vaspin was demonstrated in the trophoblast of the fetal villi, the labyrinth (Fig. 2D1, 2D2). Vaspin immunostaining in human placenta was localized in cytotrophoblast and syncytiotrophoblast in the first trimester placentas (Fig. 2D3) while in the third trimester vaspin was localized in the syncytiotrophoblast



(Fig. 2D4). Negative controls, obtained by substitution of primary antiserum with normal rabbit serum, showed no immunostaining in rat placenta (data not shown). Cytokeratin 7 (CK7) was used as positive marker of vaspin in trophoblast and decidual cells (Fig. 2E,2F).

Regarding amylin, positive immunostaining of rat (Fig. 3A1, 3A2) and human (Fig. 3A3, 3A4) stomach, used as positive controls, showed an intense expression of immunoreactive amylin localized in the gastric glands of the fundic mucosa, the parietal cells being intensely stained in both species. Respect the other cell types; they were negative in the rat mucosa, while in the human gastric mucosa chief cells were also immunostained (although less intense, Fig. 3A4). The omission of amylin antibody or its substitution for non-immune rabbit serum in the gastric mucosa resulted in a lack of immunostaining (data not shown). Rat placenta of 16 days of gestational age showed an intense immunostaining mainly localized in the labyrinth (Fig. 3B1, 3B2). The villi, trophospongium and giant cells were negative for amylin. On the other hand, in the human third trimester placenta, amylin immunoreactivity was intense in the syncytiotrophoblast of the chorionic villi (Fig. 3B3) and in decidual cells (Fig. 3B4). Cytokeratin 7 (CK7) was used as a positive marker of amylin in trophoblast and decidual cells (Fig. 3C,D).

Western blotting analysis

Placenta vaspin and amylin proteins were detected in rat and human tissues. The protein pattern was similar to that in WAT and stomach mucosa for vaspin and stomach for amylin, used as positive controls for vaspin and amylin respectively. A band at approximately 49 KDa molecular weight for vaspin and 14 KDa for amylin were identified in the protein extracts of rat (Fig. 4A,C) and human (Fig. 4B,D) placenta tissue at term. Very interestingly, we detected the presence of more than one band after assaying rat placenta with anti-vaspin antibodies; whether this is related to placental-specific posttranscriptional modifications will require further investigation.

Gene expression pattern of vaspin and amylin mRNA in rat placenta throughout pregnancy and the effect of chronic food restriction

Details on food intake and body weight data have been previously reported (Gualillo et al., 2001; Caminos et al., 2005b). A considerable increase in placenta vaspin mRNA expression was detected during the last part of pregnancy in both *ad libitum* and food restricted rats, but on day 12 of gestation vaspin levels are undetectable. Food restriction induced a further increase in vaspin mRNA and protein expression, but only on day 21 (Fig. 5A,B). On the other hand, rat placenta amylin mRNA expression levels were elevated during early pregnancy and then drastically decreased at the end of pregnancy, in both fed and food restricted rats. Food restriction decreased amylin mRNA and protein expression, but only on day 12 (Fig. 5C,D).

Effect of acute food restriction on placenta vaspin and amylin mRNA expression

Vaspin mRNA and protein levels showed a non-significant statistical tendency to be up-regulated by fasting in rat placenta (Fig. 6A,B). On the other hand, placenta amylin mRNA and protein levels were significantly reduced in pregnant fasted rats at the

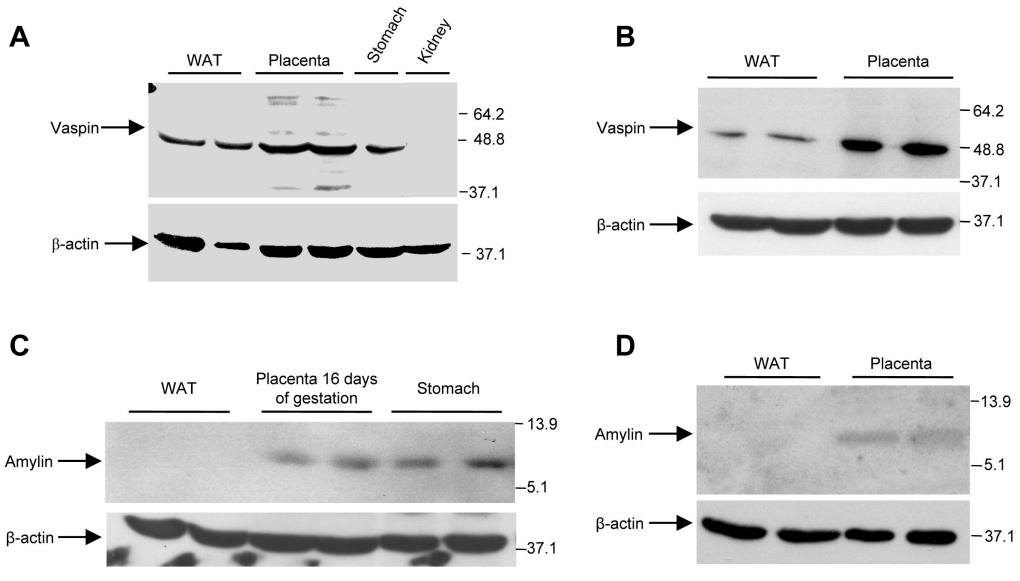


Fig. 4. Vaspin and amylin western blotting analysis. **A.** Western blot analysis of rat vaspin expression in WAT, placenta, mucosa, and kidney tissue. **B.** Western blot analysis of human vaspin expression in WAT and placenta tissue at term (third trimester). **C.** Western blot analysis of rat amylin expression in WAT, placenta (16 days of gestation) and stomach mucosa tissue. **D.** Western blot analysis of human amylin expression in WAT and placenta tissue at term (third trimester). In all cases, to confirm equal loading, the same blot was stripped and incubated with monoclonal ,-actin antibody. Numeric labels on the right show molecular weights in KDa. The analysis was performed on 7-8 human placentas and 10 rat placentas dissected from 10 different dams.

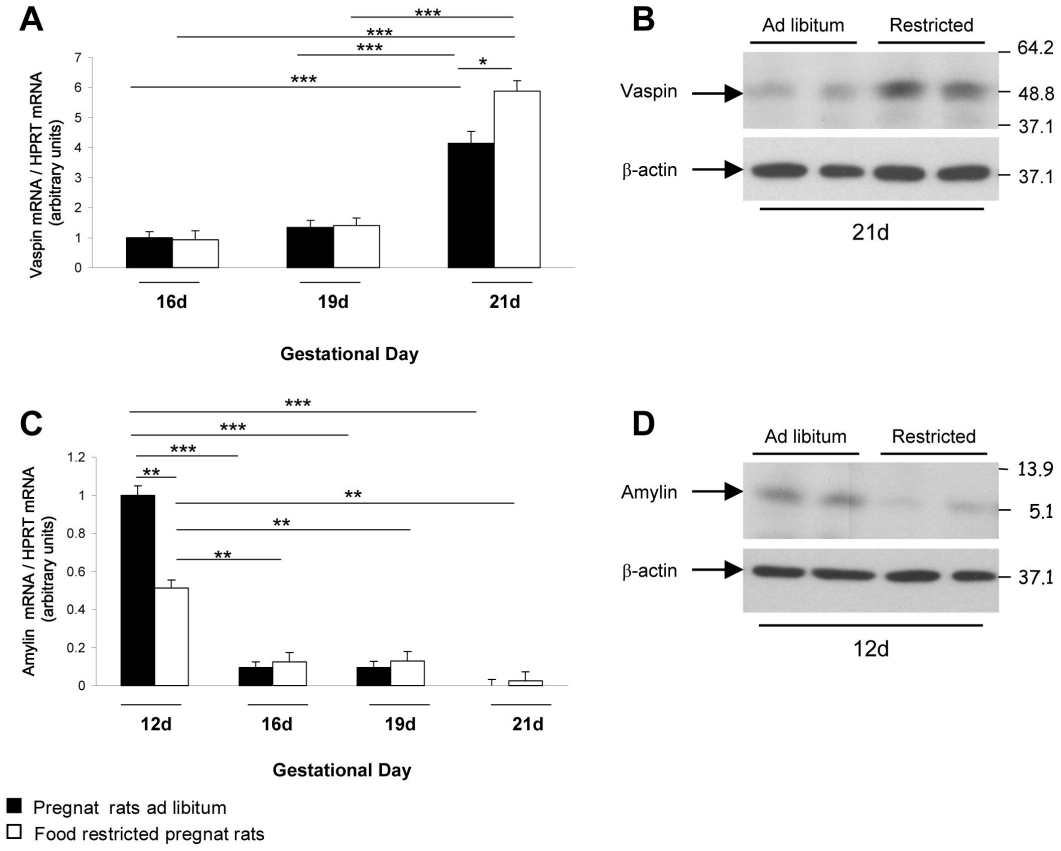


Fig. 5. Effect of chronic food restriction on vaspin and amylin expression in rat placenta Vaspin **(A and B)** and amylin **(C and D)** mRNA and protein expression in the chronic food-restricted rat placenta during the course of pregnancy compared with control animals fed ad libitum. Values are expressed relative to HPRT expression (mean±SEM; n=8 placentas per group dissected from 8 different dams).*: P<0.05 vs. ad libitum; **: P<0.01 vs. ad libitum; ***: P<0.001 vs. ad libitum.

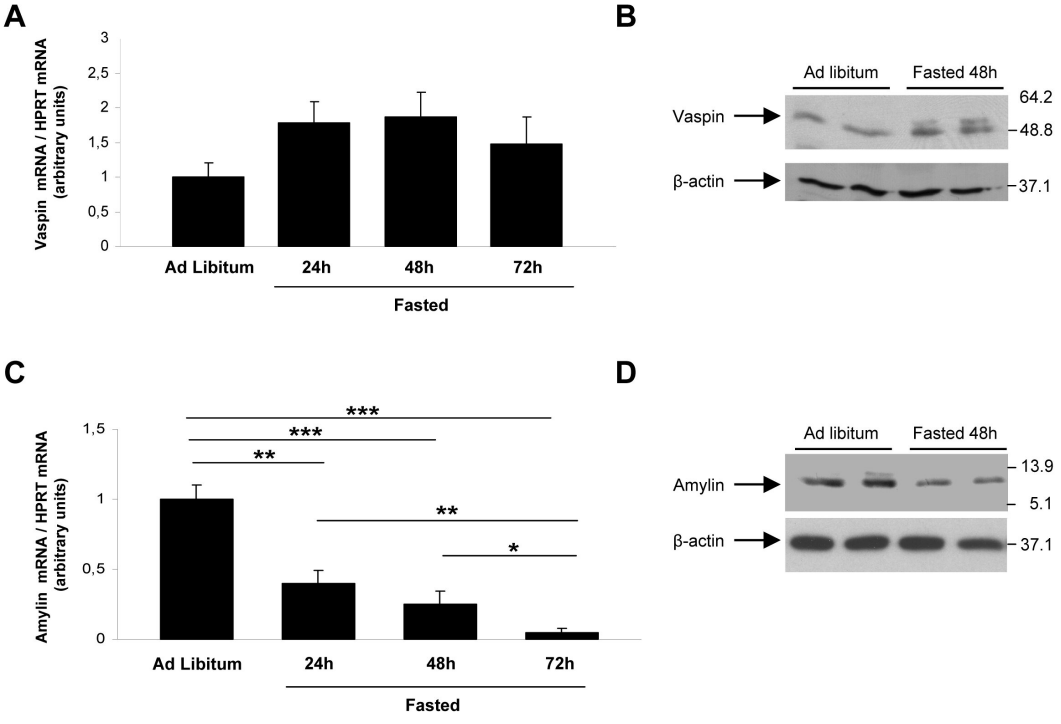


Fig. 6. Effect of acute food restriction on vaspin and amylin expression in rat placenta Vaspin **(A and B)** and amylin **(B and D)** mRNA and protein expression in rat placenta on 16th day of pregnancy food restricted for 24, 48 or 72h, compared with control animals fed ad libitum. Values are expressed relative to HPRT expression (mean±SEM; n=8 placentas per group dissected from 8 different dams). *: P< 0.05 vs. ad libitum; **: P<0.01 vs. ad libitum; ***: P<0.001 vs. ad libitum.

studied times (Fig. 6C,D).

Discussion

The interactions among fetal, placental and maternal hormones are largely unknown. In the present study we decided firstly, to determine the spatial expression of vaspin and amylin in human and rat placenta; secondly, we analyzed the changes of rat placenta vaspin and amylin messenger and protein in different gestational ages. Finally we assessed the effects of chronic and acute food restriction on their expression levels.

Here, we demonstrated for the first time that vaspin and amylin were unambiguously expressed in both rat and human placenta. The identification was obtained using several different approaches. The first one using a RT-PCR analysis showed that both amylin and vaspin mRNAs were normally expressed in the placenta. The validity of those data was further confirmed by immunohistochemistry. Taking into account that the placenta comsist of a heterogeneous cell population it was important to determine which cell type expresses these two proteins. Immunohistochemistry analyses have shown that vaspin protein is present within the placenta structure, located in the trophoblast of the fetal villi, the labyrinth. In addition, vaspin was localized in human placenta within cytotrophoblast and syncytiotrophoblast in the first trimester placenta, while in the third trimester vaspin was localized just in the syncytiotrophoblast. Regarding amylin, rat placenta of 16 days of gestational age showed an intense immunostaining, mainly localized in the labyrinth. Furthermore, in human third trimester placenta amylin immunoreactivity was intense in the syncytiotrophoblast of the chorionic villi and in decidual cells.

Placenta produces a number of molecules which are secreted into the maternal circulation, and have profound effects on maternal intermediary metabolism in order to attend the fetal growth energy demand. Previous studies have shown that amylin which is co-secreted from the pancreatic beta-cell with insulin, enter the brain and interact with satiety signals on neurons of the area postrema (AP) in the hindbrain, and appears to be involved in control of body weight (Riediger et al., 2004; Osto et al., 2007). Furthermore, peripheral or central administration of amylin dose-dependently reduces food intake in rats and mice by decreasing meal size (Osto et al., 2007). Other studies had shown that the first two trimesters of pregnancy in human (or an equivalent period in rat), are considered to be anabolic and the final trimester catabolic (Herrera, 2000). In the present study we show that amylin mRNA expression levels changed along the gestational age with a specific pattern of expression. Previous studies failed to detect amylin mRNA expression in human term placenta using northern blot analysis after a long exposure (Grigorakis et al., 1997). These results are in accordance with our data, which explain their failure since the highest levels of amylin were detected in early placenta samples, whereas the lowest levels were detected at the end of pregnancy. Furthermore, amylin mRNA levels were significantly lower in food restricted placenta when compared to fed ad libitum rats in the early pregnancy period of study. Taken together our data suggest that placenta amylin might play an important maternal and fetal metabolic role at early pregnancy, since mRNA placenta amylin levels get lower as gestation is moving ahead. Those lower levels might imply a positive maternal-fetal energetic balance, since adiposity and body weight are increased at the end of pregnancy. It is likely to be associated with higher anabolic rates concomitant with leptin, resistin and insulin resistance. In this sense, recent findings showed that throughout pregnancy maternal adipose tissue is metabolically active, producing adipocytokines involved in the process of insulin resistance (Mastorakos et al., 2007). Moreover, during normal pregnancy in non-obese and non-diabetic rats, adipose tissue increases, accompanied by a significant progressive increase of insulin resistance (Reis and Petraglia, 2001).

Our results show that vaspin mRNA expression levels changed along the gestational age with a specific pattern of expression, which resembles the one previously reported for leptin, resistin and adiponectin (Garcia et al., 2000; Caminos et al., 2005b; Caja et al., 2005) and in clear contrast to amylin. Over this period of gestation, the lowest expression values for placenta vaspin mRNA levels were detected in early placenta samples, whereas the highest levels were observed in late gestation. Furthermore, placenta vaspin mRNA levels were significantly elevated in pregnancy with intrauterine growth restriction compared with control pregnancies rats at the end of pregnancy. These changes in vaspin mRNA expression in the rat placenta during pregnancy may contribute to the concentration levels of these proteins in both the fetal and maternal circulations. Since late pregnancy is characterized by the development of insulin resistance, hyperleptinemia, hyperresistinemia, increased body weight and adiposity both in human and rat (Mitchell et al., 2005), these results are in agreement with our findings, showing higher levels of vaspin and lower levels of amylin mRNA expression in the placenta of late pregnant rats. Thus, altogether these data might suggest that amylin plays an important role involved in the anabolic control of food intake, whereas Vaspin has a catabolic function during pregnancy.

In conclusion, the results of the current investigation show for the first time that amylin and vaspin are expressed in human and rat placenta. Furthermore, we have determined the pattern of gene expression in the rat placenta of these genes in normal pregnancy, as well as the effect of acute and chronic food restriction. Our data demonstrate that human and rat placenta are an important source of vaspin and amylin and these results suggest that both genes may have an important role in the placenta, fetal and maternal function, maybe related with the control of energy balance during gestation.

Thus, placental vaspin and amylin may have physiological effects during pregnancy and these factors might contribute to the endocrine changes in energy balance which occur throughout gestation.

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